Wydział Farmaceutyczny
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Ocena możliwości wykorzystania ekstraktu z owoców *Aronia melanocarpa* L. w profilaktyce uszkodzenia nerek przez kadm – badania w modelu doświadczalnym *in vivo*

Rozprawa doktorska w oparciu o cykl publikacji naukowych
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Pragnę złożyć najszczersze wyrazy wdzięczności
Pani prof. dr hab. n. med. Małgorzacie Michalinie Brzóska
za poświęcony czas, życzliwość, wyrozumiałość, wsparcie,
porady merytoryczne oraz nieocenioną pomoc na każdym etapie
przygotowania niniejszej pracy doktorskiej.

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Spis treści strona

1. Wykaz publikacji będących podstawą rozprawy doktorskiej

Podstawą niniejszej rozprawy doktorskiej jest **cykl trzech** niżej wymienionych **publikacji naukowych** (jedna praca przeglądowa – Publikacja I i dwie prace oryginalne – Publikacja II i Publikacja III) **o łącznym współczynniku oddziaływania (*Impact Factor* – IF)** równym **11,6** i **punktacji Ministerstwa Edukacji i Nauki (MEiN)** wynoszącej **420**.

Publikacja I (praca przeglądowa)

Smereczański N.M., Brzóska M.M.: Current levels of environmental exposure to cadmium in industrialized countries as a risk factor for kidney damage in the general population: A comprehensive review of available data. International Journal of Molecular Sciences, 2023, 24 (9), 8413; doi.: 10.3390/IJMS24098413
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Publikacja II (praca oryginalna)

Smereczański N.M., Brzóska M.M., Rogalska J., Hutsch T.: The protective potential of *Aronia melanocarpa* L. berry extract against cadmium-induced kidney damage: A study in an animal model of human environmental exposure to this toxic element. International Journal of Molecular Sciences, 2023, 24 (14), 11647; doi.: 10.3390/ijms241411647
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Publikacja III (praca oryginalna)

Smereczański N.M., Brzóska M.M., Rogalska J.: Protective effect of the extract from *Aronia melanocarpa* L. berries against cadmium-induced oxidative stress in the kidney: A study in an *in vivo* experimental model. Acta Poloniae Pharmaceutica – Drug Research, 2023, 80 (4), praca w druku; doi.: 10.32383/appdr/169782
IF = 0,4; MEiN = 140

W Publikacji I i suplemencie do tej publikacji przedstawiono podstawy teoretyczne podjętej tematyki badawczej w zakresie nefrotoksyczności kadmu i ryzyka uszkodzenia nerek w konsekwencji narażenia środowiskowego na ten ksenobiotyk.

W Publikacji II i suplemencie do tej publikacji oraz w Publikacji III uzasadniono celowość podjęcia badań będących przedmiotem rozprawy doktorskiej, scharakteryzowano model doświadczalny, w którym przeprowadzono badania, omówiono metodykę badań i podano szczegółowe wyniki badań oraz przeprowadzono ich dyskusją w świetle dostępnych danych literaturowych i sformułowano wnioski końcowe z przeprowadzonych badań.

2. Wprowadzenie. Przegląd literatury dotyczący tematu rozprawy

2.1. *Aronia melanocarpa* L. jako roślina lecznicza

Rośliny lecznicze oraz produkty uzyskiwane na ich bazie od dawna budzą zainteresowanie zarówno środowiska naukowego, jak również społeczeństwa, w aspekcie możliwości wykorzystania substancji w nich występujących w profilaktyce i leczeniu różnych schorzeń, w tym chorób cywilizacyjnych [50, 55, 59, 64]. Ostatnio coraz więcej uwagi poświęca się również możliwości wykorzystania produktów pochodzenia roślinnego w celu zapobiegania niekorzystnym dla zdrowia skutkom wynikającym z narażenia na substancje toksyczne oraz łagodzenia i leczenia tych efektów [4, 7, 39]. Rośliną zasługującą na szczególną uwagę, zarówno ze względu na liczne właściwości prozdrowotne i przydatność w profilaktyce i wspomaganiu leczenia niektórych schorzeń [4, 27, 44, 50, 55, 60, 68], jak również z powodu wyników badań doświadczalnych wskazujących na możliwość jej wykorzystania w zapobieganiu skutkom działania toksycznego niektórych ksenobiotyków, w tym leków oraz zanieczyszczeń chemicznych środowiska i żywności, wliczając toksyczne metale ciężkie [4, 5, 7–12, 14, 16, 29, 30, 39–41, 70–72], jest *Aronia melanocarpa* L. ((Michx.) Elliott; aronia czarnoowocowa).

2.1.1. Charakterystyka ogólna rośliny

A. melanocarpa jest krzewem liściastym (Rycina 1) należącym do rodziny *Rosaceae*. Roślina ta pochodzi z Ameryki Północnej, ale obecnie jest rozpowszechniona na całym świecie [59], a jej niskie wymagania środowiskowe ułatwiają uprawę [2]. Krzewy aronii mogą dorastać do około 2 m wysokości. Pędy rośliny są przylegająco owłosione lub nagie, a naprzemianlegle ułożone liście o barwie ciemnozielonej (jesienią czerwieniejące) osiągają długość do 6 cm. W maju i czerwcu krzewy wytwarzają baldachogrona zawierające około 30 drobnych białych kwiatów (Rycina 1), z których dojrzewają ciemnopurpurowe jagody (Rycina 1) o średnicy około 10 mm i słodkawym, silnie aromatycznym miąższu, pokryte szarawym nalotem [59]. Zbiór owoców aronii odbywa się w sierpniu i wrześniu. Jakość jagód aronii oraz zawartość w nich składników aktywnych biologicznie zależą od warunków klimatycznych panujących w czasie wegetacji oraz nawodnienia, stosowania pestycydów, terminu zbioru owoców i warunków ich przechowywania [2]. Pomimo niskiej kumulacji w owocach aronii substancji szkodliwych dla zdrowia, w tym pestycydów i metali ciężkich takich jak ołów czy kadm (Cd), uprawa tej rośliny do celu wykorzystania prozdrowotnego powinna odbywać się na terenach o niskim poziomie industrializacji, z zachowaniem najwyższych standardów uprawy ekologicznej. Surowcem użytkowym *A. melanocarpa* są przede wszystkim owoce, ale również liście, jednak ich zastosowanie nie jest tak powszechne, jak owoców [4, 55, 64, 69].

Rycina 1. Krzew, kwiatostan i owoce *A. melanocarpa.*

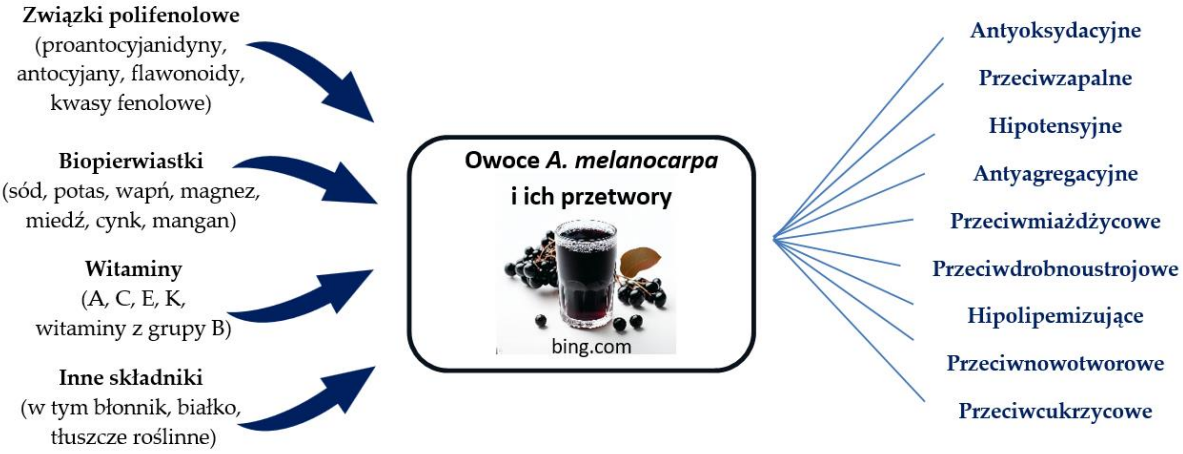
2.1.2. Właściwości prozdrowotne owoców *A. melanocarpa* i ich przetworów

Owoce aronii czarnoowocowej zawierają wiele substancji o właściwościach prozdrowotnych i są jednym z najbogatszych źródeł naturalnych związków polifenolowych charakteryzujących się silnymi właściwościami antyoksydacyjnymi [2, 4, 50, 55, 59]. Związki polifenolowe, takie jak proantocyjanidyny, antocyjany, flawonoidy i kwasy fenolowe (Tabela 1) są najważniejszymi, z punktu widzenia działania prozdrowotnego, składnikami występującymi w owocach *A. melanocarpa* [4, 50, 55, 59]. Proantocyjanidyny są oligomerycznymi i polimerycznymi katechinami, których obecność nadaje owocom aronii cierpki smak. Antocyjany występują w zewnętrznych częściach skórki owoców i odpowiadają za ich kolor. Owoce aronii są także źródłem biopierwiastków, witamin, triterpenów, fitosteroli, karotenoidów, pektyn, alkoholi cukrowych (parasorbozyd i sorbitol), garbników, błonnika pokarmowego, kwasów organicznych (kwas L-jabłkowy i kwas cytrynowy), węglowodanów i białek (Tabela 1) [4, 59].

Substancje zawarte w jagodach aronii wykazują m.in. silne właściwości przeciwutleniające, przeciwcukrzycowe, przeciwzapalne, przeciwdrobnoustrojowe i antymutagenne (Rycina 2) [4, 16, 50, 55, 59]. Spożywanie produktów z jagód aronii, takich jak soki, dżemy, konfitury, syropy, herbaty i nalewki oraz sproszkowane owoce w postaci suplementów, ma wpływ kardioprotekcyjny, gastroprotekcyjny, hepatoprotekcyjny, radioprotekcyjny i immunomodulujący, w związku z czym może skutecznie chronić przed rozwojem niektórych chorób przewlekłych, w tym zaburzeń metabolicznych, chorób układu krążenia i nowotworów, oraz jest stosowane wspomagająco w leczeniu schorzeń takich jak cukrzyca typu II, nadciśnienie, otyłość, infekcje bakteryjne, czy zaburzenia pracy wątroby [4, 27, 44, 50, 55, 59, 60, 68]. Ze względu na wielokierunkowe właściwości prozdrowotne owoców *A. melanocarpa* zaleca się wprowadzanie produktów na ich bazie do diety codziennej [4, 50, 55]. Na rynku dostępne są liczne suplementy zawierające w składzie te owoce (Tabela 2).

Tabela 1. Główne składniki owoców *A. melanocarpa* i ich zawartość.

Składnik	Zawartość (mg/100 g świeżych owoców)	Źródło
Białka	600 – 810	66
Węglowodany	13730 – 15060	66
Tłuszcze	90 – 170	66
Związki polifenolowe	247,70 – 693,05	24
proantocyjanidyny	663,7 – 1645,64	61, 74
antocyjany	150,09 – 619,2	24, 73
cyjanidyno–3–O–arabinozyd	36,72 – 544	24, 67
cyjanidino–3–O–galaktozyd	41 – 1243	37, 67
cyjanidyno 3–O–glukozyd	3,41 – 46,2	24, 67
cyjanidyno–3–O–ksylozyd	4,43 – 73	24, 67
flawonole	7,643 – 71	61, 66
kemferol	53	61
kwercetyno–3–O–galaktozyd	8,31 – 28,3	49, 67
kwercetyno–3–O–glukozyd	4,03 – 20,8	49, 67
kwercetyno–diheksozyd	4,4 – 5,67	69, 73
kwercetyno–3–O–rutynozyd	3,9 – 40,30	24, 73
kwercetyno–3–O–glukuronid	56	42
kwercetyno–3–O–wicianozyd	2,36 – 8,5	49, 67
kwercetyno–3–O–ksylozyd	0,15 – 0,40	18, 42
kwercetyna	0,03 – 7,113	18, 24, 73
epikatechina	0,12 – 86,250	18, 61
kwasy fenolowe	63,9 – 96	18, 36
kwas kawowy	60 – 75	36
kwas chlorogenowy	41,69 – 218	66, 67
kwas neochlorogenowy	32,24 – 189	24, 67
kwas 4–hydroksycynamonowy	0,02 – 7,61	18, 36
kwas ferulowy	0,01 – 2,8	18, 36
kwas elagowy	1,57	18
Biopierwiastki		
sód	1,25 – 3,7	51, 66
potas	135,63 – 497,7	51, 65
wapń	11,90 – 116,7	51, 65
magnez	8,33 – 57,8	51, 65
fosfor	15,9 – 95,6	51, 66
cynk	0,090 – 0,840	51, 66
żelazo	0,33 – 1,68	66
selen	0,021 – 0,028	51
miedź	0,035 – 0,211	51, 66
mangan	0,132 – 1,789	51, 66
Witaminy		
witamina C	1,9 – 31	49, 61
witamina E	1,35 – 1,47	66
witamina A	0,77	31
witamina K	0,017 – 0,028	66
witamina B1	0,017 – 0,019	66
witamina B2	0,016 – 0,027	66
witamina B3	0,27 – 0,34	66
witamina B5	0,225 – 0,382	66
witamina B6	0,024 – 0,029	66
witamina B11	0,002 – 0,004	66
Karotenoidy – β-karoten	0,495 – 0,887	66



Rycina 2. Właściwości prozdrowotne owoców *A. melanocarpa* i ich przetworów.

Skuteczność owoców *A. melanocarpa* i ich przetworów w profilaktyce i wspomaganiu leczenia szeregu stanów chorobowych została wykazana w wielu badaniach [4, 17, 23, 27, 43, 44, 46, 50, 55, 60, 68]. Czterotygodniowe spożywanie przez pacjentów ze zdiagnozowanym zespołem metabolicznym standaryzowanego ekstraktu z owoców *A. melanocarpa* (Alixir 400 PROTECT, Pharmanova, Belgrad, Serbia), trzy razy dziennie po 30 mL (przed lub w trakcie posiłku) [68] lub 100 mL w jednej porcji dziennie preparatu uzyskanego z czystego soku z aronii wzbogaconego w błonnik (Nutrika d.o.o., Belgrad, Serbia) [27] przyczyniało się do obniżenia masy ciała (średnio o 2,2 – 2,5 kg) i zmniejszenia obwodu talii (średnio o 3,2 – 4,2 cm) [27, 68]. Ponadto wprowadzenie do diety produktów z owoców aronii czarnoowocowej (sok w ilości 250 mL dziennie przez 12 tygodni lub 100 mL dziennie przez 4 tygodnie) poprawiało profil lipidowy u osób z hipercholesterolemią, co znajdowało odzwierciedlenie w obniżeniu stężenia triglicerydów, cholesterolu całkowitego i lipoprotein o niskiej gęstości (LDL) w krwi [60] oraz obniżało ciśnienie tętnicze krwi i zmniejszało ryzyko epizodów zakrzepowo - zatorowych, dzięki obniżeniu stężenia fibrynogenu [60]. Spożywanie przez zdrowych mężczyzn 116 mg ekstraktu z owoców lub 12 mg sproszkowanych owoców *A. melanocarpa* dziennie przez 12 tygodni skutkowało poprawą składu mikroflory jelitowej oraz funkcji śródbłonka naczyniowego, zmniejszając tym samym ryzyko epizodów sercowo - naczyniowych [23]. U osób z zespołem metabolicznym picie dwa razy dziennie po 100 mL ekstraktu z owoców *A. melanocarpa* przez okres 2 miesięcy spowodowało obniżenie stężenia dialdehydu malonowego (MDA) oraz cholesterolu całkowitego i jego frakcji LDL, a także zwiększyło całkowity status antyoksydacyjny (TAS) osocza [17]. Tasic i wsp. [68] stwierdzili, iż stosowanie ekstraktu z owoców aronii (trzy razy dziennie po 30 mL przez 4 tygodnie) przez pacjentów z zespołem metabolicznym poprawiało profil lipidowy oraz obniżało glikemię i ciśnienie tętnicze krwi.

Tabela 2. Suplementy dostępne na rynku polskim sporządzone na bazie owoców *A. melanocarpa*. [1]

Nazwa suplementu	Producent	Zawartość ekstraktu z owoców *A. melanocarpa*
Kapsułki zawierające suchy ekstrakt (zawartość w kapsułce)		
Melarginol	AronPharma	nie podano
Aronia ekstrakt	Yango	470 mg
Aronia Black Chokeberry Fruit	Swansson	400 mg
Aronia czarna	Pharmovit	200 mg
Aronia Powder	Pharmovit	450 mg
Aronia Gold	Pharmovit	350 mg (200 mg standaryzowany suchy ekstrakt + 150 mg wytłoki z owoców aronii)
Błonnik + Aronia	Vitama	100 mg
Aronia +	Herbapol w Krakowie	200 mg
Aronia ekstrakt	Bioherbs	410 mg
Immun + Aronia	DoppelHerz	50 mg
Berroxin	AronPharma	245 mg (mieszanka owoców aronii i czarnego bzu)
Rutyna Immuno+	Aura Herbals	200 mg
BioAronia	Kolagenum	nie podano przez producenta
Aronia	Fairvital	300 mg
Aronia	Organis	545 mg
Retico Aronvit Zdrowie Oczu	AronPharma	75 mg
Lutezan Premium	Adamed	50 mg
Suchy ekstrakt		
Organic Aronia Freezed Dried Powder	Yagoody Superfood	100%
Aronia 100% Organic	This is BIO®	100%
Beta Cruenta Plus	Guardian International	(mieszanka buraka i owoców aronii)
Witamina C z aronią	Visanto	mieszanka zawierająca 100 mg ekstraktu z owoców aronii/1 g proszku
Aronia	BrainMax Pure	100%

[1] opracowano na podstawie przeglądu suplementów dostępnych na rynku (w lipcu 2023 r.)

Wykazano także korzystny wpływ spożywania soku z owoców *A. melanocarpa* przez osoby z cukrzycą typu II (3 razy dziennie po 50 mL przez 3 miesiące) [44] i hipercholesterolemią (250 mL dziennie przez 18 tygodni) [60] na gospodarkę węglowodanową organizmu, o czym świadczyło obniżenie stężenia glukozy [44] lub hemoglobiny glikowanej (HbA1c) [60] w krwi. Ponadto u pacjentów z cukrzycą typu II odnotowano obniżenie stężenia kreatyniny w surowicy [44]. Stwierdzono, iż regularne picie 100 mL soku z owoców aronii dziennie poprawiało także funkcjonowanie wątroby u osób z zespołem metabolicznym już po czterech tygodniach stosowania [68]. Spożywanie produktów z owoców aronii czarnoowocowej przez osoby po zawale serca (3 razy dziennie po 85 mL ekstraktu przez 6 tygodni) obniżało stężenie cytokin prozapalnych, takich jak czynnik martwicy nowotworów alfa (TNF-α) i interferon gamma (IFN-γ) [46]. Podawanie pacjentom hemodializowanym, u

których rozwinęła się anemia, ekstraktu z owoców *A. melanocarpa* w ilości 30 mL dziennie przez miesiąc obniżało stężenie prozapalnego białka C-reaktywnego i rodników tlenowych oraz zwiększało stężenie glutationu zredukowanego (GSH) i aktywność katalazy (CAT) w krwi [43]. Handeland i wsp. [20] stwierdzili, iż dzienne spożywanie przez osoby w wieku podeszłym 200 mL soku z owoców *A. melanocarpa* (zawierającego 715 mg związków polifenolowych w 100 mL) przez 3 miesiące zmniejszało częstość występowania infekcji bakteryjnych dróg moczowych oraz skracało czas ich przebiegu objawowego, zarówno w trakcie spożywania soku, jak i w okresie do 3 miesięcy po zakończeniu jego przyjmowania.

2.2. Ekspozycja środowiskowa na kadm jako czynnik ryzyka uszkodzenia nerek w populacji generalnej

Kadm jest metalem ciężkim charakteryzującym się wysoką toksycznością dla organizmu człowieka i zwierząt [6, 13, 32, 38, 48, 56, 58, 75]. Pierwiastek ten został zaliczony do najbardziej niebezpiecznych ksenobiotyków, na które człowiek może być narażony w środowisku życia [48]. Postęp technologiczny w ostatnich dziesięcioleciach i związane z nim rosnące wykorzystanie kadmu i jego związków prowadzi do wzrostu zawartości tego pierwiastka w środowisku, co skutkuje nieuchronnym narażeniem środowiskowym na ten ksenobiotyk [47, 48, 75]. Głównym źródłem narażenia populacji generalnej na ten pierwiastek toksyczny jest żywność, zwłaszcza produkty pochodzenia roślinnego [38, 47, 48, 75], a w przypadku osób nałogowo palących tytoń – dym tytoniowy [48, 62].

Z przeglądu aktualnych danych wynika, iż narażenie środowiskowe na kadm w krajach uprzemysłowionych jest obecnie na ogół niskie lub umiarkowane [19, 35, 38, 62, 63], nie mniej jednak wykazuje tendencję wzrostową [22, 58, 75]. Badania epidemiologiczne dostarczają coraz więcej dowodów na to, że przewlekła, nawet niska, ekspozycja na ten pierwiastek toksyczny może skutkować uszkodzeniem wielu narządów i układów, w tym nerek i wątroby, układu kostnego, układu sercowo - naczyniowego i układu nerwowego, a także pogorszeniem słuchu i wzroku oraz przyczyniać się do rozwoju chorób nowotworowych [26, 34, 38, 48, 54, 56, 58].

Nerka, jako główny organ, w którym kadm ulega kumulacji w organizmie, a jednocześnie narząd odpowiedzialny za detoksykację i eliminację tego pierwiastka z ustroju, jest szczególnie podatna na uszkodzenie przez ten ksenobiotyk. Organ ten jest narządem tarczowym (narząd uszkadzany jako pierwszy) dla kadmu podczas narażenia przewlekłego [58, 62]. Ponieważ kadm jest bardzo wolno eliminowany z organizmu, pierwiastek ten stopniowo ulega nagromadzaniu w nerce wraz z czasem narażenia [8]. Średni okres półtrwania kadmu w nerkach u ludzi wynosi 14 lat, ale niektóre dane sugerują, że może on sięgać nawet 45 lat [48, 62]. Zawartość kadmu w nerkach wzrasta wraz z wiekiem osiągając szczyt w wieku około 60 lat [48].

Jony kadmu (Cd^{2+}), po wchłonięciu do krwioobiegu, wiążą się z grupami tiolowymi (grupy sulfhydrylowe, grupy -SH) białek w błonach erytrocytów i osoczu (głównie z albuminami) i w tej formie są transportowane wraz z krwią głównie do wątroby, gdzie indukują syntezę metalotioneiny (MT) i tworzą z tym białkiem kompleksy (Cd-MT), w formie których ulegają kumulacji. Kompleksy Cd-MT mogą być stopniowo uwalniane z wątroby do krwioobiegu i są łatwo filtrowane przez kłębuszki nerkowe, a następnie wchłaniane zwrotnie przez kanaliki proksymalne na drodze endocytozy lub rozkładane w komórkach nabłonka kanalików na jony Cd^{2+} i aminokwasy [53]. Następnie jony te indukują syntezę MT w nerkach i tworzą z tym białkiem nietoksyczne (w przestrzeni wewnątrzkomórkowej) kompleksy (Cd-MT), w formie których ulegają kumulacji w tym narządzie. Wiązanie jonów Cd^{2+} przez MT w nerce jest procesem detoksykacji tego pierwiastka [25, 28, 53]. Kompleksy Cd-MT charakteryzują się krótkim czasem półtrwania (około 3 dni) [53] i ulegają rozkładowi z uwolnieniem jonów Cd^{2+}, które indukują syntezę MT, a następnie wiążą się z tym białkiem. Jednak zdolność nerek do biosyntezy MT i kumulacji kadmu w postaci nietoksycznych kompleksów z tym białkiem jest ograniczona. Gdy jony Cd^{2+} nie mogą być już dłużej detoksykowane poprzez wiązanie z MT, zaczynają wiązać się z grupami -SH innych białek, w tym białek strukturalnych i czynnościowych oraz grupami funkcyjnymi innych makrocząsteczek, wywierając w ten sposób działanie toksyczne, co w rezultacie prowadzi do uszkodzenia kanalików proksymalnych [53].

Według aktualnej wiedzy mechanizm działania toksycznego kadmu na nerkę jest wielokierunkowy i obejmuje indukcję procesów zapalnych, rozwój stresu oksydacyjnego, zmiany w adhezji komórek, stymulację proliferacji komórek oraz indukcję zmian epigenetycznych (uszkodzenie kwasu dezoksyrybonukleinowego – DNA, zahamowanie zdolności naprawy DNA, metylacja genów i zaburzenie ekspresji genów) [53, 62].

Działanie nefrotoksyczne kadmu, ze szczególnym zwróceniem uwagi na ryzyko uszkodzenia nerek na skutek narażenia środowiskowego, zostało szeroko omówione w **Publikacji I** wchodzącej w skład niniejszej rozprawy doktorskiej [62]. Na podstawie kompleksowego przeglądu dostępnych danych dotyczących ryzyka uszkodzenia nerek na skutek aktualnego narażenia na kadm w krajach rozwiniętych gospodarczo (**Publikacja I**) stwierdzono, że najniższe stężenie kadmu w krwi i moczu przy którym może dochodzić do dysfunkcji kłębuszków nerkowych, która stanowi klinicznie istotne uszkodzenie tego narządu (wartość LOAEL; ang. *the lowest observed adverse effect level*), przekracza odpowiednio 0,18 μg/L i 0,27 μg/g kreatyniny. Wartości te mieszczą się w dolnym przedziale stężeń tego pierwiastka aktualnie notowanych w krwi (0,02 – 4,40 μg/L) i moczu (0,04 – 3,39 μg/g kreatyniny) mieszkańców krajów uprzemysłowionych [62], co wskazuje iż ekspozycja środowiskowa na kadm stanowi czynnik ryzyka dysfunkcji nerek. Ryzyko uszkodzenia tego narządu na skutek niskiego i umiarkowanego narażenia nie zostało jednak w pełni oszacowane, a mechanizm tego działania jest niewystarczająco poznany [62]. Przegląd dostępnej literatury (**Publikacja I)** wykazał, iż aktualne narażenie środowiskowe na kadm może stwarzać ryzyko nie tylko

uszkodzenia kanalików nerkowych, ale także istotnego klinicznie zaburzenia, którym jest dysfunkcja kłębuszków nerkowych, co rzuciło nowe światło na ten ksenobiotyk jako możliwy czynnik etiologiczny przewlekłej niewydolności nerek. Narażenie środowiskowe na kadm w krajach uprzemysłowionych stanowi zatem istotny problemem zdrowia publicznego. W związku z tym powinno być ono uważnie monitorowane i należy prowadzić dalsze badania w celu dokładniejszej oceny ryzyka uszkodzenia nerek w przypadku niskiego narażenia na kadm i poznania mechanizmu nefrotoksycznego działania tego pierwiastka.

2.3. Owoce *A. melanocarpa* i ich przetwory jako potencjalna strategia w zapobieganiu niekorzystnym dla zdrowia skutkom narażenia na ksenobiotyki lub ich łagodzeniu

Wielokierunkowy korzystny wpływ przetworów z owoców *A. melanocarpa* na zdrowie człowieka [4, 20, 23, 27, 31, 43, 44, 46, 50, 55, 60, 68], w tym zwłaszcza ich wysoki potencjał antyoksydacyjny i zdolność kompleksowania jonów metali ciężkich [4, 24, 43, 59, 64, 66, 74] spowodowały, że zwrócono uwagę również na możliwość wykorzystania tych produktów w przeciwdziałaniu niekorzystnym skutkom narażenia na substancje szkodliwe dla zdrowia. Chociaż badania w tym zakresie są dotychczas nieliczne, to ich wyniki są bardzo obiecujące. Wykazano bowiem skuteczność soku i ekstraktu z owoców aronii czarnoowocowej w zapobieganiu działaniu toksycznemu niektórych zanieczyszczeń chemicznych środowiska i leków lub łagodzeniu skutków tego działania [4, 5, 7–12, 14, 16, 29, 30, 39–41, 70–72].

Valcheva-Kuzmanova i wsp. [71] prawie 20 lat temu opublikowali wyniki badań wskazujące, iż podawanie soku z owoców *A. melanocarpa* (5, 10 lub 20 mL/kg masy ciała – m.c. dziennie przez 4 dni) znosiło indukowane przez tetrachlorek węgla ostre uszkodzenie wątroby u szczurów, o czym świadczył powrót do wartości prawidłowych aktywności biomarkerów hepatotoksyczności, takich jak aminotransferaza asparaginianowa (AST) i aminotransferaza alaninowa (ALT) w osoczu oraz stężenia MDA i GSH w wątrobie. Stwierdzono również, iż sok podawany w dawce 10 mL/kg m.c. przez 28 dni całkowicie chronił płuca szczurów przed negatywnymi skutkami stosowania amiodaronu, takimi jak wzrost liczby limfocytów, stężenia prozapalnej interleukiny 6 (IL-6) i aktywności ALP w popłuczynach oskrzelowo - pęcherzykowych oraz stężenia MDA w tkance płucnej [72]. Należy podkreślić, iż dotychczasowe badania na ogół ograniczały się do oceny wpływu przetworów z owoców aronii na wybrane efekty działania toksycznego ksenobiotyków [16, 29, 70–72]. Pierwsze, i jedyne, jak dotychczas, kompleksowe badania w zakresie możliwości wykorzystania przetworów z owoców aronii czarnoowocowej w ochronie przed skutkami narażenia na ksenobiotyki dotyczą kadmu i zostały podjęte przez zespół badaczy z Uniwersytetu Medycznego w Białymstoku [5, 8–12, 14, 30, 40, 41].

Uwagę na możliwość wykorzystania substancji zawartych w owocach aronii w zapobieganiu skutkom narażenia na kadm po raz pierwszy zwrócili Kowalczyk i wsp. [29]. Autorzy w swoich badaniach ograniczyli się jednak do oceny wpływu na wątrobę i nerki.

Wykazali, że podanie antocyjanów z jagód *A. melanocarpa* w dawce 10 mg/kg m.c. podczas 30-dniowego narażenia na chlorek kadmu ($CdCl_2 \times 2{,}5\ H_2O$) w dawce 4 μg/kg m.c. zmniejszało kumulację kadmu w nerkach i wątrobie (odpowiednio o około 68% i 31%) oraz zapobiegało wzrostowi stężenia mocznika i aktywności ALT w surowicy, nie chroniło natomiast przed indukowanym przez ten ksenobiotyk wzrostem aktywności AST oraz stężenia bilirubiny i kreatyniny w surowicy. Poza badaniami przeprowadzonymi przez Kowalczyka i wsp. [29] oraz badaczy z Uniwersytetu Medycznego w Białymstoku [5, 8–12, 14, 30, 40, 41] w dostępnym piśmiennictwie brak jest badań dotyczących możliwości wykorzystania przetworów z owoców *A. melanocarpa* w łagodzeniu skutków narażenia na kadm. Brak jest również danych wskazujących na skuteczność tych produktów w odniesieniu do uszkodzenia nerek indukowanego przez inne ksenobiotyki. W badaniach na zdrowych komórkach nerki (linia HEK293T) stwierdzono jednak, że sok z owoców *A. melanocarpa* (w stężeniu 0,01 i 0,05 mg/mL) zmniejszał cytotoksyczność cisplatyny wobec tych komórek [70].

W badaniach realizowanych w Zakładzie Toksykologii Uniwersytetu Medycznego w Białymstoku podjęto ocenę możliwości wykorzystania ekstraktu z owoców *A. melanocarpa* jako czynnika ochronnego podczas narażenia przewlekłego na kadm. W tym celu stworzono model doświadczalny na samicach szczura odzwierciedlający aktualne niskie i umiarkowane narażenie człowieka na ten ksenobiotyk w krajach uprzemysłowionych (odpowiednio 1 i 5 mg Cd/kg paszy przez okres do 24 miesięcy), w którym oceniono wpływ ekstraktu z owoców aronii czarnoowocowej, podawanego w formie 0,1% roztworu wodnego, w odniesieniu do różnych efektów działania toksycznego kadmu. W dotychczas przeprowadzonych badaniach wykazano, że podawanie tego ekstraktu podczas niskiej (1 mg Cd/kg paszy) i umiarkowanej (5 mg Cd/kg paszy) ekspozycji na ten ksenobiotyk znacząco poprawiało enzymatyczną i nieenzymatyczną barierę antyoksydacyjną, obniżało stężenie związków o właściwościach prooksydacyjnych, chroniło przed rozwojem stresu oksydacyjnego i jego następstwami, takimi jak uszkodzenia oksydacyjne lipidów, białek i DNA w wątrobie oraz zmianami w strukturze morfologicznej tego narządu, a także normalizowało aktywność wskaźnikowych enzymów wątrobowych w surowicy [40, 41]. Ponadto, ekstrakt z owoców *A. melanocarpa* łagodził wywołane przez kadm zmiany w ekspresji kolagenu typu I i III na poziomie matrycowego kwasu rybonukleinowego (mRNA) i białka, a także zwiększał stężenie metaloproteinaz macierzy pozakomórkowej 1 i 2 (MMP-1 i MMP-2) i ich inhibitorów tkankowych (TIMP-1 i TIMP-2) w wątrobie, co świadczy o ochronie przed zaburzeniem homeostazy kolagenu indukowanym przez ten pierwiastek toksyczny w tym narządzie [30]. Podawanie ekstraktu z owoców aronii podczas narażenia na kadm w obu badanych stężeniach chroniło przed wywołanym przez ten ksenobiotyk stresem oksydacyjnym i peroksydacją lipidów oraz uszkodzeniami oksydacyjnymi białek i DNA również w tkance kostnej, a także przed zaburzeniami w metabolizmie kostnym i zmianami zawartości minerałów w kościach [10, 11]. Ponadto ekstrakt poprawiał biosyntezę kolagenu kostnego i właściwości biomechaniczne kości [12]. Podawanie ekstraktu z owoców *A. melanocarpa*

przeciwdziałało rozwojowi stresu oksydacyjnego i modyfikacjom oksydacyjnym makrocząsteczek komórkowych nie tylko w wątrobie [40, 41] i tkance kostnej [11], ale również w śliniankach podjęzykowych [14]. Suplementacja ekstraktem z owoców aronii zmniejszała kumulację kadmu w organizmie i zapobiegała lub przynajmniej częściowo chroniła przed większością indukowanych przez ten pierwiastek toksyczny zmian w metabolizmie cynku [5], miedzi [5] i manganu [9], a także łagodziła zmiany w aktywności zależnej od manganu dysmutazy ponadtlenkowej (Mn-SOD) i stężeniu tego biopierwiastka oraz chroniła przed kumulacją kadmu w mitochondriach, głównie w wątrobie [9].

3. Cel pracy z uzasadnieniem podjętej tematyki badawczej będący odniesieniem do publikacji stanowiących rozprawę doktorską

Dostępne wyniki badań dostarczają coraz więcej wiarygodnych dowodów świadczących o możliwości skutecznego stosowania substancji pochodzenia roślinnego w terapii i profilaktyce wielu schorzeń, w tym chorób cywilizacyjnych [4, 20, 23, 27, 31, 43, 44, 46, 50, 55, 60, 68]. W związku z tym coraz większa uwaga naukowców koncentruje się na możliwości wykorzystania surowców roślinnych o dobrze znanym działaniu prozdrowotnym również w zapobieganiu zagrożeniom dla zdrowia wynikającym z narażenia na ksenobiotyki. Surowcem roślinnym zasługującym na szczególną uwagę w aspekcie możliwości wykorzystania w minimalizowaniu skutków narażenia na kadm są owoce *A. melanocarpa*, które są cennym i powszechnie dostępnym surowcem farmaceutycznym.

Liczne właściwości prozdrowotne przetworów z owoców aronii czarnoowocowej, w tym zwłaszcza ich wysoki potencjał antyoksydacyjny oraz obecność związków polifenolowych, które mogą kompleksować jony metali dwuwartościowych [4, 24, 43, 59, 64, 66, 74], zwróciły uwagę zespołu badaczy z Zakładu Toksykologii Uniwersytetu Medycznego w Białymstoku na możliwość wykorzystania tego surowca w zapobieganiu skutkom narażenia na kadm [5, 8–12, 14, 30, 40, 41]. Ekspozycja na ten pierwiastek jest obecnie nieunikniona w krajach uprzemysłowionych, a prognozy wskazują, iż będzie ona wzrastała [22, 58, 75]. Z przeglądu wyników dostępnych badań epidemiologicznych wynika natomiast, iż aktualne narażenie środowiskowe na kadm w krajach uprzemysłowionych może zwiększać ryzyko kliniczne istotnego uszkodzenia nerek (dysfunkcja kłębuszków nerkowych) i prowadzić lub przyczyniać się do rozwoju przewlekłej niewydolności tego narządu (**Publikacja I**) [35, 48, 58, 62]. Ze względu na rolę, jaką nerki odgrywają w organizmie, prawidłowa ich funkcja warunkuje ogólny stan zdrowia [3, 45]. Przewlekła niewydolność tego narządu jest jedną z głównych przyczyn zgonów zarówno w krajach rozwiniętych, jak i rozwijających się [33, 52]. Biorąc pod uwagę rosnącą częstość występowania chorób nerek [21, 33, 52] należy rozpoznać wszystkie czynniki etiologiczne, a w świetle najnowszych danych epidemiologicznych kadm może być jednym z nich (**Publikacja I**) [3, 6, 19, 35, 45, 57, 62]. Dlatego też konieczne jest zarówno poznanie ryzyka uszkodzenia nerek na skutek aktualnego narażenia na kadm, jak również znalezienie skutecznego czynnika, który będzie mógł zostać wykorzystany w profilaktyce uszkodzenia tego narządu.

Ryzyko dysfunkcji poszczególnych narządów i układów, w tym nerek, w warunkach niskiego narażania populacji generalnej na kadm jest trudne do oceny ze względu na prawdopodobieństwo współwystępowania wielu czynników, które mogą wpływać na stan zdrowia. Wpływ ten można natomiast dobrze oszacować w modelach eksperymentalnych, które pozwalają na wykluczenie czynników zakłócających. Modele takie są również odpowiednie do poszukiwania skutecznych strategii ochronnych przed skutkami działania toksycznego kadmu. W Zakładzie Toksykologii Uniwersytetu Medycznego w Białymstoku

stworzono model doświadczalny na szczurach niskiego i umiarkowanego narażania populacji generalnej na kadm pozwalający ocenić nie tylko szkodliwy wpływ tego ksenobiotyku na zdrowie, ale również możliwość wykorzystania ekstraktu z owoców *A. melanocarpa* w ochronie przed skutkami narażenia na ten ksenobiotyk. W dotychczas przeprowadzonych badaniach wykazano, iż podawanie ekstraktu z tych owoców chroni przed kumulacją kadmu w organizmie, w tym nagromadzaniem się tego pierwiastka w nerkach i wątrobie oraz jego działaniem uszkadzającym na wątrobę, układ kostny i ślinianki, jak również zaburzeniem metabolizmu miedzi, cynku i manganu [5, 8–12, 14, 30, 40, 41]. Wyniki dotychczasowych badań pozwalają sądzić, iż przetwory z owoców aronii mogą być skuteczną strategią w ochronie przed skutkami narażenia środowiskowego na kadm. Zastosowanie ich w profilaktyce u ludzi wymaga jednak wcześniejszego kompleksowego poznania wpływu na różne efekty działania toksycznego kadmu, w tym zwłaszcza nerki, które na ogół jako pierwsze ulegają uszkodzeniu w wyniku narażania przewlekłego na ten ksenobiotyk.

HIPOTEZA BADAWCZA

Biorąc pod uwagę dotychczasowe wyniki badań przeprowadzonych w modelu doświadczalnym *in vivo* niskiego i umiarkowanego narażenia populacji generalnej na kadm oraz wielokierunkowe działanie prozdrowotne owoców *A. melanocarpa*, postawiono hipotezę, iż długotrwałe, nawet niskie, narażenie na ten pierwiastek toksyczny może prowadzić do uszkodzenia nerek, będących narządem tarczowym dla tego ksenobiotyku, a podawanie ekstraktu z owoców aronii może chronić przed jego działaniem nefrotoksycznym. Ponadto, uwzględniając silne właściwości prooksydacyjne kadmu oraz wysoki potencjał antyoksydacyjny ekstraktu z owoców aronii czarnoowocowej uznano, iż uszkadzający wpływ tego ksenobiotyku na nerki może być spowodowany stresem oksydacyjnym, a ewentualny efekt ochronny tego ekstraktu może wynikać z zapobiegania działaniu prooksydacyjnemu kadmu.

CEL PRACY

Celem pracy było:

- **zbadanie, w modelu doświadczalnym *in vivo*, czy narażenie na kadm stanowiące odpowiednik niskiej i umiarkowanej ekspozycji środowiskowej populacji generalnej w krajach uprzemysłowionych może prowadzić do uszkodzenia struktury i funkcji nerek, a podawanie ekstraktu z owoców *A. melanocarpa* podczas narażenia na ten pierwiastek toksyczny może chronić przed uszkodzeniem tego narządu**
- **wyjaśnienie, czy uszkodzenie nerek przez kadm może wynikać z działania prooksydacyjnego tego ksenobiotyku, a potencjalny efekt ochronny ekstraktu z owoców *A. melanocarpa* może być spowodowany jego właściwościami antyoksydacyjnymi.**

Cele szczegółowe badań

Aby zrealizować cele badawcze i rozstrzygnąć postawioną hipotezę sformułowano następujące cele szczegółowe badań:

- ✓ ocena wpływu kadmu i/lub ekstraktu z owoców *A. melanocarpa* na funkcję kanalików i kłębuszków nerkowych w oparciu o pomiar w moczu i surowicy biomarkerów uszkodzenia tego narządu (**Publikacja II**)
- ✓ ocena wpływu kadmu i/lub ekstraktu z owoców aronii na obraz morfologiczny nerki (**Publikacja II**)
- ✓ ocena wpływu podawania ekstraktu z owoców aronii na wydalanie kadmu z moczem dobowym (**Publikacja II**)
- ✓ ocena wpływu kadmu i/lub ekstraktu z owoców *A. melanocarpa* na wybrane biomarkery stanu zapalnego w nerce (**Publikacja II**)
- ✓ ocena wpływu narażenia na kadm i/lub podawania ekstraktu z owoców aronii na równowagę oksydacyjno - redukcyjną i stopień nasilenia stresu oksydacyjnego w nerce (**Publikacja III**)
- ✓ ocena zależności pomiędzy biomarkerami funkcji nerek a markerami stanu zapalnego i równowagą oksydacyjno - redukcyjną w nerce oraz badanymi parametrami a stężeniem kadmu w krwi, moczu i nerce oznaczonym we wcześniejszych badaniach (**Publikacja II** i **Publikacja III**).

Badań będących przedmiotem niniejszej rozprawy doktorskiej dotychczas nie prowadzono.

4. Realizacja celów naukowych, zwięzłe omówienie materiałów i metod badawczych, wyniki badań, podsumowanie i dyskusja będąca odniesieniem do publikacji stanowiących rozprawę z podaniem perspektyw dalszego rozwoju tematu

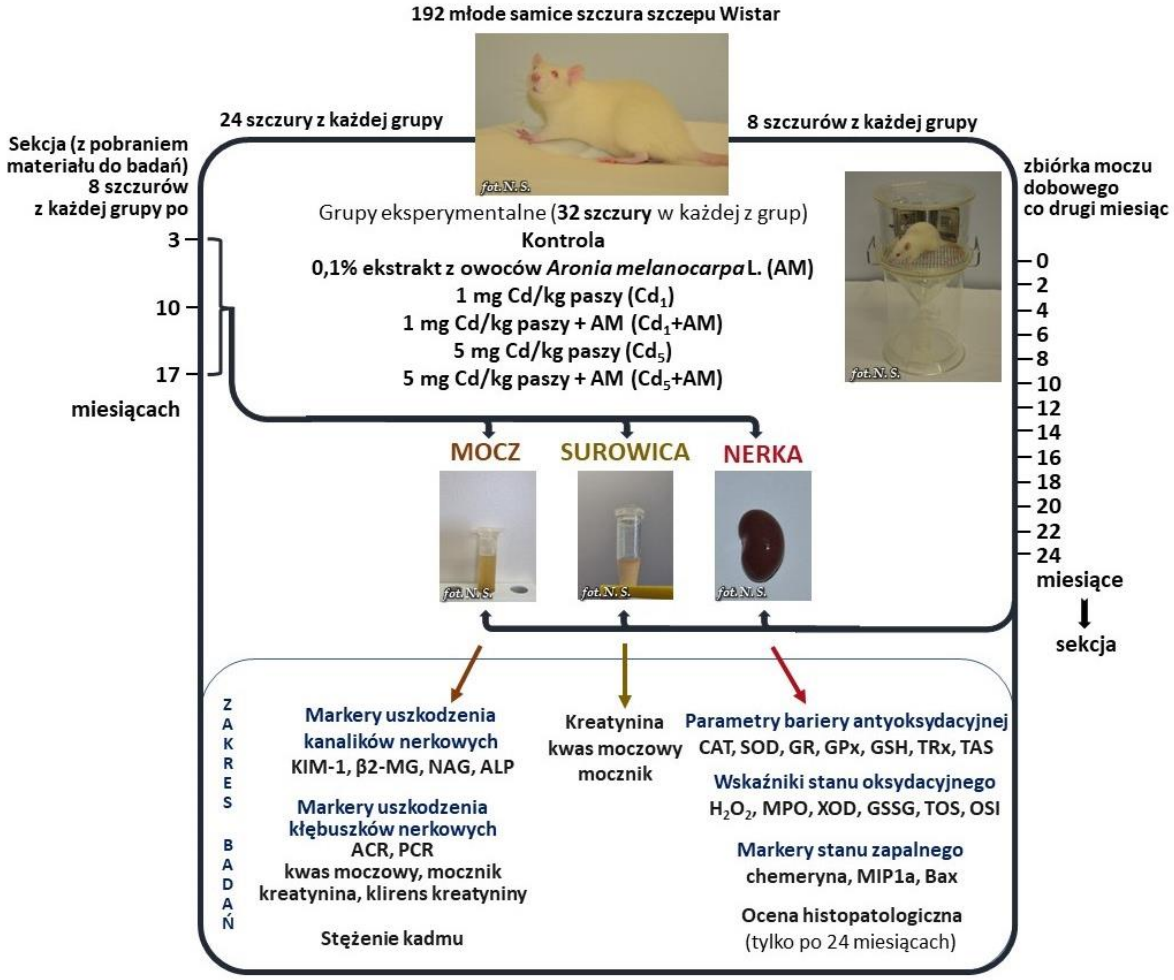
4.1. Model badawczy

Na przeprowadzenie badań z wykorzystaniem zwierząt laboratoryjnych uzyskano zgodę Lokalnej Komisji Etycznej do Spraw Doświadczeń na Zwierzętach w Białymstoku (zgoda nr 60/2009 z dnia 21 września 2009 r.).

Badania przeprowadzono w stworzonym w Zakładzie Toksykologii Uniwersytetu Medycznego w Białymstoku modelu doświadczalnym narażenia środowiskowego człowieka na kadm. Młodym samicach szczura szczepu Wistar podawano kadm w paszy (Wytwórnia Pasz „Morawski", Kcynia, Polska) w stężeniu 1 mg Cd/kg (odpowiednik niskiego narażenia środowiskowego człowieka) lub 5 mg Cd/kg (odpowiednik umiarkowanego narażenia człowieka) i/lub 0,1% wodny roztwór ekstraktu z owoców *A. melanocarpa* (firmy Adamed, Tuszyn, Polska, który wg procenta zawierał 65,74% związków polifenolowych i 18,65% antocyjanów) przez okres od 3 do 24 miesięcy. Zwierzęta z grupy kontrolnej żywiono paszą standardową i wodą pitną, które były pozbawione zanieczyszczeń.

Stężenie kadmu w krwi, moczu i nerce szczurów narażanych na ten pierwiastek toksyczny mieściło się w zakresie stężeń obecnie notowanych w populacji generalnej krajów uprzemysłowionych [62] i wynosiło 0,1030 – 0,3240 μg Cd/L krwi, 0,0852 – 0,2820 μg/g kreatyniny w moczu i 0,2626 – 2,8322 μg/g nerki u zwierząt narażanych na 1 mg Cd/kg paszy oraz 0,7350 – 1,3320 μg Cd/L krwi, 0,2839 – 0,6949 μg/g kreatyniny w moczu i 0,9739 – 14,8705 μg/g nerki w przypadku narażenia na 5 mg Cd/kg paszy [8]. Spożycie przez zwierzęta związków polifenolowych, oszacowane na podstawie spożycia ekstraktu (63,1 – 154,7 mg/kg m.c.), wynosiło 41,5 – 101,7 mg/kg m.c. [8] i było kilkakrotnie wyższe niż przeciętne spożycie tych związków w populacji generalnej (1000 mg/dobę, czyli 14,29 mg/kg m.c., przy założeniu masy ciała 70 kg) [15]. 24-miesięczne badanie na szczurach stanowi odzwierciedlenie kilkudziesięcioletniego okresu życia człowieka. Według danych dostępnych w literaturze, wiek szczura po 3 miesiącach doświadczenia stanowił odpowiednik około 18 lat życia człowieka, po 10 miesiącach odpowiadał wiekowi około 30 lat, po 17 miesiącach odzwierciedlał wiek około 45 lat, natomiast okres 24 miesięcy jest odpowiednikiem około 60 lat życia człowieka [1]. Zastosowany model badawczy jest zatem właściwy zarówno do oceny wpływu narażenia odzwierciedlającego przewlekłą ekspozycję populacji generalnej na kadm, jak również potencjalnego efektu ochronnego ekstraktu z owoców *A. melanocarpa*. Model ten został szczegółowo opisany we wcześniejszych publikacjach [5, 8–12, 14, 30, 40, 41], jak również jest omówiony w **Publikacji II** i **Publikacji III**, które są podstawą niniejszej rozprawy doktorskiej.

Materiałem do badań własnych była surowica i nerki uzyskane po 3, 10, 17 i 24 miesiącach doświadczenia, a także mocz pobrany zarówno po 3, 10, 17 i 24 miesiącach, jak również uzyskany od 8 zwierząt z każdej grupy przed rozpoczęciem doświadczenia i co drugi miesiąc w ciągu 24-miesięcznego badania. Schemat modelu doświadczalnego wraz z zakresem wykonanych badań przedstawiono na Rycinie 3.



Zwierzętom podawano kadm w paszy w stężeniu 0, 1 i 5 mg Cd/kg (grupa kontrolna, grupa Cd_1 i grupa Cd_5) i/lub 0,1% wodny roztwór ekstraktu z owoców *A. melanocarpa* L. (grupa AM, grupa Cd_1+AM i grupa Cd_5+AM) przez 3, 10, 17 i 24 miesiące. Poszczególne parametry, z wyjątkiem stężenia kadmu, zostały oznaczone w surowicy, moczu lub nerce po 3, 10, 17 i 24 miesiącach. Ponadto β2-MG, NAG, ALP, ACR i PCR, jak również stężenie kadmu, oceniano w moczu pobranym przed rozpoczęciem doświadczenia i co dwa miesiące jego 24-miesięcznego trwania. KIM-1, białko uszkodzenia nerek-1; β2-MG, beta2-mikroglobulina; NAG, N-acetylo-β-D-glukozaminidaza; ALP, fosfataza alkaliczna; ACR, stężenie albuminy w moczu wyrażone w przeliczeniu na kreatyninę; PCR, stężenie białka całkowitego w moczu wyrażone w przeliczeniu na kreatyninę; CAT, katalaza; SOD, dysmutaza ponadtlenkowa; GR, reduktaza glutationowa; GPx, peroksydaza glutationowa; GSH, glutation zredukowany; TRx, tioredoksyna; TAS, całkowity status antyoksydacyjny; H_2O_2, nadtlenek wodoru; MPO, mieloperoksydaza; XOD, oksydaza ksantynowa; GSSG, glutation utleniony; TOS, całkowity status oksydacyjny; OSI, indeks stresu oksydacyjnego; MIP1a, białko zapalne makrofagów-1; Bax, białko z rodziny białek Bcl-2.
Schemat stanowi modyfikację schematu przedstawionego w Publikacji II, polegającą na uzupełnieniu o badania, których wyniki przedstawiono w Publikacji III.

Rycina 3. Schemat przedstawiający zastosowany model doświadczalny oraz zakres przeprowadzonych badań.

4.2. Zakres przeprowadzonych badań

Aby zrealizować cele szczegółowe badań i rozstrzygnąć postawioną hipotezę przeprowadzono badania, które obejmowały:

- ❑ **ocenę stanu funkcjonalnego nerek w oparciu o pomiar biomarkerów uszkodzenia kanalików i kłębuszków nerkowych, w tym czułych biomarkerów nefrotoksyczności kadmu** (**Publikacja II**)
  - ✓ **markery uszkodzenia kanalików nerkowych**
  - **stężenie białka uszkodzenia nerek-1 (KIM-1) w moczu** – zestawem KIM-1 ELISA Kit firmy MyBioSource, Inc. (San Diego, USA)
  - **stężenie β2-microglobuliny (β2-MG) w moczu** – zestawem B2M ELISA Kit firmy EIAAB Science Inc. (Wuhan, Chiny)
  - **aktywność N-acetylo-β-D-glukozaminidazy (NAG) w moczu** – zestawem Rat N-acetyl-beta-D-glucosaminidase ELISA Kit firmy EIAAB Science Inc. (Wuhan, Chiny)
  - **aktywność fosfatazy zasadowej (ALP) w moczu** – zestawem firmy BioMaxima (Lublin, Polska)
  - ✓ **markery uszkodzenia kłębuszków nerkowych**
  - **stężenie albuminy w moczu wyrażone w przeliczeniu na stężenie kreatyniny (ACR)** – stężenie albuminy oznaczono zestawem firmy BioMaxima (Lublin, Polska)
  - **stężenie białka całkowitego w moczu wyrażone w przeliczeniu na stężenie kreatyniny (PCR)** – stężenie białka całkowitego oznaczono zestawem firmy BioMaxima (Lublin, Polska)
  - **stężenie kreatyniny w surowicy i moczu oraz klirens kreatyniny** – stężenie kreatyniny oznaczono zestawem firmy BioMaxima (Lublin, Polska)
  - **stężenie mocznika w surowicy i moczu** – zestawem BioMaxima (Lublin, Polska)
  - **stężenie kwasu moczowego w surowicy i moczu** – zestawem BioMaxima (Lublin, Polska)
- ❑ **ocenę struktury histologicznej nerki (Publikacja II)**
  - ✓ ocena makroskopowa podczas badania sekcyjnego
  - ✓ ocena mikroskopowa (barwienie hematoksylina - eozyna i barwienie Massona)
- ❑ **ocenę markerów stanu zapalnego w nerce (Publikacja II)**
  - ✓ **stężenie chemeryny** – zestawem Rat Chemerin ELISA kit firmy MyBioSource, Inc. (San Diego USA)
  - ✓ **stężenie białka zapalnego makrofagów-1 alfa (MIP1a)** – zestawem ELISA Kit for Macrophage Inflammatory Protein 1 Alpha (MIP1a) firmy Cloud-Clone Corp. (Katy, USA)
  - ✓ **stężenie białka z rodziny białek Bcl-2 (Bax)** – zestawem ELISA Kit for Bcl2 Associated X Protein (Bax) firmy Cloud-Clone Corp. (Katy, USA)
- ❑ **ocenę równowagi oksydacyjno - redukcyjnej w tkance nerkowej (Publikacja III)**
  - ✓ **całkowity status antyoksydacyjny (TAS)** – zestawem ImAnOx (TAS) ELISA kit firmy Immundiagnostik AG (Bensheim, Niemcy)

- ✓ **aktywność enzymów antyoksydacyjnych**
- **dysmutaza ponadtlenkowa (SOD)** – zestawem SOD Assay kit firmy Cayman Chemical Company (Ann Arbor, USA)
- **katalaza (CAT)** – metodą spektrofotometryczną wg Aebi (Methods Enzymol., 1984, 105, 121-126)
- **peroksydaza glutationowa (GPx)** – zestawem Bioxytech GPx-340 kit firmy Percipio Biosciences (Burlingame, USA)
- **reduktaza glutationowa (GR)** – zestawem Bioxytech GR-340 kit firmy Percipio Biosciences (Burlingame, USA)
- ✓ **stężenie antyoksydantów nieenzymatycznych**
- **glutation zredukowany (GSH)** – zestawem Glutathione Assay Kit firmy Cayman Chemical Company (Ann Arbor, USA)
- **tioredoksyna (TRx)** – zestawem Rat (Trx) ELISA KIT, firmy SunRed (Szanghaj, Chiny)
- ✓ **stan oksydacyjny**
- **stężenia glutationu utlenionego (GSSG)** – zestawem Glutathione Assay Kit firmy Cayman Chemical Company (Ann Arbor, USA) oraz **stosunek stężenia GSH i GSSG**
- **stężenie nadtlenku wodoru (H_2O_2)** – zestawem Bioxytech H_2O_2-560 kit firmy Percipio Biosciences (Burlingame, USA)
- **stężenie oksydazy ksantynowej (XOD)** – zestawem Rat (XOD) ELISA KIT firmy SunRed (Szanghaj, Chiny)
- **stężenie mieloperoksydazy (MPO)** – zestawem Rat (MPO) ELISA KIT firmy SunRed (Szanghaj, Chiny)
- **całkowity status oksydacyjny (TOS**) – zestawem PerOx (TOS) ELISA kit firmy Immundiagnostik AG (Bensheim, Niemcy)
- ✓ **stopień nasilenia stresu oksydacyjnego w oparciu o indeks stresu oksydacyjnego (OSI = TOS/TAS)**

Pomiary z wykorzystaniem zestawów komercyjnych wykonano zgodnie z zaleceniami producentów.

4.3. Aparatura

Podczas oznaczeń spektrometrycznych i immunoenzymatycznych (ELISA) biomarkerów nefrotoksyczności, markerów stanu zapalnego oraz parametrów stanu oksydacyjo - redukcyjnego wykorzystano spektrofotometry MULTISCAN GO (Thermo Scientific, Vantaa, Finlandia), Epoch (Bio Tek Instruments Inc, Winooski, USA) oraz Specord 50 Plus (Analityk Jena, Jena, Niemcy). Oznaczenie stężenia kadmu w moczu przeprowadzono przy użyciu spektrometru atomowo - absorpcyjnego Hitachi Z-5000 (Tokio, Japonia). Podczas badań histopatologicznych wykorzystano półautomatyczny mikrotom rotacyjny Histocore Multicut 1860 (Leica Biosystems, Nußloch, Niemcy) i mikroskop Axiolab 5 (Carl Zeiss

Microscopy GmbH, Jena, Niemcy), a zdjęcia preparatów histologicznych wykonano z użyciem aparatu Axiocam i oprogramowania ZEN 2.0 (Zeiss, Halle, Niemcy).

4.4. Analiza statystyczna

Wszystkie obliczenia statystyczne przeprowadzono przy użyciu oprogramowania Statistica 13.3 (StatSoft; Tulsa, USA). Normalność rozkładu danych sprawdzono testem Shapiro-Wilka. W związkiem z brakiem rozkładu normalnego danych, celem stwierdzenia, czy występują różnice statystycznie znamienne ($p < 0,05$) pomiędzy grupami doświadczalnymi przeprowadzono nieparametryczny test Kruskala-Wallisa z testem mediany. Wykonano także nieparametryczny test Friedmana w celu sprawdzenia, czy w poszczególnych grupach występują różnice w wynikach pomiarów powtarzanych u tych samych zwierząt co drugi miesiąc w trakcie 24 miesięcy doświadczenia. Gdy test Friedmana wykazał statystycznie istotne różnice ($p < 0,05$) pomiędzy punktami czasowymi, przeprowadzono test Wilcoxona w celu porównania danych sparowanych.

W celu oceny występowania zależności pomiędzy wartościami mierzonych parametrów przeprowadzono analizę regresji liniowej, której wyniki przedstawiono jako współczynnik β (procent zmiany zmiennej zależnej na każdą jednostkę zmiany zmiennej niezależnej), R^2 (procent jednej zmiennej odpowiedzialny za zmienność drugiej zmiennej) i poziom istotności statystycznej (p). Oceniano również zależności pomiędzy parametrami oznaczonymi w badaniach własnych, których wyniki przedstawiono w Publikacji II i Publikacji III a stężeniem kadmu w krwi, moczu i nerce oznaczonym we wcześniejszych badaniach [8]. Zależność pomiędzy dwiema zmiennymi uznano za statystycznie istotną przy wartości współczynnika β, dla którego $p < 0,05$.

4.5. Wyniki badań

4.5.1. Wpływ narażenia na kadm i/lub podawania ekstraktu z owoców *A. melanocarpa* na strukturę i funkcję nerek

Szczegółowe wyniki oceny wpływu niskiego i umiarkowanego narażenia na kadm i/lub podawania ekstraktu z owoców *A. melanocarpa* na strukturę i funkcję nerek przedstawiono w **Publikacji II i Suplemencie do Publikacji II** oraz podsumowano w Tabeli 3 i 4.

Ocena funkcji nerek, przeprowadzona w oparciu o pomiar wielu biomarkerów uszkodzenia kanalików i kłębuszków nerkowych, w tym czułe biomarkery nefrotoksyczności kadmu, wraz z oceną struktury histologicznej tego narządu wykazały, iż powtarzana, zarówno umiarkowana (5 mg Cd/kg paszy), jak również niska (1 mg Cd/kg paszy) ekspozycja samic szczura na kadm prowadziła do uszkodzenia funkcji i rozwoju zmian patologicznych

w strukturze morfologicznej kanalików i kłębuszków nerkowych. Do uszkodzenia kanalików nerkowych dochodziło wcześniej, niż do dysfunkcji kłębuszków nerkowych, a pierwsze oznaki szkodliwego wpływu kadmu na nerki stwierdzono już po 3 miesiącach niskiego narażenia (Tabela 3 i 4). Po 24 miesiącach narażenia na 1 i 5 mg Cd/kg odnotowano zmiany patologiczne w kanalikach nerkowych, takie jak wakuolizacja i poszerzenie światła kanalików (obie zmiany wystąpiły tylko na skutek narażenia na 1 mg Cd/kg paszy), hialinizacja (zwyrodnienie szkliste), hiperplazja (rozrost) i hipertrofia (przerost) nabłonka kanalików krętych oraz proliferacja tkanki śródmiąższowej nerek, jak również zapalenie kłębuszków nerkowych i przekrwienie na granicy kora - rdzeń oraz obrzęk okołonaczyniowy (obrzęk wystąpił tylko na skutek narażenia na 5 mg Cd/kg paszy).

Tabela 3. Podsumowanie wyników oceny wpływu narażenia na kadm (1 i 5 mg Cd/kg paszy) oraz podawania 0,1% wodnego roztworu ekstraktu z owoców *A. melanocarpa* podczas narażenia na ten pierwiastek toksyczny na wartości biomarkerów uszkodzenia kanalików i kłębuszków nerkowych w moczu i surowicy szczurów.

Parametr	Grupa	Miesiące doświadczenia 3	10	17	24	Parametr	Grupa	Miesiące doświadczenia 3	10	17	24
Biomarkery uszkodzenia kanalików nerkowych						**Biomarkery uszkodzenia kłębuszków nerkowych**					
KIM-1	Cd_1	↑	↑	↑	↔	ACR	Cd_1	↔	↔	↑	↑
	Cd_1+AM	↔↘	↔↘	↔↘	↔↘		Cd_1+AM	↔	↔	↑	↔↘
	Cd_5	↑	↑	↑	↑		Cd_5	↔	↔	↑	↑
	Cd_5+AM	↔↘	↔↘	↔↘	↔↘		Cd_5+AM	↔	↔↘	↔↘	↔↘
β2-MG	Cd_1	↔	↔	↑	↑	PCR	Cd_1	↔	↑	↑	↑
	Cd_1+AM	↔	↔	↔↘	↔↘		Cd_1+AM	↔	↔↘	↔↘	↔↘
	Cd_5	↔	↔	↑	↑		Cd_5	↔	↑	↑	↑
	Cd_5+AM	↔	↔	↔↘	↔↘		Cd_5+AM	↔	↔↘	↔↘	↔↘
NAG	Cd_1	↔	↔	↑	↑	Klirens kreatyniny	Cd_1	↔	↔	↔	↔
	Cd_1+AM	↔	↔	↔↘	↔↘		Cd_1+AM	↔	↔	↔	↔
	Cd_5	↔	↑	↑	↑		Cd_5	↔	↔	↓	↓
	Cd_5+AM	↔	↔↘	↔↘	↔↘		Cd_5+AM	↔	↔	↔↗	↔↗
ALP	Cd_1	↔	↑	↑	↑	Kwas moczowy w surowicy	Cd_1	↑	↔	↔	↔
	Cd_1+AM	↔	↔	↔	↔		Cd_1+AM	↑	↔	↔	↔
	Cd_5	↔	↑	↑	↑		Cd_5	↔	↔	↔	↑
	Cd_5+AM	↔	↔↘	↔↘	↔↘		Cd_5+AM	↔	↔	↔	↔
						Mocznik w surowicy	Cd_1	↔	↔	↔	↔
							Cd_1+AM	↔	↔	↔	↔
							Cd_5	↔	↔	↑	↑
							Cd_5+AM	↔	↔	↔	↔↘

↑ – wzrost, ↓ – obniżenie w stosunku do grupy kontrolnej
↗ – wzrost, ↘ – obniżenie w stosunku do odpowiedniej grupy narażanej na kadm (Cd_1 lub Cd_5)
↔ – brak zmiany względem grupy kontrolnej
↔ – brak zmiany względem grupy kontrolnej i odpowiedniej grupy narażanej na kadm (Cd_1 lub Cd_5)

Indukowane przez kadm uszkodzenie kanalików, stwierdzone w oparciu o pomiar czułych biomarkerów (podwyższone stężenie KIM-1 i β2-MG oraz zwiększona aktywność NAG i ALP w moczu) stopniowo postępowało wraz z czasem ekspozycji, szczególnie przy

wyższym narażeniu (Tabela 3 i 4). Chociaż odnotowano brak różnic w wartościach biomarkerów uszkodzenia kanalików w zależności od poziomu narażenia na kadm, to zmiany wartości tych parametrów (z wyjątkiem KIM-1) następowały wcześniej w przypadku narażenia na 5 mg Cd/kg paszy. Ponadto dodatnie zależności stwierdzone pomiędzy prawie wszystkimi wskaźnikami uszkodzenia kanalików nerkowych a stężeniem kadmu w krwi, moczu i nerkach zwierząt narażanych na ten pierwiastek wskazują, iż stopień uszkodzenia kanalików nerkowych zwiększał się wraz ze wzrostem obciążenia organizmu tym ksenobiotykiem. Również badania histologiczne wykazały, że zmiany w strukturze morfologicznej kanalików (z wyjątkiem wakuolizacji i poszerzenia światła kanalików, których nie stwierdzono w przypadku narażenia na 5 mg Cd/kg paszy) były bardziej zaawansowane na skutek ekspozycji umiarkowanej niż niskiego narażenia.

Tabela 4. Podsumowanie wyników oceny wpływu narażenia na kadm (1 i 5 mg Cd/kg paszy) oraz podawania 0,1% wodnego roztworu ekstraktu z owoców *A. melanocarpa* podczas narażenia na ten pierwiastek toksyczny na wartości biomarkerów uszkodzenia kanalików i kłębuszków nerkowych w moczu szczurów oceniane co dwa miesiące w trakcie 24-miesięcznego doświadczenia.

Parametr	Grupa	Miesiące doświadczenia											
		2	4	6	8	10	12	14	16	18	20	22	24
β2-MG	Cd_1	↔	↔	↔	↔	↔	↔	↑	↑	↑	↑	↑	↑
	Cd_1+AM	↔	↔	↔↘	↔↘	↔	↔↘	↔	↔↘	↔↘	↔↘	↔↘	↔↘
	Cd_5	↔	↔	↔	↔	↔	↑	↑	↑	↑	↑	↑	↑
	Cd_5+AM	↔	↔	↔	↔↘	↔	↔↘	↔↘	↔↘	↔↘	↔↘	↔↘	↔↘
ALP	Cd_1	↔	↔	↔	↔	↑	↑	↑	↑	↑	↑	↑	↑
	Cd_1+AM	↔	↔	↔	↔	↔	↔	↔	↔	↔	↔	↔	↔
	Cd_5	↔	↑	↑	↑	↑	↑	↑	↑	↑	↑	↑	↑
	Cd_5+AM	↔	↔	↑	↔↘	↔↘	↔↘	↔↘	↔↘	↔↘	↔↘	↔↘	↔↘
NAG	Cd_1	↔	↔	↔	↔	↔	↑	↑	↑	↑	↑	↑	↑
	Cd_1+AM	↔	↔	↔	↔	↔	↔↘	↔↘	↔↘	↔↘	↔↘	↔↘	↔↘
	Cd_5	↔	↔	↔	↔	↑	↑	↑	↑	↑	↑	↑	↑
	Cd_5+AM	↔	↔	↔	↔	↔↘	↔↘	↔↘	↔↘	↔↘	↔↘	↔↘	↔↘
ACR	Cd_1	↔	↔	↑	↔	↔	↔	↔	↔	↑	↑	↑	↑
	Cd_1+AM	↔	↔	↔↘	↔	↔	↔	↔	↔	↔↘	↔↘	↔↘	↔↘
	Cd_5	↔	↔	↔	↔	↔	↔	↔	↑	↑	↑	↑	↑
	Cd_5+AM	↔	↔	↔↘	↔↘	↔↘	↔↘	↔↘	↔↘	↔↘	↔↘	↔↘	↔↘
PCR	Cd_1	↔	↔	↑	↑	↑	↑	↑	↑	↑	↑	↑	↑
	Cd_1+AM	↔	↔	↔↘	↔↘	↔↘	↔↘	↔↘	↔↘	↔↘	↔↘	↔↘	↔↘
	Cd_5	↔	↔	↑	↑	↑	↑	↑	↑	↑	↑	↑	↑
	Cd_5+AM	↔	↔	↔	↔↘	↔↘	↔↘	↔↘	↔↘	↔↘	↔↘	↔↘	↔↘

↑ – wzrost w stosunku do grupy kontrolnej
↘ – obniżenie w stosunku do odpowiedniej grupy narażanej na kadm (Cd_1 lub Cd_5)
↔ – brak zmiany względem grupy kontrolnej
↔ – brak zmiany względem grupy kontrolnej i odpowiedniej grupy narażanej na kadm (Cd_1 lub Cd_5)

Wzrost ACR i PCR oraz stężenia kwasu moczowego i mocznika w surowicy, a także obniżenie klirensu kreatyniny (co świadczy o zmniejszeniu filtracji kłębuszkowej)

odzwierciedla upośledzenie funkcji kłębuszków nerkowych (Tabela 3 i 4). Narażenie na kadm nie miało wpływu na zawartość kwasu moczowego i mocznika w moczu dobowym (Suplement do Publikacji II). Chociaż nie stwierdzono różnic w wartościach poszczególnych markerów uszkodzenia kłębuszków nerkowych pomiędzy grupami narażanymi na 1 i 5 mg Cd/kg (z wyjątkiem niższego stężenia kwasu moczowego w surowicy po 3 miesiącach i wyższego stężenia mocznika po 17 miesiącach w grupie Cd_5), to fakt, że długotrwała, niska ekspozycja na ten pierwiastek toksyczny nie miała wpływu na klirens kreatyniny, a także stężenie kwasu moczowego i mocznika wskazuje, że szkodliwy wpływ tego ksenobiotyku na funkcję kłębuszków nerkowych był silniejszy w przypadku narażenia umiarkowanego (Tabela 3 i 4). Ponadto w badaniach histologicznych wykazano bardziej zaawansowane zapalenie kłębuszków nerkowych przy wyższej ekspozycji na kadm.

Suplementacja ekstraktem z owoców *A. melanocarpa* podczas narażenia na 1 i 5 mg Cd/kg paszy zapobiegała wszystkim powodowanym przez kadm zmianom w stężeniu KIM-1 i β2-MG oraz aktywności NAG i ALP w moczu z wyjątkiem wzrostu aktywności ALP po 6 miesiącach narażenia umiarkowanego. Poza tym jednym wyjątkiem, wartości wszystkich biomarkerów uszkodzenia kanalików nerkowych u zwierząt, którym podczas narażenia na kadm podawano ekstrakt z owoców aronii czarnoowocowej mieściły się w zakresie wartości oznaczonych w grupie kontrolnej (Tabela 3 i 4).

Podawanie ekstraktu z owoców aronii czarnoowocowej chroniło również przed powodowanym przez kadm zaburzeniem funkcji kłębuszków nerkowych. Wartości wszystkich ocenianych biomarkerów uszkodzenia kłębuszków nerkowych, tj. ACR, PCR, stężenie kreatyniny w surowicy i moczu (Suplement do Publikacji II) oraz klirens kreatyniny, a także stężenie kwasu moczowego i mocznika w surowicy oraz ich zawartość w moczu dobowym u zwierząt, którym podczas narażenia na kadm podawano ekstrakt z owoców aronii mieściły się w zakresach wartości oznaczonych w grupie kontrolnej, z wyjątkiem ACR w grupie Cd_1+AM po 17 miesiącach oraz stężenia kwasu moczowego po 3 miesiącach, które były podwyższone w porównaniu z grupą kontrolną, ale nie różniły się w porównaniu z grupą Cd_1. Ponadto stężenie kreatyniny w surowicy w grupie Cd_5+AM po 3, 17 i 24 miesiącach było niższe niż w grupie Cd_5 (Suplement do Publikacji II).

U zwierząt, które podczas narażenia na kadm suplementowano ekstraktem z owoców *A. melanocarpa* intensywność zmian patologicznych w obrazie morfologicznym nerki była łagodniejsza w porównaniu z odpowiednimi grupami, które nie otrzymywały ekstraktu podczas ekspozycji na ten pierwiastek toksyczny. Podawanie ekstraktu z owoców aronii zwierzętom eksponowanym na 1 mg Cd/kg paszy całkowicie chroniło przed wakuolizacją i hialinizacją kanalików nerkowych, poszerzeniem światła kanalików oraz hiperplazją i przerostem nabłonka kanalików krętych, a także osłabiało proliferację tkanki śródmiąższowej nerki. Podawanie ekstraktu w czasie narażenia na 5 mg Cd/kg paszy zapobiegało hiperplazji nabłonka kanalików krętych i osłabiało inne zmiany w strukturze histologicznej kanalików, takie jak hialinizacja, przerost nabłonka kanalików krętych i proliferacja śródmiąższowa.

Stosowanie ekstraktu z owoców aronii podczas narażenia na kadm zapobiegało rozwojowi kłębuszkowego zapalenia nerek lub osłabiało stopień jego nasilenia oraz zmniejszało przekrwienie na granicy kora - rdzeń nerki. Suplementowanie ekstraktem podczas ekspozycji na 5 mg Cd/kg paszy skutkowało także zmniejszeniem obrzęku okołonaczyniowego w nerce. W przypadku zastosowania ekstraktu podczas niskiego narażenia zmianę tego rodzaju odnotowano u 25% zwierząt, których nerkę poddano ocenie mikroskopowej, podczas gdy w grupie zwierząt otrzymujących jedynie kadm zmiana ta nie wystąpiła. Ponadto u 50% zwierząt, którym podawano jednocześnie 5 mg Cd/kg paszy i ekstrakt z owoców aronii, stwierdzono niewielkiego stopnia poszerzenie światła kanalików nerkowych, podczas gdy u osobników nie otrzymujących ekstraktu zmiana tego rodzaju nie została odnotowana.

Podawanie ekstraktu z owoców *A. melanocarpa* zwierzętom, które nie były narażane na kadm nie miało wpływu na wartości oznaczanych biomarkerów uszkodzenia kanalików i kłębuszków nerkowych. W obrazie morfologicznym nerki tych zwierząt stwierdzono wprawdzie zmiany takie jak wakuolizacja i poszerzenie światła kanalików, rozrost nabłonka kanalików krętych i zapalenie kłębuszków nerkowych. Zmiany te były jednak nieznaczne i występowały jedynie u pojedynczych osobników. Mogły być one związane ze zmianami starczymi zachodzącymi w tym narządzie.

4.5.2. Wpływ podawania ekstraktu z owoców *A. melanocarpa* na stężenie kadmu w moczu

Szczegółowe wyniki oceny wpływu podawania ekstraktu z owoców *A. melanocarpa* na stężenie kadmu w moczu szczurów przedstawiono w **Publikacji II** i **Suplemencie do Publikacji II** oraz podsumowano w Tabeli 5.

Stężenie kadmu w moczu (główny wskaźnik narażenia przewlekłego na ten pierwiastek toksyczny) u zwierząt narażanych na 1 i 5 mg Cd/kg przez okres od 2 do 24 miesięcy mieściło się odpowiednio w zakresie 0,1114 – 0,6386 µg/g kreatyniny i 0,1664 – 0,9785 µg/g kreatyniny.

Tabela 5. Podsumowanie wyników oceny wpływu podawania 0,1% wodnego roztworu ekstraktu z owoców *A. melanocarpa* podczas narażenia na kadm (1 i 5 mg Cd/kg paszy) na stężenie tego pierwiastka toksycznego w moczu szczurów.

Parametr	Grupa	Miesiące doświadczenia											
		2	4	6	8	10	12	14	16	18	20	22	24
Cd	Cd_1	↔	↔	↑	↔	↔	↔	↔	↔	↔	↔	↔	↔
	Cd_1+AM	↑	↔	↔	↑	↔	↑	↑	↔	↔	↔	↑	↔
	Cd_5	↑	↑	↑	↑	↑	↑	↑	↑	↑	↑	↑	↑
	Cd_5+AM	↑	↑	↑	↑	↑	↑	↑	↑↗	↑	↑↗	↑↗	↑↗

↑ – wzrost w stosunku do grupy kontrolnej
↗ – wzrost w stosunku do grupy Cd_5
↔ – brak zmiany względem grupy kontrolnej
↔ – brak zmiany względem grupy kontrolnej i grupy Cd_1

Podawanie ekstraktu z owoców aronii czarnoowocowej szczurom utrzymywanym na diecie zawierającej 1 mg Cd/kg nie miało wpływu na stężenie kadmu w moczu dobowym. U zwierząt, którym podczas narażenia na 5 mg Cd/kg paszy podawano ekstrakt z owoców aronii, stężenie kadmu w moczu po 16 miesiącach doświadczenia oraz pomiędzy 20-tym a 24-tym miesiącem było wyższe o 17–45% w porównaniu do osobników, którym nie podawano ekstraktu (Tabela 5).

4.5.3. Wpływ narażenia na kadm i/lub podawania ekstraktu z owoców *A. melanocarpa* na wybrane biomarkery stanu zapalnego w nerce

Szczegółowe wyniki oceny wpływu niskiego i umiarkowanego narażenia na kadm oraz podawania ekstraktu z owoców aronii czarnoowocowej podczas ekspozycji na ten pierwiastek toksyczny na wybrane biomarkery stanu zapalnego w nerce przedstawiono w **Publikacji II** i **Suplemencie do Publikacji II** oraz podsumowano w Tabeli 6.

Pomiary stężeń ocenianych biomarkerów stanu zapalnego w nerce wykazały, iż zarówno niskie, jak i umiarkowane, długotrwałe narażenie na kadm może indukować rozwój zmian zapalnych w tkance nerkowej, a podawanie ekstraktu z owoców aronii może łagodzić te zmiany (Tabela 6).

Tabela 6. Podsumowanie wyników oceny wpływu narażenia na kadm (1 i 5 mg Cd/kg paszy) oraz podawania 0,1% wodnego roztworu ekstraktu z owoców *A. melanocarpa* podczas narażenia na ten pierwiastek toksyczny na wartości biomarkerów stanu zapalnego w nerce.

Parametr	Grupa	Miesiące doświadczenia			
		3	10	17	24
Chemeryna	Cd_1	↔	↔	↔	↑
	Cd_1+AM	↔	↔	↔	↔ ↘
	Cd_5	↔	↔	↑	↑
	Cd_5+AM	↓ ↘	↓ ↘	↔ ↘	↔ ↘
MIP1a	Cd_1	↔	↔	↔	↔
	Cd_1+AM	↔	↔	↓ ↘	↓ ↘
	Cd_5	↓	↔	↔	↔
	Cd_5+AM	↓	↓ ↘	↓ ↘	↓ ↘
Bax	Cd_1	↔	↔	↔	↔
	Cd_1+AM	↔	↔ ↘	↔ ↘	↔ ↘
	Cd_5	↔	↔	↔	↑
	Cd_5+AM	↓	↔	↔ ↘	↔ ↘

↑ – wzrost, ↓ – obniżenie w stosunku do grupy kontrolnej
↘ – obniżenie w stosunku do odpowiedniej grupy narażanej na kadm (Cd_1 lub Cd_5)
↔ – brak zmiany względem grupy kontrolnej
↔ – brak zmiany względem grupy kontrolnej i odpowiedniej grupy narażanej na kadm (Cd_1 lub Cd_5)

4.5.4. Wpływ narażenia na kadm i/lub podawania ekstraktu z owoców *A. melanocarpa* na równowagę oksydacyjno - redukcyjną i stopień nasilenia stresu oksydacyjnego w nerce

Szczegółowe wyniki oceny wpływu niskiego i umiarkowanego narażenia na kadm oraz podawania ekstraktu z owoców aronii czarnoowocowej podczas ekspozycji na ten pierwiastek toksyczny na równowagę oksydacyjno - redukcyjną i stopień nasilenia stresu oksydacyjnego w nerce przedstawiono w **Publikacji III** oraz podsumowano w Tabeli 7.

Tabela 7. Podsumowanie wyników oceny wpływu narażenia na kadm (1 i 5 mg Cd/kg paszy) oraz podawania 0,1% wodnego roztworu ekstraktu z owoców *A. melanocarpa* podczas narażenia na ten pierwiastek toksyczny na równowagę oksydacyjno - redukcyjną i stopień nasilenia stresu oksydacyjnego w nerce.

Parametr	Grupa	Miesiące doświadczenia 3	10	17	24	Parametr	Grupa	Miesiące doświadczenia 3	10	17	24
Markery stanu antyoksydacyjnego						**Markery stanu oksydacyjnego**					
TAS	Cd_1	↔	↔	↓	↓	TOS	Cd_1	↔	↔	↑	↑
	Cd_1+AM	↔	↔	↔	↔↗		Cd_1+AM	↔	↔	↔	↔
	Cd_5	↓	↓	↓	↓		Cd_5	↔	↔	↑	↑
	Cd_5+AM	↓	↓	↔	↔		Cd_5+AM	↔	↔	↔	↔↘
SOD	Cd_1	↔	↔	↔	↔	OSI	Cd_1	↔	↔	↑	↑
	Cd_1+AM	↔	↔↗	↔↗	↔		Cd_1+AM	↔	↔	↔↘	↔↘
	Cd_5	↓	↓	↓	↓		Cd_5	↔	↔	↑	↑
	Cd_5+AM	↔	↔↗	↔	↔↗		Cd_5+AM	↔	↔	↔	↔↘
CAT	Cd_1	↓	↓	↔	↔	GSSG	Cd_1	↔	↑	↔	↑
	Cd_1+AM	↔	↔	↔	↔		Cd_1+AM	↔↘	↔	↔↘	↔↘
	Cd_5	↓	↓	↓	↓		Cd_5	↔	↔	↔	↑
	Cd_5+AM	↔↗	↓	↔↗	↔↗		Cd_5+AM	↔	↔	↓↘	↔↘
GPx	Cd_1	↓	↓	↓	↓	GSH/GSSG	Cd_1	↔	↓	↓	↓
	Cd_1+AM	↔	↔	↔	↔		Cd_1+AM	↔	↔↗	↔↗	↔↗
	Cd_5	↓	↓	↓	↓		Cd_5	↓	↓	↓	↓
	Cd_5+AM	↔↗	↔↗	↔	↔		Cd_5+AM	↔	↔↗	↔↗	↔↗
GR	Cd_1	↔	↔	↔	↑	H_2O_2	Cd_1	↔	↔	↔	↑
	Cd_1+AM	↔	↔	↔	↔↘		Cd_1+AM	↓↘	↔↘	↔↘	↔↘
	Cd_5	↔	↔	↑	↔		Cd_5	↔	↔	↔	↑
	Cd_5+AM	↔	↔	↔↘	↔↘		Cd_5+AM	↓↘	↔↘	↔↘	↔↘
GSH	Cd_1	↔	↔	↓	↓	XOD	Cd_1	↔	↔	↔	↔
	Cd_1+AM	↔	↔	↔	↔		Cd_1+AM	↔	↔↘	↔	↔
	Cd_5	↓	↓	↓	↓		Cd_5	↔	↔	↑	↑
	Cd_5+AM	↓	↔↗	↔↗	↔		Cd_5+AM	↔↘	↓↘	↔↘	↔↘
TRx	Cd_1	↔	↔	↔	↔	MPO	Cd_1	↔	↔	↑	↑
	Cd_1+AM	↔	↓	↔	↔↘		Cd_1+AM	↔	↔↘	↔↘	↔↘
	Cd_5	↓	↔	↔	↑		Cd_5	↔	↔	↑	↑
	Cd_5+AM	↓	↔	↔	↔↘		Cd_5+AM	↔↘	↔↘	↔↘	↔↘

↑ – wzrost, ↓ – obniżenie w stosunku do grupy kontrolnej
↗ – wzrost, ↘ – obniżenie w stosunku do odpowiedniej grupy narażanej na kadm (Cd_1 lub Cd_5)
↔ – brak zmiany względem grupy kontrolnej
↔ – brak zmiany względem grupy kontrolnej i odpowiedniej grupy narażanej na kadm (Cd_1 lub Cd_5)

Ekspozycja na kadm, w sposób zależny od jej poziomu i czasu trwania, osłabiała enzymatyczną (obniżanie aktywności SOD, CAT i GPx) i nieenzymatyczną barierę antyoksydacyjną (głównie obniżenie stężenia GSH) nerki prowadząc do obniżenia jej całkowitego statusu antyoksydacyjnego (TAS), a także podwyższała status oksydacyjny tego narządu (wzrost stężenia H_2O_2, MPO i XOD oraz podwyższony TOS), co skutkowało rozwojem stresu oksydacyjnego (wzrost OSI). Podawanie ekstraktu z owoców aronii czarnoowocowej podczas niskiego i umiarkowanego narażenia na kadm chroniło przed wywołanym przez ten ksenobiotyk zaburzeniem równowagi oksydacyjno - redukcyjnej i rozwojem stresu oksydacyjnego w tym narządzie (Tabela 7).

4.5.5. Zależności pomiędzy biomarkerami funkcji nerek a markerami stanu zapalnego i równowagą oksydacyjno - redukcyjną w nerce

Dokonano oceny wzajemnych zależności pomiędzy biomarkerami uszkodzenia kanalików i kłębuszków nerkowych a markerami procesów zapalnych w tym narządzie i głównymi wskaźnikami stanu oksydacyjno - redukcyjnego nerek (TAS, TOS i OSI) u zwierząt, którym podczas śladowego (grupa kontrolna), niskiego (1 mg Cd/kg paszy) i umiarkowanego (5 mg Cd/kg paszy) narażenia na kadm nie podawano ekstraktu z owoców *A. melanocarpa*. Oceniono również zależności pomiędzy tymi parametrami u zwierząt, którym podawano ekstrakt z owoców aronii (grupa AM, grupa Cd_1+AM i grupa Cd_5+AM) (**Publikacja II** i **Publikacja III**). Ponadto oceniono zależności pomiędzy parametrami oznaczonymi w badaniach własnych a stężeniem kadmu w krwi, moczu i nerce oznaczonymi we wcześniejszych badaniach prowadzonych u tych zwierząt [8] (**Publikacja II** i **Publikacja III**).

Zależności, które odnotowano pomiędzy biomarkerami uszkodzenia kanalików i kłębuszków nerkowych oraz markerami procesów zapalnych w tym narządzie, jak również TAS, TOS i OSI u zwierząt, którym podczas śladowego, niskiego i umiarkowanego narażenia na kadm nie podawano ekstraktu z owoców aronii wskazują, iż uszkadzający wpływ kadmu na nerkę może być związany z jego działaniem prozapalnym, jak również z właściwościami prooksydacyjnymi, co skutkowało zaburzeniem równowagi oksydacyjno - redukcyjnej i rozwojem stresu oksydacyjnego w nerce.

Ujemne zależności pomiędzy biomarkerami uszkodzenia kanalików (stężenie β2-MG i aktywność ALP w moczu) i kłębuszków nerkowych (ACR i PCR) a TAS nerki oraz pozytywne zależności pomiędzy ACR i stężeniem kwasu moczowego w surowicy a TOS nerki i OSI odnotowane u zwierząt, którym podawano ekstrakt z owoców *A. melanocarpa* wskazują na związek pomiędzy stanem funkcjonalnym tego narządu a jego statusem oksydacyjno - redukcyjnym.

4.6. Podsumowanie i dyskusja

Badania będące przedmiotem niniejszej rozprawy doktorskiej są pierwszymi, w których, w modelu eksperymentalnym *in vivo*, dokonano zarówno oceny ryzyka uszkodzenia nerek na skutek niskiej i umiarkowanej ekspozycji na ten pierwiastek toksyczny, jak również zaproponowano skuteczną strategię ochronną przed uszkodzeniem tego narządu.

Badania obejmujące ocenę działania nefrotoksycznego kadmu jednoznacznie wykazały, iż przewlekła, nawet niska ekspozycja na ten pierwiastek szkodliwy dla zdrowia może prowadzić do zaburzenia funkcji kanalików, a następnie również kłębuszków nerkowych oraz zmian strukturalnych w nerce. Stwierdzenie w zastosowanym modelu eksperymentalnym niekorzystnego wpływ kadmu na funkcję i obraz morfologiczny nerek przy stężeniach tego pierwiastka w moczu (0,0852 – 0,2820 μg/g kreatyniny) mieszczących się w dolnym przedziale wartości notowanych obecnie w populacji generalnej pozwala sądzić, iż nawet niskie narażenie środowiskowe na ten ksenobiotyk może stwarzać ryzyko uszkodzenia nerek. W świetle wyników badań własnych uszkadzający wpływ kadmu na nerki w przypadku niskiego i umiarkowanego narażenia jest związany z rozwojem stanu zapalnego i działaniem prooksydacyjnym tego pierwiastka prowadzącym do rozwoju stresu oksydacyjnego w tym narządzie.

Kolejnym, obok stwierdzenia, iż nawet niskie narażenie na kadm stanowi czynnik ryzyka uszkodzenia nerek, a zarazem najważniejszym, osiągnięciem badań własnych jest wykazanie ochronnego wpływu ekstraktu z owoców *A. melanocarpa* w odniesieniu do działania nefrotoksycznego kadmu. Wyniki przeprowadzonych badań pozwalają wnioskować, iż przetwory z owoców aronii mogą stanowić skuteczną strategią ochronną przed uszkodzeniem nerek powodowanym przez niskie i umiarkowane narażenie przewlekłego na kadm.

Biorąc pod wyniki badań własnych, jak również rezultaty badań wcześniej przeprowadzonych w zastosowanym modelu doświadczalnym [5, 8–12, 14, 30, 40, 41] oraz prozdrowotne właściwości owoców *A. melanocarpa* [4, 15, 29, 50], ochronny wpływ ekstraktu z tych owoców w odniesieniu do działania nefrotoksycznego kadmu można wytłumaczyć antyoksydacyjnymi, przeciwzapalnymi, przeciwapoptotycznymi i antyproliferacyjnymi właściwościami składników ekstraktu, a przede wszystkim związków polifenolowych. Chociaż na obecnym etapie badań nie można jeszcze w pełni wyjaśnić mechanizmów ochronnego wpływu ekstraktu z owoców aronii, to fakt, że suplementacja tym ekstraktem przy obu poziomach ekspozycji na kadm prowadziła do obniżenie stężenia chemeryny, MIP1a i Bax w porównaniu ze zwierzętami, które nie otrzymały tego ekstraktu podczas narażenia na ten pierwiastek toksyczny świadczy o jego przeciwzapalnym i antyapoptotycznym działaniu. Efekt ten wraz z antyproliferacyjnym działaniem ekstraktu z owoców aronii czarnoowocowej, wykazanym w badaniach histopatologicznych, oraz mniejszym obciążeniem organizmu

kadmem, w tym jego mniejszą kumulacją w nerkach [8], może w pewnym stopniu wyjaśniać działanie ochronne ekstraktu.

Główną grupą składników ekstraktu z owoców *A. melanocarpa* odpowiedzialną za jego działanie nefroprotekcyjne wydają się być związki polifenolowe, które obok wielu działań prozdrowotnych, a przede wszystkim wysokiego potencjału antyoksydacyjnego [39, 43, 59, 66], co potwierdzono zarówno w badaniach własnych, jak też we wcześniejszych badaniach prowadzonych w zastosowanym modelu doświadczalnym [9, 11, 14, 40, 41], wykazują również zdolność kompleksowania jonów Cd^{2+} [4, 39]. Należy jednak podkreślić, iż ochronny wpływ ekstraktu z owoców aronii na nerki mógł wynikać także z obecności innych składników skutecznie zmniejszających toksyczność kadmu, takich jak β-karoten, triterpeny, błonnik, pektyny, witamina C i witamina E oraz biopierwiastki, a w tym cynk i selen [4, 15, 29, 50]. Fakt, iż zaburzeniu funkcji nerek u zwierząt narażanych na kadm towarzyszył stres oksydacyjny, a liczne korelacje pomiędzy biomarkerami funkcji nerek a wskaźnikami równowagi oksydacyjno - redukcyjnej potwierdziły związek przyczynowo - skutkowy pomiędzy stresem oksydacyjnym a uszkodzeniem nerek pozwala sądzić, iż ekstrakt z owoców aronii chroniąc przed rozwojem stresu oksydacyjnego w nerce skutecznie chronił przed uszkodzeniem tego narządu.

Należy podkreślić, iż wpływ protekcyjny ekstraktu z owoców aronii na nerkę można również, przynajmniej częściowo, tłumaczyć działaniem pośrednim jego składników skutkującym mniejszą kumulacją kadmu w tym narządzie [8]. We wcześniejszych badaniach prowadzonych w tym modelu doświadczalnym stwierdzono, że stężenie kadmu w nerce zwierząt, którym podczas narażenia na 1 mg Cd/kg paszy przez 3 i 10 miesięcy podawano ekstrakt było niższe o odpowiednio 29% i 9,5% w porównaniu do zwierząt, które nie otrzymały tego ekstraktu, natomiast w przypadku narażenia na 5 mg Cd/kg paszy przez okres od 3 do 24 miesięcy stężenie tego pierwiastka w nerce było niższe o 5,6 – 14% [8]. Związki polifenolowe występujące w dużych ilościach w ekstrakcie, ze względu na obecność grup hydroksylowych (-OH), są zdolne do chelatowania jonów Cd^{2+}, a co za tym idzie mogą zmniejszać wchłanianie tego ksenobiotyku z przewodu pokarmowego [4, 8, 39]. Ponadto, polifenole mogą komplekować jony Cd^{2+} już wchłonięte z przewodu pokarmowego i zwiększać ich wydalanie z moczem, przyśpieszając ich eliminację z organizmu, co odnotowano w badaniach własnych u zwierząt narażonych na 5 mg Cd/kg paszy, jak również we wcześniejszych badaniach prowadzonych u tych zwierząt [8]. Ze względu na mniejszą zawartość tego pierwiastka toksycznego w organizmie, w tym niższe jego stężenie w nerkach, również skutki jego działania toksycznego na ten narząd były mniejsze.

Wyniki badań własnych mają nie tylko istotną wartość naukową, ale również istotny aspekt praktyczny. Wykazanie, iż niskie narażenie na kadm może uszkadzać nerki wskazuje na konieczność zwrócenia większej uwagi na ten ksenobiotyk jako środowiskowy czynnik ryzyka dla zdrowia populacji generalnej i podejmowania zarówno dalszych działań mających na celu ograniczanie narażenia, jak również wdrażanie skutecznych strategii ograniczania

niekorzystnych dla zdrowia skutków wynikających z narażenia na ten ksenobiotyk. Wyniki badań będących przedmiotem niniejszej rozprawy doktorskiej łącznie z wynikami badań wcześniej przeprowadzonych w tym modelu doświadczalnym [5, 8–12, 14, 30, 40, 41] wskazują, iż taką strategią mogą być przetwory z owoców *A. melanocarpa*, które mogą, chronić nie tylko przed uszkodzeniem nerek, ale również innymi efektami działania toksycznego kadmu. Należy jednak podkreślić, iż zastosowanie owoców aronii czarnoowocowej w zapobieganiu skutkom narażenia na kadm, w tym jego działaniu nefrotoksycznemu, u ludzi wymaga dalszych badań.

5. Wnioski

- Umiarkowana, a nawet niska ekspozycja przewlekła samic szczura na kadm, stanowiąca odpowiednik aktualnego narażenia środowiskowego populacji generalnej na ten ksenobiotyk, prowadzi do zaburzenia funkcji kanalików i kłębuszków nerkowych oraz zmian patologicznych w obrazie morfologicznym tego narządu, co wskazuje, iż niskie narażenie na ten pierwiastek toksyczny może stwarzać ryzyko uszkodzenia nerek.

- Indukowane przez kadm uszkodzenie kanalików nerkowych poprzedza uszkodzenie kłębuszków, a zmiany ulegają nasileniu wraz z czasem trwania narażenia i kumulacją tego pierwiastka toksycznego w nerce.

- Mechanizm działania nefrotoksycznego kadmu w warunkach niskiego i umiarkowanego narażenia jest związany z indukcją zmian zapalnych oraz działaniem prooksydacyjnym prowadzącym do rozwoju stresu oksydacyjnego w nerce.

- Podawanie ekstraktu z owoców *A. melanocarpa* podczas niskiego i umiarkowanego narażenia na kadm chroni przed powodowanym przez ten pierwiastek toksyczny uszkodzeniem funkcji nerek i zmianami patologicznymi w strukturze tego narządu.

- Efekt nefroprotekcyjny ekstraktu z owoców aronii czarnoowocowej podczas narażenia na kadm wynika z jego właściwości antyoksydacyjnych oraz wpływu na metabolizm kadmu w organizmie.

- Przetwory z owoców *A. melanocarpa* mogą stanowić skuteczną strategię w zapobieganiu uszkodzeniu nerek podczas niskiego i umiarkowanego narażenia przewlekłego na kadm.

6. Skróty zastosowane w rozprawie doktorskiej

ACR	stężenie albuminy w moczu wyrażone w przeliczeniu na stężenie kreatyniny
ALP	fosfataza zasadowa
ALT	aminotransferaza alaninowa
AM	0,1% wodny roztwór ekstraktu z owoców *A. melanocarpa* L.
AST	aminotransferaza asparaginianowa
Bax	białko z rodziny białek Bcl-2
β2-MG	β2-mikroglobulina
CAT	katalaza
Cd	kadm
Cd^{2+}	jon kadmu
Cd-MT	kompleks kadm-metalotioneina
DNA	kwas deoksyrybonukleinowy
ELISA	test immunoenzymatyczny (ang. *enzyme-linked immunosorbent assay*)
GPx	peroksydaza glutationowa
GR	reduktaza glutationowa
GSH	glutation zredukowany
GSSG	glutation utleniony
grupa AM	grupa doświadczalna, której podawano 0,1% wodny roztwór ekstraktu z owoców *A. melanocarpa* L.
grupa Cd_1	grupa narażana na kadm w paszy w stężeniu 1 mg/kg
grupa Cd_1+AM	grupa doświadczalna, której podczas narażana na kadm w paszy w stężeniu 1 mg/kg podawano 0,1% wodny roztwór ekstraktu z owoców *A. melanocarpa* L.
grupa Cd_5	grupa narażana na kadm w paszy w stężeniu 5 mg/kg
grupa Cd_5+AM	grupa doświadczalna, której podczas narażana na kadm w paszy w stężeniu 5 mg/kg podawano 0,1% wodny roztwór ekstraktu z owoców *A. melanocarpa* L.
H_2O_2	nadtlenek wodoru
KIM-1	białko uszkodzenia nerek-1
LDL	lipoproteiny o niskiej gęstości
m.c.	masa ciała
MDA	dialdehyd malonowy
MIP1a	białko zapalne makrofagów-1
MPO	mieloperoksydaza
MT	metalotioneina
NAG	N-acetylo-β-D-glukozaminidaza
OSI	indeks stresu oksydacyjnego
PCR	stężenie białka całkowitego w moczu wyrażone w przeliczeniu na stężenie kreatyniny
-SH	grupa tiolowa, grupa sulfhydrylowa
SOD	dysmutaza ponadtlenkowa
TAS	całkowity status antyoksydacyjny
TOS	całkowity status oksydacyjny
TRx	tioredoksyna
XOD	oksydaza ksantynowa

7. Literatura

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8. Streszczenie w języku polskim

W ostatnich latach obserwuje się wzrost zainteresowania możliwością wykorzystania substancji pochodzenia roślinnego w terapii i profilaktyce wielu schorzeń, w tym chorób cywilizacyjnych. Ponadto coraz większa uwaga naukowców koncentruje się na możliwości wykorzystania surowców roślinnych o dobrze znanym działaniu prozdrowotnym w profilaktyce zagrożeń dla zdrowia wynikających z narażenia na ksenobiotyki. Do grupy tej zaliczamy owoce aronii czarnoowocowej (*Aronia melanocarpa* L.), która jest krzewem z rodziny różowate (*Rosaceae*).

Liczne właściwości prozdrowotne przetworów z owoców *A. melanocarpa*, w tym zwłaszcza ich wysoki potencjał antyoksydacyjny oraz obecność związków polifenolowych, które mogą kompleksować jony metali dwuwartościowych zwróciły uwagę zespołu badaczy z Zakładu Toksykologii Uniwersytetu Medycznego w Białymstoku na możliwość wykorzystania tego surowca w zapobieganiu skutkom narażenia na kadm (Cd), który należy do głównych zanieczyszczeń chemicznych środowiska w krajach uprzemysłowionych, w związku z czym ekspozycja na ten pierwiastek jest obecnie nieunikniona, a prognozy wskazują, iż będzie ona wzrastała. Badania epidemiologiczne dostarczają natomiast coraz więcej dowodów na to, że nawet niskie narażenie środowiskowe na kadm może stwarzać zagrożenie dla zdrowia, w tym ryzyko uszkodzenia nerek, które są narządem tarczowym dla tego ksenobiotyku. Dlatego też konieczne jest zarówno poznanie ryzyka uszkodzenia nerek na skutek aktualnego narażenia na kadm, jak również znalezienie skutecznego czynnika, który będzie mógł zostać wykorzystany w profilaktyce uszkodzenia tego narządu.

W dotychczasowych badaniach, prowadzonych w modelu doświadczalnym na samicach szczura szczepu Wistar, wykazano, iż podawanie 0,1% wodnego roztworu ekstraktu z owoców *A. melanocarpa* (zawierającego 65,74% związków polifenolowych i 18,65% antocyjanów) podczas narażenia na kadm stanowiącego odpowiednik niskiej i umiarkowanej ekspozycji środowiskowej człowieka na ten pierwiastek toksyczny (odpowiednio 1 i 5 mg Cd/kg paszy) przez okres od 3 do 24 miesięcy chroniło przed kumulacją kadmu w organizmie, w tym nagromadzaniem się tego pierwiastka w nerkach i wątrobie oraz wieloma skutkami jego działania wliczając działanie hepato- i osteotoksyczne.

Mając powyższe na uwadze postawiono hipotezę, iż ekstrakt z owoców aronii może chronić również przed działaniem uszkadzającym kadmu na nerki. W związku z tym podjęto badania własne mające na celu stwierdzenie, czy narażenie na kadm stanowiące odpowiednik niskiej i umiarkowanej ekspozycji środowiskowej człowieka może prowadzić do uszkodzenia struktury i funkcji nerek, a podawanie ekstraktu z owoców *A. melanocarpa* podczas tego narażenia może chronić przed uszkodzeniem tego narządu. Ponadto badania miały na celu wyjaśnienie, czy uszkodzenie nerek przez kadm może wynikać z działania prooksydacyjnego tego ksenobiotyku, a potencjalny efekt ochronny ekstraktu z owoców aronii może być spowodowany jego właściwościami antyoksydacyjnymi.

Badania obejmowały ocenę stanu funkcjonalnego nerek w oparciu o pomiar biomarkerów uszkodzenia kanalików (stężenie białka uszkodzenia nerek-1 – KIM-1 i beta2-mikroglobuliny – β2-MG, aktywność N-acetylo-β-D-glukozaminidazy – NAG i fosfatazy alkalicznej – ALP) i kłębuszków nerkowych (stężenie albuminy i białka całkowitego w moczu wyrażone w przeliczeniu na stężenie kreatyniny, klirens kreatyniny, stężenie mocznika i kwasu moczowego w surowicy i moczu), ocenę markerów stanu zapalnego (chemeryna, białko zapalne makrofagów-1 – MIP1a i białko z rodziny białek Bcl-2 – Bax) oraz równowagi oksydacyjno - redukcyjnej (w tym wskaźniki enzymatycznej i nieenzymatycznej bariery antyoksydacyjnej, całkowity status antyoksydacyjny – TAS, całkowity status oksydacyjny – TOS, indeks stresu oksydacyjnego – OSI), a także ocenę struktury histologicznej tego narządu.

W przeprowadzonych badaniach wykazano, iż umiarkowana (5 mg Cd/kg paszy), a nawet niska (1 mg Cd/kg paszy) ekspozycja przewlekła samic szczura na kadm prowadzi do zaburzenia funkcji kanalików i kłębuszków nerkowych oraz zmian patologicznych w obrazie morfologicznym tego narządu. Uszkodzenie kanalików nerkowych poprzedzało uszkodzenie kłębuszków, a zmiany ulegały nasileniu wraz z czasem trwania narażenia i kumulacją tego pierwiastka toksycznego w nerce. Stwierdzono, iż mechanizm działania nefrotoksycznego kadmu w warunkach niskiego i umiarkowanego narażenia jest związany z indukcją zmian zapalnych oraz działaniem prooksydacyjnym prowadzącym do rozwoju stresu oksydacyjnego w nerce. Podawanie 0,1% wodnego roztworu ekstraktu z owoców *A. melanocarpa* podczas niskiego i umiarkowanego narażenia na kadm chroniło przed uszkodzeniem funkcji nerek i zmianami patologicznymi w strukturze tego narządu, osłabiało indukowany przez kadm stan zapalny oraz zapobiegało zaburzeniu równowagi oksydacyjno - redukcyjnej i rozwojowi stresu oksydacyjnego.

Wykazanie niekorzystnego wpływ kadmu na funkcję i obraz morfologiczny nerek przy stężeniach tego pierwiastka w moczu szczurów (0,0852 – 0,2820 μg/g kreatyniny) mieszczących się w dolnym przedziale wartości notowanych w populacji generalnej pozwala sądzić, iż nawet niskie narażenie na ten pierwiastek może stwarzać ryzyko uszkodzenia nerek, co wskazuje na konieczność zwrócenia większej uwagi na ten ksenobiotyk jako środowiskowy czynnik ryzyka dla zdrowia populacji generalnej i podejmowania zarówno dalszych działań mających na celu ograniczanie narażenia, jak również wdrażanie skutecznych strategii zapobiegania niekorzystnym dla zdrowia skutkom wynikającym z narażenia na ten ksenobiotyk. Wykazanie ochronnego wpływu ekstraktu z owoców aronii w odniesieniu do działania nefrotoksycznego kadmu łącznie z rezultatami wcześniejszych badań przeprowadzonych w tym modelu doświadczalnym pozwala sądzić, iż taką strategią mogą być przetwory z owoców *A. melanocarpa*. Ewentualne zastosowanie produktów aroniowych w profilaktyce zagrożeń dla zdrowia wynikających z narażenia na kadm, w tym jego działania nefrotoksycznego, u ludzi wymaga jednak dalszych badań.

9. Streszczenie w języku angielskim/Summary

In recent years, there has been an increase in interest in the possibility of using plant-based substances in the treatment and prevention of many diseases, including civilizational diseases. The growing attention of scientists is focused on the possibility of using plant materials with well-known health-promoting properties in the prevention of health risks resulting from exposure to various xenobiotics. This group includes the fruits of the black chokeberry (*Aronia melanocarpa* L.), which is a shrub from the *Rosaceae* family.

Numerous health-promoting effects of *A. melanocarpa* fruit preserves, especially their high antioxidative potential and the presence of polyphenolic compounds that can complex divalent metal ions drew the attention of researchers from the Department of Toxicology of the Medical University of Bialystok to the possibility of using this raw material in the prevention of the effects of exposure to cadmium (Cd), which is one of the main chemical pollutants of the environment in industrialized countries, therefore exposure to this element is now inevitable and forecasts indicate that it will increase. Epidemiological studies provide a growing number of evidence, that even low environmental exposure to cadmium may pose a health risk, including the risk of damage to the kidneys, which are the target organ for this xenobiotic. Therefore, it is necessary both to know the risk of kidney damage due to current exposure to cadmium, as well as to find an effective factor that can be used in the prevention of damage to this organ.

In previous studies, conducted in an experimental model on female Wistar rats, it was shown that the administration of a 0.1% extract from the berries of *A. melanocarpa* (containing 65.74% of polyphenolic compounds and 18.65% of anthocyanins) during exposure to cadmium equivalent of low and moderate human environmental exposure to this toxic element (1 and 5 mg Cd/kg of feed, respectively) for 3 to 24 months, protected against cadmium accumulation in the body (including its content in the kidneys and liver) and many effects of its action, including hepato- and osteotoxicity.

Considering the above, it was hypothesized that chokeberry extract may also protect against the damaging impact of cadmium on the kidney. Therefore, own research was undertaken to determine whether exposure to cadmium at levels equal to low and moderate human environmental exposure can lead to damage to the structure and function of the kidney and whether the administration *of A. melanocarpa* fruit extract during this exposure can protect against damage to this organ. Moreover, the study was aimed at clarifying whether kidney damage caused by cadmium may result from the pro-oxidative effect of this xenobiotic and whether the potential protective effect of chokeberry extract may be due to its antioxidative properties.

The studies included the assessment of the function of the kidneys based on the measurement of biomarkers of tubular damage (the concentrations of kidney damage protein-1 – KIM-1 and beta2-microglobulin – β2-MG and the activities of N-acetyl-β-D-

glucosaminidase – NAG and alkaline phosphatase – ALP) and renal glomeruli (albumin and total protein concentration in the urine adjusted for creatinine concentration, creatinine clearance, urea and uric acid concentrations in the serum and urine), assessment of inflammatory markers (chemerin, macrophage inflammatory protein-1 – MIP1a, and protein from the Bcl-2 family – Bax) and oxidative - reductive balance (including indicators of the enzymatic and non-enzymatic antioxidative barrier, total antioxidative status – TAS, total oxidative status – TOS, and oxidative stress index – OSI), as well as assessment of the histological structure of this organ.

The studies showed that moderate (5 mg Cd/kg of feed) and even low-level (1 mg Cd/kg of feed) chronic exposure of female rats to cadmium led to disturbances in the function of the renal tubules and glomeruli and pathological changes in the morphological structure of this organ. Damage to the renal tubules preceded damage to the glomeruli, and the changes intensified with the duration of exposure and the accumulation of this toxic element in the kidney. It was found that the mechanism of nephrotoxic action of cadmium in the conditions of low and moderate exposure is associated with the induction of inflammatory changes and prooxidative activity leading to the development of oxidative stress in the kidney. The administration of a 0.1% extract from the berries of *A. melanocarpa* during low and moderate exposure to cadmium protected against damage to the kidney function and pathological changes in the structure of this organ, weakened inflammation induced by cadmium and prevented disruption of the oxidative - reductive balance and the development of oxidative stress.

The revealing of the negative impact of cadmium on the function and morphological structure of the kidney at the concentrations of this element in the urine of rats (0.0852 – 0.2820 μg/g creatinine), which are in the lower range of values noted currently in the general population, allows us to believe that even low exposure to this element may pose a risk of kidney damage, emphasizing the need to pay more attention to this xenobiotic as an environmental risk factor for the health of the general population and to take both further measures to reduce exposure and implement effective strategies to prevent negative outcomes resulting from exposure to this xenobiotic. Demonstration of the protective effect of chokeberry extract in relation to the nephrotoxic effect of cadmium, together with the results of previous studies conducted in this experimental model, suggests that *A. melanocarpa* fruit preparations may be an effective strategy. However, the possible use of aronia products in the prevention of health risks resulting from exposure to cadmium in humans, including its nephrotoxic effects, requires further research.

10. Kopie publikacji wchodzących w skład rozprawy doktorskiej

10.1. Publikacja I – Current levels of environmental exposure to cadmium in industrialized countries as a risk factor for kidney damage in the general population: A comprehensive review of available data

10.2. Publikacja II – The protective potential of *Aronia melanocarpa* L. berry extract against cadmium-induced kidney damage: A study in an animal model of human environmental exposure to this toxic element

10.3. Publikacja III – Protective effect of the extract from *Aronia melanocarpa* L. berries against cadmium-induced oxidative stress in the kidney: A study in an *in vivo* experimental model

10.1. Publikacja I

Current levels of environmental exposure to cadmium in industrialized countries as a risk factor for kidney damage in the general population: A comprehensive review of available data.

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Review

Current Levels of Environmental Exposure to Cadmium in Industrialized Countries as a Risk Factor for Kidney Damage in the General Population: A Comprehensive Review of Available Data

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Abstract: The growing number of reports indicating unfavorable outcomes for human health upon environmental exposure to cadmium (Cd) have focused attention on the threat to the general population posed by this heavy metal. The kidney is a target organ during chronic Cd intoxication. The aim of this article was to critically review the available literature on the impact of the current levels of environmental exposure to this xenobiotic in industrialized countries on the kidney, and to evaluate the associated risk of organ damage, including chronic kidney disease (CKD). Based on a comprehensive review of the available data, we recognized that the observed adverse effect levels (NOAELs) of Cd concentration in the blood and urine for clinically relevant kidney damage (glomerular dysfunction) are 0.18 μg/L and 0.27 μg/g creatinine, respectively, whereas the lowest observed adverse effect levels (LOAELs) are >0.18 μg/L and >0.27 μg/g creatinine, respectively, which are within the lower range of concentrations noted in inhabitants of industrialized countries. In conclusion, the current levels of environmental exposure to Cd may increase the risk of clinically relevant kidney damage, resulting in, or at least contributing to, the development of CKD.

Keywords: cadmium; kidney; nephrotoxicity; biomarkers of nephrotoxicity; chronic kidney disease; environmental exposure; general population; LOAEL; NOAEL; odds risk

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1. Introduction

Epidemiological studies have provided a growing amount of reliable evidence that long-term moderate and, sometimes, even low-level environmental exposure to certain chemicals poses a risk to health and can result in damage to various organs and systems, including the kidneys [1–3]. Therefore, an important task in the area of public health is to estimate the threat posed by chemical substances present in the living and work environments of humans, as well as in food, in economically developed and developing countries. The proper assessment of the health risks posed by these pollutants is necessary in order to find and implement appropriate preventive strategies and effective methods for treating their unfavorable effects. Recognizing the risk factors for kidney damage in the general population is also important because of the possibility of simultaneous exposure to two or more nephrotoxic factors that could interact, leading to an intensification of the injurious impact on this organ [4–7].

Among the xenobiotics to which the general population is exposed throughout their lifetimes and that can be harmful to the kidney, special attention has been paid to toxic heavy metals, including cadmium (Cd). Cd was ranked seventh on the 2022 priority list of hazardous substances of the Agency for Toxic Substances and Disease Registry [8], and forecasts indicate that the exposure of the general population to this element is increasing in industrialized and developing countries [9,10]. The results of epidemiological studies,

especially recent findings, show that even low-level chronic exposure to this xenobiotic can result in damage to the kidney, liver, skeletal system, cardiovascular system, and nervous system, as well as deterioration in hearing and sight (for a review, see [2,11–15]). Moreover, exposure to this element has been suggested to contribute to the development of cancer [16].

The kidney is the main location of Cd accumulation in the body, as well as the target organ (i.e., the organ damaged first) for this element during chronic intoxication [15,17–19]. The fact that repeated high or moderate exposure to this xenobiotic injures this organ is well known [20–25]. However, for an assessment of the health hazard faced by the general population, clarifying whether and to what extent the low-level lifetime exposure to Cd that is currently common and unavoidable in industrialized countries [11,13,26,27] could increase the risk of damage to various organs and systems, especially the kidney, is particularly important. This is also important because evidence suggests that the level of exposure to this metal that is currently recognized as safe may be too high and should be revised [2,28–30].

Due to its role in the elimination of exo- and endogenous substances and products of their biotransformation from organisms, the kidney is an organ whose proper function determines the general state of health [31–33]. Chronic kidney disease (CKD), also called chronic kidney failure, is a global problem, as it is a leading cause of death in both developed and developing countries [34,35]. Therefore, considering the increasing prevalence of kidney diseases in the world's population [34–36], we must identify all causative factors, including Cd, which deserves special attention among the potential risk factors for kidney dysfunction [20,22,31,37]. In the case of xenobiotics, estimating the exposure levels at which this effect occurs is also crucial.

Thus, the aim of this paper was to provide a critical review of the literature available worldwide on the influence of current human exposure to Cd in economically developed and developing countries on the kidneys. In addition, we aimed to assess, based on reliable data, whether this exposure poses a substantial risk of clinically relevant damage to this organ. For this purpose, ample evidence from recent epidemiological studies on this topic is presented and discussed. Since the adequate assessment of the impact of Cd on the kidney and the risk of injury to this organ at low exposure levels requires the measurement of early and sensitive biomarkers, special attention was also paid to providing an overview of the available data on biomarkers of Cd nephrotoxicity in terms of their usefulness in detecting early changes under low exposure levels. We attempted to select the earliest and most useful of these biomarkers. A critical review of the methods of estimating the intensity of exposure to this element, including biomarkers of exposure, is also provided. From a public health perspective, evaluating the concentration of Cd in the urine and blood of the general population that poses a substantial risk of damage to the kidney is crucial. Hence, we assessed, based on the available epidemiological data, the no observed adverse effect level (NOAEL) and lowest observed adverse effect level (LOAEL) of the Cd concentration in the blood and urine for clinically relevant kidney damage. This article is the first review focused on evaluating the risk of kidney damage at low-to-moderate levels of environmental exposure to Cd, which occur in numerous countries worldwide.

The following literature databases were searched to prepare this review article: Pubmed, Scopus, Elsevier, and Taylor & Francis Online. We used keywords such as cadmium and kidney, nephrotoxicity, general population, environmental exposure, occupational exposure, concentration, blood, urine, health risk, health effects, accumulation, target organ, NOAEL, LOAEL, threshold level, mechanisms of action, damage, injury, dysfunction, disease, proximal tubules, glomerulus, nephron, markers of nephrotoxicity, oxidative stress, apoptosis, metallothionein (MT), β2-microglobulin (β2-MG), retinol-binding protein (RBP), α1-microglobulin (α1-MG), N-acetyl-β-D-glucosaminidase (NAG), kidney injury molecule-1 (KIM-1), glomerular filtration rate (GFR), estimated glomerular filtration rate (eGFR), and albuminuria. The assessment of whether the current levels of environmental exposure to Cd in industrialized countries may be a risk factor for kidney damage, including CKD, was based on an overview of data published within the last 10 years (the search

strategy is presented in Table 1). Reports from studies conducted among inhabitants of highly polluted areas, presenting high concentrations of Cd in the blood and urine, were excluded from this review because this exposure level was unrepresentative of the general population worldwide.

Table 1. The data search strategy regarding the impact of the current environmental exposure to cadmium (Cd) on the kidneys.

Database	Total Number of Articles Found (Published in 2013–2023)	Number of Articles Excluded (Duplicates, Papers Out of Our Scope, or Papers Older than 10 Years)	Number of Articles Included in This Study
Pubmed	3971	3896	75
Scopus	8571	8546	25
Elsevier	3064	3041	23
Taylor & Francis Online	2491	2482	9

2. The Kidney as One of the Most Important Organs in the Body

The kidney is one of the most important organs in the body, playing a multidirectional role (Figure 1) [31,38]. The function of the kidney in the process of detoxification mainly consists of the elimination of toxic substances and their metabolites, as well as the biotransformation of xenobiotics [33,39]. Although the liver is the main organ responsible for the biotransformation of xenobiotics in the body, the kidney is also involved in this process. Moreover, the kidney is the location for the accumulation of numerous substances, including toxic heavy metals such as Cd [17,19,40–42]. The accumulation of toxic substances in the kidneys is, to some extent, a process of detoxification (e.g., Cd retention in the MT-bound form), because the substances accumulated in this organ are excluded from systemic circulation; however, the ability of the kidneys to accumulate xenobiotics is limited, and this process generally has negative outcomes for this organ [27,40]. Furthermore, substances accumulated in the kidneys can be released into the bloodstream and exert a toxic effect [43].

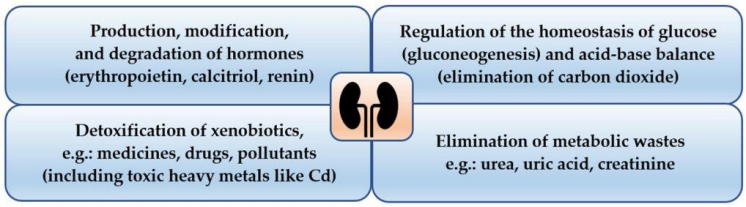

Figure 1. Functions of the kidney.

The kidney is at a particularly high risk of being damaged by xenobiotics because of the crucial role of this organ in the detoxification of chemical substances and the elimination of unnecessary compounds from the body [31,32,44]. On the one hand, exposure to xenobiotics can damage the kidney. On the other hand, the functional state of this organ determines the unfavorable effects of the toxic substances to which the body is exposed, as well as their metabolites [20,45]. Regardless of the cause, the dysfunction of the kidney leads, or at least contributes, to the development of anemia, cardiovascular diseases, and diabetes, and damages mineral and bone metabolism [46]. Additionally, kidney failure enhances the toxicity of chemical substances, mainly by prolonging their halftime in the body through slower excretion [31,47].

3. Main Causes of Kidney Dysfunction in the General Population

Kidney dysfunction (kidney failure) is a gradual loss in the functionality of this organ due to endo- or exogenous causes. This condition may include morphological and

functional changes, and may be acute or chronic [38,47,48]. CKD is a long-term condition in which the kidneys are damaged and cannot filter blood as they should. It is diagnosed based on albuminuria and a decreased renal filtration rate (GFR or eGFR). CKD is a major worldwide health problem, with a prevalence of 11–13% globally, of which only 10% are diagnosed and receive proper treatment [36,49].

The destructive impact of various factors on the kidney involves changes in the tubules and glomeruli of the nephrons, such as modifications to the glomerular hemodynamics, oxidative injury to tubular and glomerular cells, thrombotic microangiopathy, and rhabdomyolysis [9,33,44]. Among the main causes of kidney dysfunction in the general population (Table S1), diabetes [50] and hypertension [51] are considered the most common; however, medicines and environmental or occupational pollutants, including Cd [32,33,48], should not be uncredited. Moreover, co-exposure to multiple nephrotoxic factors increases the risk of kidney damage [4,6], and Cd may be one of these factors [5,52], as explained later in this review.

4. The Current Cd Exposure Level in Industrialized Countries

The technological progress in recent decades is the main reason for the increased use of Cd worldwide, contaminating the environment and dietary products and resulting in inevitable lifelong human exposure to this xenobiotic [2,9,26,53,54]. Naturally, Cd is present in the lithosphere at low concentrations (0.15 mg/kg in the Earth's crust and 1.1×10^{-4} mg/L in seawater) [9], but numerous industrial activities (e.g., mining and smelting) have increased its presence in the environment and enhanced human exposure [9]. Every year, thousands of tons of Cd-contaminated wastes are discarded into the environment worldwide [9,21]. Despite the actions taken to remove Cd from and decrease the amount of Cd released into the lithosphere, the contamination of the natural environment with this xenobiotic shows an increasing trend, as this metal is not biodegradable and persists in the environment for hundreds of years [55].

Foods, especially plant products, are the main source of exposure to this heavy metal in the non-smoking portion of the general population [2,9,12], while for habitual tobacco smokers, tobacco smoke is a serious additional and often main source of intoxication with this xenobiotic [56,57]. The available data indicate that the current dietary intake of Cd worldwide sometimes exceeds the levels acknowledged to be safe [11,58,59]. The provisional tolerable monthly intake (PTMI) for this heavy metal is 25 μg/kg body weight (b.w.) [59], while its provisional tolerable weekly intake (PTWI) according to the European Food Safety Authority (EFSA) is 2.5 μg/kg b.w. [25]. Currently, the dietary intake of Cd in populations inhabiting areas considered to be non-polluted varies from 10 to 70 μg/day [25,26,29,54,60–63]. Assuming an average body weight of 70 kg, the weekly and monthly intake of this heavy metal would reach 1–7 and 4.2–30 μg/kg b.w., respectively. This proves that even for inhabitants of areas that are not polluted with Cd, the safe intake levels of this toxic element (the PTWI and PTMI) may be exceeded, in some cases by about threefold (PTWI). The lowest daily intake of Cd, with an arithmetic mean (AM) oscillating around 10 μg (1 μg/kg b.w./week; 99th percentile—2.1 μg/kg b.w./week), was noted in Sweden [61]. The highest oral exposure to this xenobiotic (exceeding the PTMI for this element by more than two-fold), which reached 55 μg/kg b.w./month in males and 53 μg/kg b.w./month in females (aged 18–39 years), was noted in industrialized regions of China [59]. The facts that the Cd concentration in commercially available dietary products sometimes exceeds the safe-limit values and that the dietary intake of this xenobiotic in some parts of the world or in certain groups exceeds the levels currently recognized as safe (the PTWI and PTMI) indicate a substantial risk of excessive intoxication with this element [54,59,62,64].

Numerous factors may increase the gastrointestinal absorption of Cd, simultaneously enhancing the burden of this xenobiotic in the body and exacerbating the risk of toxic effects. The efficiency of the absorption of this xenobiotic from the gastrointestinal tract is low, reaching only 1 to 8% in humans, and it depends mainly on diet, age, and sex [26,27,65–68].

Enhanced Cd absorption is noted particularly in women of reproductive age and children [67,68]. Among the nutritional factors influencing the gastrointestinal absorption of this heavy metal, the presence of essential elements (mainly zinc, magnesium, selenium, calcium, and iron); vitamins; and other bioactive compounds, such as polyphenols, phytates, and carotenoids, is the most important (for a review, see [69,70]). The bioavailability of Cd from the digestive tract may be increased by up to 20% due to the insufficient consumption of these nutritional factors [65,66].

Habitual tobacco smoking significantly increases the burden of Cd in the body, as each cigarette contains approximately 1 µg of this element, 25–35% of which undergoes absorption into the bloodstream [47]. Substantial data show that tobacco smoking is a source of exposure to large quantities of Cd. Concentrations of this heavy metal found in the blood and urine of active smokers were two to eight times higher compared to non-smokers who were gender- and ethnicity-matched and/or living in the same area (Table 2). Exposure to second-hand cigarette smoke also leads to (2–3-fold) higher Cd concentrations in the blood and urine compared to individuals who are neither active nor passive smokers [71,72].

Table 2. The concentration of cadmium (Cd) in the blood and urine of tobacco smokers compared to people who have never smoked [a].

Country	*n*	Expression of Cd Concentration	Cd in the Blood (µg/L) and Urine [b] (µg/g Creatinine)		Reference
			Smokers	Non-Smokers	
Canada	10,099	GM (SE)	1.63 (0.06) † *0.56 (0.02)* †	0.22 (0.01) *0.33 (0.01)*	[73]
	144	GM (P95)	1.62 (3.75) * *0.467 (1.21)* *	0.265 (1.88) *0.333 (0.937)*	[74]
Iran	140	Mean (IQR)	0.87 (0.67–1.31) NS	0.81 (0.59–1.30)	[75]
Serbia	81	Mean ± 95% Cl	2.41 ± 0.04 ‡	0.67 ± 0.04	[76]
South Korea	200	Mean (SD)	1.67 (0.68) NP	0.83 (4.23)	[77]
	4744	GM (SD)	1.06 (0.02) ‡	0.89 (0.01)	[78]
Sweden	4304	Median (P5, P95)	1.00 (0.22–2.46) NP	0.20 (0.09–0.46)	[79]
USA	2325	GM (95% Cl)	1.17 (0.77–1.81) NP	0.86 (0.54–1.36)	[80]
	6761	GM (95% Cl)	1.02 (0.97–1.06) ‡ *0.39 (0.36–0.41)* ‡	0.24 (0.24–0.25) *0.20 (0.19–0.21)*	[57]

GM, geometric mean; IQR, interquartile range; *n*, number of individuals; P5, 5th percentile; P95, 95th percentile; SD, standard deviation; SE, standard error; 95% CI, 95% confidence interval; NS, no statistically significant difference compared to non-smokers; NP, data regarding the statistical significance of differences were not provided; * $p < 0.05$, † $p < 0.01$, and ‡ $p < 0.001$ compared to non-smokers; [a] based on studies published in the last 10 years; [b] values in italics represent Cd concentration in the urine.

The exposure of the general population to Cd may be monitored by evaluating the concentration of this xenobiotic in food and its total daily dietary intake [18,47]. However, due to the difficulty of precisely evaluating the daily intake of Cd, the influence of various factors on its absorption, the uncertainty as to whether a person is an active and/or passive tobacco smoker, and the possibility of additional exposure from sources other than the diet (i.e., the workplace or passive tobacco smoking), calculating the daily intake of this element is not considered a credible method for estimating Cd exposure. Measuring the Cd concentration in the blood and urine is the most reliable method for quantifying the exposure to this xenobiotic because its levels in these biological fluids reflect the exposure from all sources. The blood concentration of this element reflects the current exposure (within the last month), while the concentration in the urine is a more effective biomarker to monitor chronic intoxication [9,27,29,81]. Since Cd is a common contaminant of the envi-

ronment and food, it is always present in the blood and urine of humans (Tables 2 and 3). Concentrations of this element below 1 μg/g creatinine in the urine and 0.5 μg/L in the blood are recognized as "normal Cd concentrations" for the general population, defined as very low and safe concentrations resulting from inevitable exposure to low levels in the natural environment and in food [2]. The most recent comprehensive report on worldwide exposure to Cd was published in 2012 [82]. Moreover, there is no global system for monitoring environmental exposure to Cd in areas recognized as unpolluted by this heavy metal. Furthermore, the available data on the current concentrations of Cd in the blood and urine of inhabitants of unpolluted areas are incomplete, and originate from studies conducted in a limited number of countries (Table 3). In addition, the concentration of this element is expressed in various forms (AM, geometric mean (GM), or median), and its values in the urine are not always adjusted for the creatinine concentration (μg/g creatinine), sometimes being expressed as μg/L. Therefore, comparing data between studies is sometimes very difficult.

According to our review of the available data, the Cd concentration in the blood of the general population in industrialized countries worldwide ranges from 0.02 to 4.40 μg/L (0.02–2.88 μg/L in males and 0.02–4.40 μg/L in females), whereas its urinary concentration reaches 0.04–3.39 μg/g creatinine (0.04–2.34 μg/g creatinine in males and 0.09–3.39 μg/g creatinine in females) and 0.01–3.00 μg/L, and is generally higher in females than in males (Table 3). The higher concentration of Cd in the biological fluids of women compared to men may be explained by its higher rate of gastrointestinal absorption in women due to the smaller iron stores in the body and frequent deficiency of this bioelement. The blood and urinary concentrations of Cd in inhabitants of industrialized countries depend on several factors, mainly including smoking habits, age, and the pollution levels in the place of residence (Tables 2 and 3). Due to the cumulative properties of this xenobiotic, its content in the body increases with age [47,57,83]. Available data in the literature show that the worldwide Cd concentration in the blood of non-smoking individuals reaches 0.09–1.88 μg/L, while in smokers it is higher, ranging from 0.22 to 3.75 μg/L (Table 2) and reaching 7 μg/L in heavy smokers (more than 20 cigarettes/day) [2]. The Cd concentration in the blood and urine increases with the extent of industrialization in the place of residence, as well as the degree of contamination with this xenobiotic [2,21,30,83–86]. According to the available data, the concentration of Cd in the blood and urine in the general population is lowest in countries such as Sweden and Canada, while the highest levels are found in South Korea and China (Table 3). According to this overview of recently published data, the current Cd exposure levels in industrialized countries worldwide, except for areas recognized as excessively polluted, are low to moderate.

Although the present article is focused on environmental exposure to Cd, one should not ignore that another source of intoxication with this element is the inhalation of airborne Cd particles in the workplace (e.g., in the production of alloys and batteries; the coating, enameling, and smelting of metals; and the printing of textiles) [87–94]. The concentration of Cd in the blood and urine of individuals occupationally exposed to this element exceeds the "normal concentration" of this heavy metal by many times, and is higher than that noted in persons who are not occupationally exposed, reaching 34 μg/L in the blood and 62 μg/g creatinine in the urine [90].

Table 3. The current concentration of cadmium (Cd) in the blood and urine of the general population [a].

Country	*n*	Expression of Cd Concentration	Cd in the Blood (μg/L)		Cd in the Urine (μg/L) (μg/g Creatinine) [b]		Reference
			Male	Female	Male	Female	
Argentina	172	Median (range)		0.36 (0.17–1.00)		0.24 (0.01–1.5)	[95]
Bangladesh	72	Median (range)				0.22 (0.01–1.5)	
Canada	10,099	GM (SE)	0.35 (0.01)	0.45 (0.01) †			[73]
	7082	GM (SE)	0.34 (0.01)	0.43 (0.02) *	*0.35 (0.01)*	*0.53 (0.01)* *	[71]
China	896	Median (P25–P75)	1.34 (0.38–2.88)	0.49 (0.31–0.65) NP	*0.38 (0.21–0.65)*	*0.42 (0.23–0.70)* NP	[84]
	78	Median (P25–P75)		1.44 (0.87–2.33)		2.20 (1.42–3.00)	[96]
Ireland	100	Median (P25–P75)			0.3 (0.2–0.6)	0.4 (0.2–0.9)	[97]
South Korea	12,099	GM (95% Cl)	0.76 (0.74–0.77)	1.01 (0.99–1.03) NP			[58]
	643	GM (GSD)	1.10 (1.77)	1.29 (1.78) *	*0.82 (2.04)*	*1.04 (2.29)* *	[29]
	1907	GM (P95)			*0.82 (2.34)*	*1.36 (3.39)* †	[37]
	3781	Median (P25–P75)			0.42 (0.19–0.77)	0.43 (0.18–0.87)	[98]
Sweden	109	Mean (range)	0.46 (0.02–2.3)	0.54 (0.02–2.9) NP	*0.23 (0.04–0.80)*	*0.34 (0.09–1.12)* NP	[30]
Thailand	392	GM (SD)			*0.28 (0.84)*	*0.23 (0.49)* NS	[99]
	81	GM (GSD)	0.9 (2.2)		0.5 (1.9)	1.1 (2.3) NP	[100]
Turkey	30	Median (min–max)		0.34 (0.11–0.84)		0.42 (0.08–0.98)	[101]
USA	3226	GM (SE)	0.49 (0.02)				[102]
	9662	Mean ± SD	0.52 ± 0.58	0.40 ± 0.47			[103]
Denmark	282	GM (95% Cl)			0.123 (0.112–0.350)		[104]
Iceland	203	GM (95% Cl)			0.135 (0.119–0.153)		
Czech Republic	300	GM (95% Cl)			0.132 (0.122–0.142)		
Poland	228	GM (95% Cl)			0.408 (0.369–0.450)		
Croatia	300	GM (95% Cl)			0.175 (0.160–0.192)		
Portugal	295	GM (95% Cl)			0.109 (0.098–0.120)		
France	393	GM (95% Cl)			0.365 (0.340–0.391)		
Luxembourg	210	GM (95% Cl)			0.316 (0.288–0.347)		
Germany	289	GM (95% Cl)			0.199 (0.186–0.213)		
China	50	GM (range)		0.99 (0.23–2.6)			[105]
Croatia	59	GM (range)		0.56 (0.15–4.4)			
Czech Republic	50	GM (range)		0.41 (0.11–2.1)			
Ecuador	25	GM (range)		0.61 (0.25–2.1)			
Morocco	49	GM (range)		0.39 (0.15–1.8)			
Slovakia	52	GM (range)		0.40 (0.17–2.1)			
Slovenia	50	GM (range)		0.49 (0.21–2.2)			
Sweden (north)	35	GM (range)		0.25 (0.08–1.8)			
Sweden (south)	55	GM (range)		0.35 (0.11–2.6)			

GM, geometric mean; GSD, geometric standard deviation; *n*, number of individuals; P25, 25th percentile; P75, 75th percentile; SD, standard deviation; SE, standard error; 95% CI, 95% confidence interval; NP, data regarding the statistical significance of differences were not provided; NS, no statistically significant difference compared to non-smokers; * $p < 0.05$ and † $p < 0.01$ compared to males; [a] based on studies published in the last 10 years; [b] values in italics represent Cd concentration in the urine expressed as μg/g creatinine.

5. Kidneys as the Main Organ of Cd Accumulation in the Body

After entering the bloodstream, the absorbed Cd binds with thiol groups (sulfhydryl groups, -SH groups) of proteins in the erythrocyte membranes and plasma (mainly with albumins), and most of it is transported with the blood into the liver. In this organ, ions of Cd (Cd^{2+}) induce the synthesis of MT and form complexes with this protein (Cd-MT complexes). Some of these complexes are released from the liver into the bloodstream and pass into the tubular fluid [43,52,106]. Moreover, small amounts of this element bound to thiol-containing compounds (e.g., GSH, L-cysteine, L-homocysteine, and N-acetyl-L-cysteine) in the plasma are carried to the kidneys and can be absorbed via the cells of the renal proximal tubules [107].

The main locations of Cd accumulation in both human and animal bodies are the liver and kidneys. During short-term intoxication, Cd is retained mainly in the liver, while long-term exposure results in the accumulation of this xenobiotic mainly in the kidneys, due to their inability to eliminate it from the renal tissues [32,42,108]. The average half-life of Cd in the kidney is 14 years (9–28 years), but some data suggest that it may reach 45 years [18,109]. Thus, the kidney Cd content increases with age, peaking at around 60 years [71,110].

The concentration of this element in the kidneys of the general population (Table 4) has not yet been precisely estimated because of the substantial difficulty of obtaining such data. The only method that allows for the determination of the Cd content in the kidney in vivo, i.e., neutron activation analysis [111], has not been used in epidemiological studies. To our knowledge, the burden of Cd on the kidneys has not been evaluated using this method in humans. Data on the Cd concentration in the kidney usually originate from studies carried out post mortem or in living donors. According to the available data, the Cd concentrations detected in the kidneys of the general population represent a wide range of values, from 1.45 to 93 μg/g wet weight (w.w.) (Table 4). The very limited data from the last 10 years show that the mean concentration of this heavy metal in this organ is 16.0 ± 13.2 μg/g w.w. in subjects aged 37.1 ± 18.7 [112]. The concentration of Cd in the kidneys of individuals occupationally exposed to this xenobiotic [2,88–90,94] may be many times higher (150–395 μg/g w.w.) than in the general population (Table 4).

Table 4. The concentration of cadmium (Cd) in the kidneys of different populations non-occupationally exposed to this heavy metal.

Region (*n*)	Expression of Cd Concentration	Cd Concentration in the Kidney (μg/g w.w.)		Reference
Australia (61)	Mean ± SD	15.45 ± 14.04		[108]
Czech Republic (70)	Mean (95% CI)	28.7 (6.61–93.0)		[113]
Greenland (95)	Mean ± SD	15.97 ± 9.26		[114]
Japan (71)	GM ± GSD	Male, cortex: 72.1 ± 1.7	Female, cortex: 83.9 ± 2.2	[115]
		Male, medulla: 18.3 ± 2.2	Female, medulla: 24.5 ± 2.1	
Japan (41)	GM ± SD	Cortex: 82.7 ± 1.99	Medulla: 36.1 ± 1.99	[19]
Norway (28)	Mean (95% CI)	20.5 (3.74–62.16)		[116]
Poland (99)	Mean ± SD	16.0 ± 13.2		[112]
South Korea (150)	Mean ± SD	35 ± 18		[117]
Spain (78)	Mean (95% CI)	10.8 (6.1–20.2)		[118]
Spain (20)	Mean	21.15		[119]
Sweden (10)	Median (95% CI)	5.18 (2.29–29.99)		[41]
Sweden (109)	Median (range)	12.9 (1.50–55.0)		[120]
		Male: 10.9 (1.6–32.0)	Female: 14.7 (1.50–55.0)	

GM, geometric mean; GSD, geometric standard deviation; *n*, number of individuals; SD, standard deviation; w.w., wet weight; 95% CI, 95% confidence interval.

Cd accumulates in the body mainly in the form of complexes with MT. The MT family is a group of cysteine-rich proteins that have a high affinity to various elements, including both necessary and toxic elements, due to the abundance of -SH groups in their cysteine residues. The physiological role of MT is to regulate the metabolism of bioelements such as copper, zinc, and selenium. Furthermore, this protein protects against the toxicity of heavy metals, including Cd, mercury, and lead [56,107,121]. MT binds Cd^{2+} ions in the kidney cells, forming Cd-MT complexes, which are non-toxic; however, their presence in the extracellular space is dangerous [122].

Since data concerning Cd accumulation in the human kidney are highly limited, the retention of this element in the kidney was investigated almost exclusively based on experimental studies conducted using laboratory animals. The available literature contains significant amounts of data on the Cd concentration in the kidneys of animals intoxicated with this element; however, most of these studies considered high exposure levels [106,123,124]. To our knowledge, the only published data on Cd accumulation in the kidney in an experimental model that accurately reflected the current environmental exposure of the general population to this heavy metal originated from our study, which was conducted on rats fed for up to 24 months with a diet containing 1 or 5 mg Cd/kg, corresponding to low or moderate lifetime human exposure, respectively (Table S2) [17]. The study showed that both the low (0.103–0.306 μg Cd/L in the blood and 0.085–0.276 μg Cd/g creatinine in the urine) and moderate (0.584–1.332 μg Cd/L in the blood and 0.284–0.820 μg/g creatinine in the urine) levels of lifelong exposure to Cd led to an increase (up to 100-fold) in the content and concentration of this heavy metal in the renal tissue of rats, and that the accumulation of this xenobiotic was dose- and time-dependent (Table S2). The finding was that under very low and low exposure to Cd (the control group, fed with a diet containing 0.098 mg Cd/kg, and the group administered with 1 mg Cd/kg of feed, respectively), the accumulation of this element in the kidneys of rats increased throughout the experiment and reached a peak after 24 months (0.084 ± 0.036 and 1.98 ± 0.509 μg/g w.w., respectively) (Table S2), when the age of the animals corresponded to the human age of 60 years [125]. This agrees with the observation that in humans, the accumulation of Cd in this organ reaches a peak at around 60 years of age [71,110]. However, the accumulation of this toxic element in the kidneys during moderate exposure reached its peak after 17 months (10.77 ± 1.936 μg/g w.w.), representing approximately 45 human years, before a plateau was reached (Table S2). The extrapolation of our findings regarding Cd accumulation in the kidneys of rats to humans could be inaccurate and should be approached with caution; yet, this study remains the only attempt to explore this process in vivo considering a lifetime exposure level comparable to that currently noted in the general population in industrialized countries. It is important to emphasize that Cd accumulation in the kidney results from both the intensity of its uptake by this organ and the rate of its elimination.

6. Cd as a Nephrotoxic Factor

Both acute and chronic intoxication with Cd may result in kidney dysfunction in humans and experimental animals (for a review, see [15,27,88]). Since acute poisoning with this toxic element is very rare nowadays, the risk of acute kidney damage is negligible on a global scale. Chronic occupational [90,93,94] and environmental [7,16,40,126,127] exposure to Cd may cause or contribute to kidney injury; however, the risk of damage to this organ in the general population at the low and moderately low exposure levels that currently occur in many developed and developing countries has not been fully estimated.

The fact that Cd damages the kidneys of humans and experimental animals has been known for a long time. The first cases of this xenobiotic exerting a harmful impact on the kidneys as an outcome of environmental exposure were reported in Japan in the mid-1950s in areas around the Jinzu River, which were polluted by this heavy metal due to the operations of the Kamioka Mine, located upriver [53,128–130]. The water of this river was used for both the irrigation of rice fields and fishing. The long-term consumption of food (mainly rice) contaminated with this heavy metal caused chronic Cd poisoning, later called

"Itai-Itai" disease. Patients suffering from this disease had a mean Cd concentration of 26.4 μg/g creatinine in the urine and between 10.7 and 46.7 μg/L in the blood [129], while its concentration in the medulla and cortex of the kidney reached 41.6 and 27.8 μg/g w.w., respectively (data presented as GM) [128]. "Itai-Itai" disease first manifested in kidney failure, accompanied by anemia, bone weakening, spinal and leg pains, and deformities, as well as idiopathic bone fractures. Kidney failure, which was a consequence of tubular dysfunction (epithelial cell damage) and glomerular dysfunction, was one of the most dangerous outcomes. This disease resulted in multiple deaths due to kidney failure. The concentration of β2-MG in the urine of "Itai-Itai" disease patients exceeded 1000 μg/g creatinine [129], indicating irreversible kidney damage. The histopathological examination of the renal tissues showed atrophy of the tubular epithelium, accompanied by dilatation of the lumen, the disappearance of renal tubules, and hyalinization and sclerosis in the glomeruli [130].

As in "Itai-Itai" disease patients, analogical changes characterized by damage to the tubules and glomeruli, including irreversible nephropathy, have been found worldwide in the kidneys of individuals chronically exposed to Cd in the workplace [84,121]. Workers employed in a nickel–cadmium battery factory presented Cd concentrations in the blood and urine reaching 10.21 ± 2.671 μg/L (mean ± standard deviation (SD)) and 5.16 μg/g creatinine (median; range: 1.93–8.76 μg/g creatinine), respectively, resulting in damage to the tubules and glomeruli [121]. More recent data show that the present occupational exposure to Cd poses a risk of developing pathological changes in the structure and function of the kidney, such as tubulointerstitial injury, the degeneration of the tubular epithelial cells in the cortex, and microproteinuria [23,89,90,94]. The NOAEL and LOAEL of the Cd concentration in the blood for kidney damage in people occupationally exposed to this heavy metal for 30 years were estimated (based on the concentration of β2-MG in the urine) to be 2.2 and 2.7 μg/L, respectively, while in the case of 40-year exposure, these values reached 1.7 and 2.0 μg/L, respectively [23].

High environmental exposure to Cd (10 μg Cd/L in the blood and higher) resulting in the development of serious kidney damage, as in "Itai-Itai" disease patients, is not found nowadays. However, as mentioned above, epidemiological studies over the years have indicated a risk of kidney injury as an outcome of even low-level exposure [2,54,99,102,131]. The influence of low to moderate exposure to this xenobiotic on renal tissue is described and discussed in detail later in this review.

The revelation of Cd's damaging impact on the kidneys of "Itai-Itai" disease patients and workers occupationally exposed to this xenobiotic prompted experimental studies using animal models or kidney cell cultures to unveil the mechanisms behind this effect and establish the threshold concentration of Cd in the kidneys, blood, and urine for nephrotoxicity. The toxic effect of this heavy metal in rodent renal tissue (Tables S3 and S4) manifests in numerous defects analogical to those observed in the human kidney, with an identical destruction process, advancing from tubular damage to glomerular disruption. The main histopathological changes in the kidneys of rodents due to Cd exposure are the hypertrophy of epithelial cells, the desquamation of tubular epithelial cells, the dilatation of tubules, and the enlargement of renal glomeruli (Tables S3 and S4) [132–134]. Although multiple studies have been conducted regarding the toxic effect of this xenobiotic on the renal tissue of laboratory animals, experiments using models that closely reflect the current lifetime human environmental exposure levels are lacking. Studies on the nephrotoxicity of Cd have mainly been conducted in animal models exposed to moderate, high, and even very high doses of this xenobiotic; furthermore, the exposure routes often do not correspond to those affecting humans (Tables S3 and S4). These studies provided important data on the impact of Cd on the kidney and the possible mechanisms underlying its nephrotoxicity; however, they did not explain the effects of Cd under low-level long-term exposure. Hence, we conducted a study to investigate the damaging impact of Cd on the kidneys and the possible risk of its occurrence in an experimental rat model that accurately reflected lifetime low and moderate human exposure levels (1 and 5 mg Cd/kg of diet for 3–24 months). To

our knowledge, the impact of such low chronic exposure to this toxic heavy metal on the kidneys has not yet been investigated in an experimental model, and the findings will be published soon.

The critical concentration of Cd in the renal cortex, endangering 10% of the population, is currently considered to be 50 µg/g w.w. and above [9,24,135]. The results of epidemiological studies suggest that damage to the kidney may occur at lower concentrations of this metal; however, evaluating the threshold level is difficult because epidemiological data on the concentration of this element in renal tissues are lacking. It is important to underline that the risk of damage to the kidney depends on not only the level of exposure to Cd, but also factors such as the exposure duration and chemical form of the xenobiotic, as well as the characteristics of the exposed person (mainly age, sex, diet, and health status), which are recognized as important determinants [2,14,47,63,67,68,126,136].

7. Mechanism of Cd Nephrotoxicity

The mechanism behind the damaging impact of Cd on the kidney has been the subject of numerous studies conducted using in vivo and in vitro experimental models (Tables S3 and S4) [39,44,107,134,137]. According to our current knowledge, the mechanism is multidirectional (Figure 2) and includes the induction of inflammatory processes; the development of oxidative stress; alterations in cell adhesion; the stimulation of cell proliferation; and the induction of epigenetic changes (damage to deoxyribonucleic acid (DNA), the suppression of the DNA repair capacity, the methylation of genes, and the disruption of gene expression) [24,39,123,134,138,139].

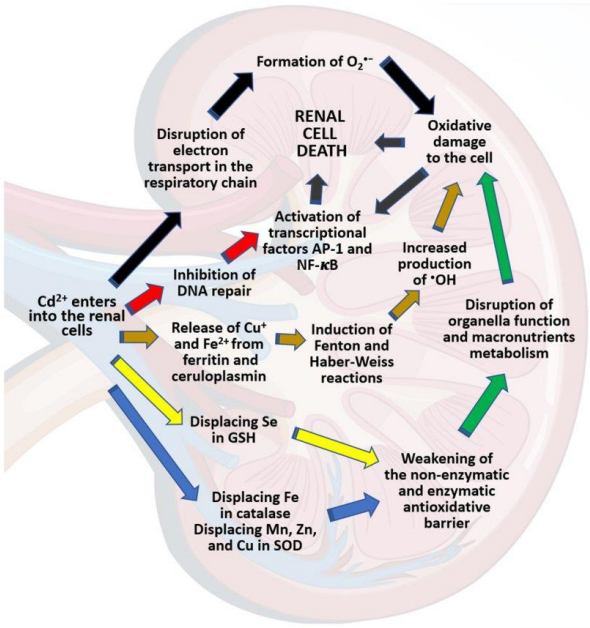

Figure 2. The mechanisms behind the nephrotoxicity of cadmium (Cd). AP-1, activator protein 1; Cd^{2+}, cadmium ions; Cu^{+}, copper (I) ions; DNA, deoxyribonucleic acid; Fe, iron; Fe^{2+}, iron (II) ions; GSH, reduced glutathione; Mn, manganese; NF-κB, nuclear factor kappa-B; $O_2^{\bullet-}$, superoxide radical; •OH, hydroxyl radical; Se, selenium; SOD, superoxide dismutase; Zn, zinc. This figure was designed using assets from Freepik.com.

The Cd-MT complexes, released from the liver, are easily filtered through the glomeruli and then reabsorbed by the proximal tubules (segment 1 and segment 2) via endocytosis or decomposed in the epithelial cells of the proximal tubules into Cd^{2+} ions and amino acids [43]. Subsequently, these ions induce the synthesis of MT in the kidneys, bind to it, and accumulate in this form. The Cd-MT complexes are characterized by a short lifetime (about 3 days) [140] and decompose to release Cd^{2+} ions, which further induce the synthesis of MT and bind to this protein. However, the ability of the kidney to biosynthesize MT and accumulate Cd in the form of complexes with this protein is limited, and when Cd^{2+} ions can no longer be detoxified by binding with MT, they begin to bind to -SH groups of other proteins, including structural and functional proteins, and the functional groups of other macromolecules, thus exerting toxic effects [43]. When Cd reaches the kidneys at a high enough concentration that the organ cannot prevent its damaging effects, the epithelial cells malfunction, resulting in injury to the proximal tubules. During low or moderate intoxication with this element, apoptotic or autophagic cell death can occur [44]. If the injury to the cells is severe and widespread, the processes of repair are insufficient, and the necrosis of the cells of the proximal tubules occurs [44]. Despite the research presented in [24,107,124,134], the mechanism of Cd-induced glomerular damage is still unknown. Glomerular damage exacerbates kidney dysfunction.

Numerous data show that Cd-induced kidney damage results from this element's pro-oxidative properties, which weaken the enzymatic and non-enzymatic antioxidative barriers of cells and increase the production of reactive oxygen species (ROS), thus intensifying the oxidative modifications of cellular macromolecules and damaging cellular organelles (Figure 2) [12,24]. Although this xenobiotic cannot generate free radicals and ROS by itself, it can indirectly generate nitryl, hydroxyl ($^{\bullet}OH$), and superoxide ($O_2^{\bullet-}$) radicals, which increase the concentration of hydrogen peroxide in cells, allowing the Fenton reaction to take place [24,139]. The generation of ROS also occurs due to a decrease in the cellular concentration of GSH in the nephrons after the disruption of the GSH redox cycle. Cd^{2+} ions replace selenium in GSH biosynthesis, causing the formation of an inactive compound. Cd^{2+} ions can also replace ions of other elements, such as iron (II) (Fe^{2+}) and copper (I) (Cu^{+}), on the membrane proteins. The release of these transition metal ions via the Fenton reaction intensifies the oxidative processes [137]. A high concentration of ROS, as a result of oxidative stress, damages crucial cellular macromolecules (lipids, proteins, and nucleic acids) and cellular structures (including cellular organelles and cellular membranes), and ultimately leads to cell death, including apoptosis, to which renal tubular cells are highly vulnerable [20,138,141]. A consequence of Cd-induced oxidative stress is the dysfunction of the renal mitochondria, which are the target cellular organelles for this xenobiotic [107]. After penetrating the membranes of mitochondria, Cd^{2+} ions interfere with the electron transport chain and lead to electron leakage and the increased production of ROS; furthermore, they disrupt the course of biochemical processes in the mitochondria, such as respiration and the Krebs cycle [142]. As a result, oxidative stress also activates transcriptional factors such as nuclear factor kappa-B (NF-κB) and activator protein 1 (AP-1), as has been shown in animal studies as well as in renal cell cultures [143]. Metabolomic studies, which have become more common in recent years [138,144,145], could provide a more detailed and precise explanation of the mechanisms behind Cd nephrotoxicity in the future.

Although Cd has not yet been shown to lead to human kidney cancer, its damaging impact on genes, as well as alterations of the expression of microribonucleic acid (microRNA) in renal tissues, have been confirmed in various studies conducted on rats [134]. This heavy metal is known to disrupt important stages of the cellular cycle, such as differentiation, proliferation, progression, DNA synthesis, and apoptosis. Cd also disrupts the processes of DNA repair, stimulates the activation of proto-oncogenes, and increases the methylation of DNA [24]. All of these effects can result in carcinogenic changes in the kidney tissue, as well as other pathological changes in the morphological structure of this organ [146,147].

8. Biomarkers of Cd-Induced Kidney Damage

In order to monitor exposure to Cd and ensure that it does not exceed the safe threshold for humans, the concentration of this metal in the blood and/or urine is measured [9,80,100]. The blood and urinary concentration of Cd is the most useful biomarker of exposure to this xenobiotic; however, monitoring the exposure level by measuring the Cd concentration in the blood and urine is insufficient due to a lack of feedback on the functional status of the body, including the kidney. The early detection of pathological changes in the kidney as a result of exposure to this toxic element, as well as the proper estimation of the risk of damage to this organ, require sensitive and specific biomarkers of its nephrotoxicity (Figure 3). Appropriate biomarkers enable one to establish the part of the nephron that has been damaged [33,84,91,148]. Due to the inevitability of environmental exposure to Cd in industrialized countries and the available epidemiological data showing that current exposure to this xenobiotic may pose a risk of kidney damage [9,37,47], identifying and applying specific and sensitive biomarkers and properly interpreting changes in their concentrations are necessary for detecting the damaging impact of this xenobiotic on the kidneys at an early stage.

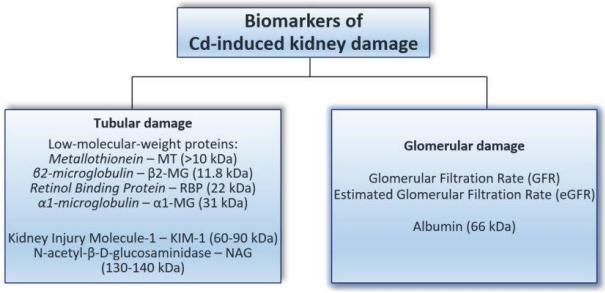

Figure 3. Biomarkers commonly used in the assessment of cadmium (Cd)-induced kidney damage.

Cd-induced damage to parts of the nephron may be detected using appropriate biomarkers (Figure 3). The determination of β2-MG, α1-MG, RBP, NAG, KIM-1, and albumin in the urine, as well as the assessment of the GFR and eGFR, have been performed in epidemiological studies to evaluate the renal function of people environmentally and occupationally exposed to Cd [4,22,66,91,92,149]. Increased concentrations of β2-MG, α1-MG, RBP, or KIM-1, and the activity of NAG in the urine, indicate tubular damage, whereas a reduced GFR or eGFR and the occurrence of albuminuria are indicators of glomerular damage [33,84,107,148]. It is important to underline that the markers of tubular and glomerular dysfunction used to detect Cd-induced kidney damage are not specific to this xenobiotic. These markers are used for evaluating kidney function in general [34,36,38,46,49,50].

β2-MG and α1-MG are low-molecular-weight proteins that form major histocompatibility complex class I molecules, which are present on the surfaces of almost all nucleated cells and are routinely shed by these cells into the blood. These proteins pass through the glomeruli and, under suitable conditions, are reabsorbed by the proximal tubules; thus, they are present in the urine only in small amounts. However, damage to the renal proximal tubules results in an increase in their concentrations in the urine [44]. A concentration of β2-MG exceeding 300 μg/g creatinine in the urine indicates defective tubular reabsorption [25]. Damage of this degree increases the risk of death due to kidney and urinary tract diseases [150]. Numerous studies have shown that the concentration of β2-MG in the urine rises with increasing exposure to Cd and an increasing concentration of this xenobiotic in the urine [9,37,40,45,145,151,152]. β2-MG is one of the main biomarkers used to evaluate the status of the kidney in "Itai-Itai" disease patients and individuals exposed to Cd occupationally [40,90,91,121,150,153,154]. It is very important to emphasize that the application of β2-MG as a biomarker of nephrotoxicity may be limited due to the degradation of this

protein in acidic urine (pH < 5.6), as well as the increase in its concentration in the urine with age and in some diseases [15,154]. The problem of the instability of β2-MG in acidic urine can be avoided by administering bicarbonate to patients before taking urine samples, but this procedure has not yet been carried out in epidemiological studies. The instability of β2-MG in acidic urine can partially explain the discrepancies in the concentration of this protein at similar Cd concentrations in the urine which have been reported by some authors [30,84,155].

Ikeda et al. [156] revealed that an increasing concentration of α1-MG in the urine positively correlated with an increase in the concentration of Cd in both the blood (median = 1.2 μg/L, range = 0.1–6.9 μg/L) and the urine (median = 1.0 μg/g creatinine, range = 0.1–9.6 μg/g creatinine). The cut-off value for this protein is age-dependent: 11.24 μg/g creatinine for ages 18–40 and 19.47 μg/g creatinine for over 40 years of age [157]. Nowadays, α1-MG is rarely used as a marker of Cd nephrotoxicity [96,127].

The use of RBP as an indicator of renal malfunction was proposed in the 1980s, as this protein presents a similar level of sensitivity as a biomarker for this effect to β2-MG [45]. RBP is synthesized mainly in the liver, and its main function is to bind retinol (vitamin A) [158]. Under physiological conditions, this protein is reabsorbed in the renal tubules, but when the reabsorptive function of the tubules is damaged, it appears in the urine. The presence of RBP in the urine is considered to be one of the most sensitive biomarkers for the failure of the proper reabsorptive function of the proximal tubules [91,158]. It is widely used to evaluate the function of the kidney in individuals occupationally exposed to Cd [91,153,154]. However, the usefulness of this marker at Cd concentrations in the urine below 1 μg/g creatinine requires further research [91,151].

The available data from epidemiological studies show that NAG, a cytotoxicity marker enzyme, is a more sensitive biomarker for Cd-induced renal tubular damage than β2-MG and RBP [45]. The activity of NAG in the urine is widely used as a biomarker in studies evaluating the impact of exposure to this heavy metal on the kidneys [81,156,159]. NAG is a lysosomal enzyme abundantly present in the cells of the renal proximal tubules. The activity of this enzyme is low under physiological conditions, and increases during renal tubular cell injury as a result of the growing Cd concentration in the renal tissue. This enzyme occurs in the kidney and urine in two major isoforms, isoenzyme A (NAG-A) and isoenzyme B (NAG-B). NAG-A is released into the urine during the physiological turnover of kidney cells. NAG-B is an intralysosomal membrane-bound enzyme released into the urine upon the disruption of lysosomal membranes. Thus, this isoenzyme is a lesional form of NAG, and is considered to be a highly sensitive indicator of tubular toxicity. However, the activity of total NAG, rather than its isoenzyme B (NAG-B), is commonly assessed in epidemiological studies as a biomarker of Cd nephrotoxicity [45,132]. Although no NAG activity level is currently defined as safe, values over 11 U/g creatinine are considered to indicate renal tubular damage [37].

KIM-1 is one of the most ubiquitous markers of renal failure. It is a transmembrane glycoprotein localized on the epithelial cells of the proximal tubules. Once damage to the epithelial cells of the proximal tubules occurs, KIM-1 is shed into the urine, and, thus, serves as a very sensitive diagnostic indicator of injury to this part of the nephron. The expression of this glycoprotein in a normal kidney is low, but it increases in the injured regions of kidney tubules [160]. Numerous studies, both in humans and experimental animals, have shown an increase in the concentration of KIM-1 due to Cd-induced damage to the proximal tubules [30,44,134,144,161], although some authors have suggested that the usefulness of this marker during low-level exposure to this heavy metal may be limited [28,81]. Recent studies suggest that a KIM-1 concentration of 1.51 μg/L in the urine (median; 0.78–2.55 μg/L) indicates an increased probability of damage to the renal proximal tubules [147,161].

CC16 can also be used to detect renal tubular dysfunction, but the postrenal secretion of this protein from the prostate in men reduces both its specificity and sensitivity. In women, determining the concentration of CC16 in the urine allows for the detection of subtle defects

in the proximal tubules that escape notice when other biomarkers are used [162,163]. CC16 is not usually applied in the estimation of kidney function during exposure to Cd, although no evidence is available to prove that CC16 is not useful for this purpose [162].

When assessing the impact of Cd on the function of the renal glomeruli, albuminuria and parameters describing glomerular filtration, such as the GFR and eGFR, are commonly evaluated [30,37,84,164,165]. Albuminuria is the occurrence of an increased amount of albumin in the urine, presenting an albumin concentration (mg/g urine) to creatinine concentration (mg/g urine) ratio over 30. Albumin is the predominant plasma protein normally present in the blood. Under suitable conditions, only trace amounts of this protein occur in the urine; however, under glomerular damage, its concentration in the urine increases [30,149]. The best method for assessing glomerular function is to estimate the rate of glomerular filtration (the amount of fluid filtered from the glomerular capillaries into the Bowman's capsule per unit of time), expressed as the GFR or eGFR. Studying the GFR involves determining the coefficient of purification of the body using a compound that is filtered in the kidneys, but does not undergo reabsorption in the renal tubules (e.g., creatinine or inulin). The GFR is usually evaluated based on the endogenous creatinine clearance, which represents the amount of creatinine filtered in the glomeruli per unit of time. The eGFR is a mathematically derived entity that is calculated based on an individual's serum creatinine concentration, age, sex, and race [37]. GFR or eGFR values < 60 mL/min/1.73 m^2 indicate glomerular damage [37].

The most sensitive biomarkers of nephrotoxicity should be used to estimate the risk of kidney damage due to low or moderate exposure to Cd, allowing for the detection of kidney injury at the earliest stage. Moreover, assessing the impact of low-level Cd exposure on kidney status should be based on determining more than one biomarker of nephrotoxicity, enhancing the possibility of detecting early lesions. Our overview of recent epidemiological studies estimating the impact of current environmental Cd exposure levels on kidney status showed that the most frequently used biomarkers are the β2-MG concentration and NAG activity in the urine, as well as the urinary concentration of albumin and the GFR or eGFR. Due to the interference of Cd in many metabolic pathways in the body, metabolomic studies would likely identify new biomarkers, allowing for a more precise assessment of the risk of kidney damage due to low-level environmental exposure compared to current biomarkers. Potential candidates are compounds such as citrate, creatine, tryptophan, adenine, and uric acid, the values of which in the urine have been found to correlate not only with the concentration of Cd in the urine, but also with commonly used biomarkers of nephrotoxicity, such as the β2-MG concentration and NAG activity [138].

9. Risk of Kidney Damage among the General Population at Current Environmental Cd Exposure Levels

Although researchers worldwide have conclusively identified a growing risk of tubular and glomerular kidney impairment due to environmental Cd exposure levels that are considered safe [37,131,155], epidemiological studies exploring the impact of the current environmental Cd exposure levels on kidney status in developed countries are scarce, and the risk of kidney damage has not been well-assessed. Reduced tubular reabsorption and tubular injury have been reported as a result of low-level and moderate environmental exposure to Cd [30,37,45,81,84,156]; however, these changes were not considered clinically relevant. In the present article, the evaluation of whether the current levels of environmental exposure to Cd in industrialized countries pose a substantial risk of clinically relevant kidney damage was based on reliable clinical biomarkers for kidney dysfunction and CKD diagnosis, such as albuminuria, the GFR, and the eGFR, as well as the odds risk (OR) of changes in these parameters. Moreover, available data on biomarkers of tubular damage and the OR of their changes upon low-level and moderate chronic exposure were considered in order to compare the risk of damage to the tubules and glomeruli. To estimate the risk of kidney damage, we considered all available literature data from the last 10 years pertaining to the values of Cd nephrotoxicity biomarkers and the OR of changes in these

parameters at Cd concentrations within the range currently found in the general population worldwide (0.02–4.40 µg Cd/L in the blood and 0.04–3.39 µg Cd/g creatinine in the urine; Table 3) (Figure 4, Tables 5 and S5).

Evaluating the risk of kidney damage at the current environmental Cd exposure levels based on the available data is difficult, because the data are limited; the impact of Cd is often assessed using different biomarkers of nephrotoxicity; the OR of changes in certain parameters is not always provided; the urinary concentration of Cd is not adjusted for the creatinine concentration (sometimes expressed as µg/L) in every study; and some studies only determine the Cd concentration in the blood. Various authors have evaluated the impact of the current Cd exposure levels on kidney status based on biomarkers such as β2-MG, α1-MG, NAG, RBP, albuminuria, and the GFR or eGFR, as well as estimating the OR of changes in these parameters (Figure 4, Tables 5 and S5) [4,45]. However, studies determining whether the current levels of environmental exposure to this xenobiotic pose a risk of kidney damage most often considered β2-MG, NAG, albuminuria, and the GFR or eGFR (Tables 5 and S5, Figure 4).

Table 5. The link between the concentration of cadmium (Cd) in the urine and biomarkers of tubular and glomerular damage [a].

Expression of Data	Cd Concentration in the Urine (µg/g Creatinine)	Biomarkers of Tubular Damage			Biomarkers of Glomerular Damage		Reference
		α1-MG (mg/g Creatinine)	β2-MG (µg/g Creatinine)	NAG (U/g Creatinine)	Albumin (mg/L) (mg/g Creatinine) [b]	eGFR (mL/min/1.73 m^2)	
Median (P25, P75)	M: 0.38 (0.21–0.65) F: 0.42 (0.23–0.70)		M: 370 (0.00, 3135) F: 280 (0.00, 2090)	M:10.31 (1.46, 199.09) F: 10.09 (2.16, 48.63)		M: 84.89 (19.30, 204.34) F: 78.56 (19.32, 239.60)	[84]
Median (P5–P95)	2.1 (0.3–5.2)		140 (40–1500)	2.50 (0.09–15.0)	*2.6 (0.5–26.3)*		[45]
GM (95% CI)	M: 0.82 (0.79, 0.86) F: 1.36 (1.31, 1.41)		M: 80.47 (72.22, 88.72) F: 79.86 (74.29, 85.43)	M: 4.17 (3.74, 4.59) F: 4.14 (3.74, 4.54)		M: 91.88 (90.63, 93.13) F: 97.89 (96.79, 98.99)	[37]
Median (IQR)	0.41 (0.195–1.26)		99.8 (71.0–186.80)			109.52 ± 17.43 [c]	[155]
Mean (range)	0.29 (0.04–1.12)	5.1 (2.0–15.2)	150 (10–1300)	1.95 (0.18–4.88)	*4.8 (0.69–23.6)*	101 (77–140) 91 (43–178)	[30]
Median (GM)	2.20 (2.10)	1.30 (1.13)		6.95 (6.13)	1.40 (1.42)		[96]
Median (P5–P95)	<2.05 ≥2.05–<3.97	3.82 (0.00–17.27) 4.89 (0.00–19.42)	60 (0.00–5000) 90 (0.00–1760)	6.51 (2.93–62.83) 10.28 (3.69–82.69)	2.84 (0.00–32.37)	66.0 ± 11.3	[164]
Median (min–max)	0.19 (0.01–2.79)	4.13 (0.47–60.1)			6.87 (3.50–11.9)		[127]
Mean (range)	0.11 (0.01–0.52)	2.0 (0.11–31)			*6.3 (1.1–78)*		[166]
Mean	<1.0 1.0–1.9 2.0–4.9		84.65 140.89 115.74	2.12 2.82 2.90			[29]
GM (GSD)	M: 0.5 (1.9) F: 1.1 (2.3)		M: 249.6 (4.0) F: 187.2 (6.6)	M: 5.2 (2.1) F: 4.8 (2.3)			[100]
Mean (SD)	1.08 (1.98)		51.4 (2.64)	4.01 (2.78)			[131]
Median (P25–P75)	2.25 (1.20–5.10) [d]	0.31 (0.07–6.49)	110 (70–2800)	5.10 (3.30–7.25)	2.86 (0.46–7.03)		[81]

The values of the urine parameters that indicate renal damage are: α1-MG—11.24 µg/g creatinine, β2-MG—300 µg/g creatinine, and NAG—11 U/g creatinine for tubular damage; and albumin—30 mg/g creatinine (or 20 mg albumin/L) and eGFR ≤ 60 mL/min/1.73 m^2 for glomerular damage. F, female; IQR, interquartile range; GM, geometric mean; GSD, geometric standard deviation; M, male; P5, 5th percentile; P75, 75th percentile; P95, 95th percentile; SD, standard deviation; 95% CI, 95% confidence interval; [a] based on studies conducted in the last 10 years; [b] values in italics represent albumin concentration in the urine expressed as mg/g creatinine, [c] mean ± SD; [d] µg/L.

Regardless of the biomarker of tubular or glomerular damage which is used, an OR > 1 indicates an increased risk of damage to the respective part of the nephron. Thus, the OR is an effective parameter to estimate the risk of Cd nephrotoxicity. Although the proximal tubule is the critical part of the nephron under exposure to Cd, researchers have focused on

evaluating the OR of glomerular damage due to current levels of environmental exposure to Cd rather than the OR of tubular damage, because damage to the glomeruli is clinically relevant and may result in the development of CKD. In most studies, even if biomarkers of tubular damage were determined, the ORs of changes in these parameters were not calculated [45,81,84,96,127,131,155,164]. Thus, the risk of tubular injury at low to moderate Cd exposure in the general population has not been thoroughly assessed; however, the available data [37] allow us to conclude that this risk is higher than that of glomerular damage and occurs at a lower concentration of Cd in the blood and urine than that at which glomerular damage occurs.

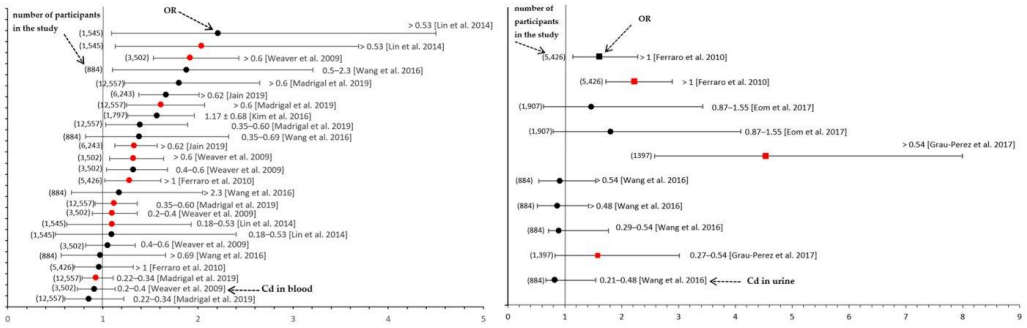

Figure 4. The odds risk (OR) of a reduced estimated glomerular filtration rate (eGFR) (black) and albuminuria (red) dependent on the cadmium (Cd) concentration in the blood (**left**; µg Cd/L) and urine (**right**; square symbols—µg Cd/g creatinine, round symbols—µg Cd/L). Detailed data on the OR values are provided in Table S5 and the following references Eom, S.Y. et al. *Arch. Environ. Contam. Toxicol.* 2017, 73, 401–409 [37]; Ferraro, P.M. et al. *BMC Public Health* 2010, 10, 304 [167]; Grau-Perez, M. et al. *Environ. Int.* 2017, 106, 27–36 [149]; Jain, R.B. *Environ. Sci. Pollut. Res.* 2019, 26, 30112–30118 [5]; Kim, N.H. et al. *J. Korean Med. Sci.* 2015, 30, 272–277 [126]; Lin, Y.S. et al. *Environ. Res.* 2014, 134, 33–38 [66]; Madrigal, J.M. et al. *Environ. Res.* 2019, 169, 180–188 [22]; Wang, D. et al. *Chemosphere* 2016, 147, 3–8 [84]; and Weaver, V. et al. *Am. J. Epidemiol.* 2009, 170, 1156–1164 [168]. The available data show that the threshold Cd concentration for a decreased eGFR and albuminuria is 0.18 µg/L in the blood and 0.27 µg/g creatinine in the urine (Tables 5 and S5).

The detailed overview of the available data suggested that the current levels of environmental exposure to Cd in developed countries have increased the risk of damage to both the tubules and glomeruli, as OR values for changes in the biomarkers of Cd nephrotoxicity exceeding 1 and reaching 13.29 have been noted in this Cd concentration range in the blood and urine of the general population (Figure 4, Table S5) [4,45]. It is important to emphasize that in some studies [45], an OR exceeding 1 was found for more than four biomarkers of kidney status (β2-MG, α1-MG, NAG, albuminuria, and the eGFR), providing clear evidence of an increased risk of damage to both tubules and glomeruli. The only available data pertaining to the OR of changes in the biomarkers of tubular damage under low-level exposure to Cd referred to β2-MG and NAG [37]. Eom et al. [37] revealed that at Cd concentrations in the urine ranging from 0.87 µg/L to 1.55 µg/L, the ORs of increases in the β2-MG concentration and NAG activity reached 4.07 (1.35–12.24) and 1.47 (0.80–2.70), respectively, while the OR of decreased eGFR was 1.46 (0.62–3.43). The finding that the ORs of changes in the β2-MG concentration and NAG activity exceeded 1 at urine Cd concentrations between 0.87 and 1.55 µg/L showed that people presenting such concentrations of this toxic element in their urine are at a higher risk of tubular damage.

Grau-Perez et al. [149] observed a reduced risk of developing albuminuria when the Cd concentration in the urine did not exceed 0.27 µg/g creatinine, whereas twice this concentration (0.54 µg/g creatinine) corresponded to a threefold higher OR for albuminuria,

with the risk being positively correlated with the Cd concentration in the urine. Other studies also showed that the risk of albuminuria rose significantly for a urine Cd concentration exceeding 1 μg/g creatinine [167]. According to studies carried out on individuals environmentally exposed to Cd, the risk of albuminuria increased when the Cd concentration was higher than 0.18 μg/L in the blood and 0.27 μg/L in the urine [22,66,167,168]. The Cd concentration in both the blood and urine seems to be a very useful parameter for evaluating the risk of developing albuminuria. An increased OR of albuminuria has been noted at blood Cd concentrations ranging from 0.18 μg/L to 1 μg/L [66,84] (Figure 4, Table S5).

Cd has been shown to impair the ability of the kidney to conduct proper glomerular filtration (evaluated according to the eGFR) at concentrations in the blood of 0.18 μg/L or higher, and the risk of a reduced eGFR correlates positively with the blood Cd concentration [66,84]. A similar relationship has been shown in other studies [124,164,165,167]. Ferraro et al. [167] reported that the OR of a reduced eGFR reached 1.48 at a Cd concentration > 1 μg/L in the blood and urine. A Korean study [126] found that the OR of a reduced eGFR was 1.57 at a blood Cd concentration of 1.17 ± 0.68 μg/L. These changes in the biomarkers of Cd nephrotoxicity and the ORs of these changes at urine Cd concentrations currently found in the general population (Figure 4, Tables 3 and 5) imply that environmental exposure to this heavy metal is a threat to kidney health. Importantly, the ORs of albuminuria and a decreased eGFR were reported to increase at Cd concentrations in the blood and urine starting from 0.18 μg/L and 0.27 μg/g creatinine, respectively, showing that the risk of glomerular damage also occurs at relatively low Cd concentrations (within the range measured in the general population; Figure 4, Table S5). Unfortunately, biomarkers of tubular damage were not determined in these studies [5,125,149,169]. However, Eom et al. [37] revealed that the OR of changes in β2-MG was higher that of glomerular damage (eGFR) at the same urine Cd concentration, confirming the higher risk of tubular injury than glomeruli injury.

The available data show that the NOAELs of the Cd concentration in the blood and urine for clinically relevant kidney damage (glomerular dysfunction expressed as albuminuria and decreased eGFR) due to environmental exposure are 0.18 μg/L and 0.27 μg/g creatinine, respectively, whereas the LOAELs are >0.18 μg/L and >0.27 μg/g creatinine, respectively (Figure 4, Tables 5 and S5). Since the blood and urinary concentrations of Cd found in the general population worldwide range from 0.02 to 4.40 μg/L and from 0.04 to 3.39 μg/g creatinine (Table 3), respectively, the current levels of environmental exposure to this heavy metal in industrialized countries may pose a substantial risk of CKD or at least contribute to its development. This conclusion was based on relatively limited epidemiological data; however, in the available literature, only these data were relevant for the current low and moderate levels of environmental exposure to Cd. To strengthen the above conclusion, it is important to underline that each study exploring the impact of low to moderate environmental Cd exposure on kidney status revealed an increased risk of kidney damage. Therefore, the claim that Cd should be considered an environmental risk factor for CKD is reasonable.

10. Exposure to Cd as a Factor Increasing the Risk of Kidney Damage Due to Other Causes

As our review of the available data revealed that the current levels of environmental exposure to Cd pose a risk of kidney damage, it is very important to underline that such exposure could exacerbate pathological changes in the kidney resulting from the action of other nephrotoxic factors; worsen the course of certain disease states; and potentiate the health effects of other xenobiotics [4,92,170–172]. Co-exposure to Cd and other compounds with and without nephrotoxic properties, such as microplastics [173] and heavy metals including chromium [174], lead [175,176], mercury [87], uranium [177], and thallium [7], was found to pose a more substantial threat to the human kidney than Cd alone. Jain [5] reported that co-exposure to Cd, mercury, and lead increased the risk of kidney malfunction

by up to twofold compared to exposure to Cd alone. Occupational exposure to mixtures of Cd and lead or Cd, lead, and chromium resulted in more serious damage to the kidneys than exposure to each metal individually [4,6,52,153], resulting in a higher risk of a decrease in the eGFR (OR: 7.9, 95% CI: 0.9–67.2) [4] and an increase in early renal biomarkers such as urinary NAG activity and albuminuria [153]. The results of an experimental study by Riaz et al. [124] suggested that kidney injury due to co-exposure to Cd and other toxic elements (lead, manganese, and arsenic) could be amplified in a chronic disease such as diabetes. Co-exposure disrupted the kidneys more seriously than Cd alone, resulting in more substantial damage to the renal cells (tubular degeneration, fibrosis, and the vacuolation of cells) and the weakening of the antioxidative barrier.

11. Conclusions and Outlook

This overview allowed us to estimate the NOAELs of the Cd concentration in the blood and urine for clinically relevant kidney damage (glomerular dysfunction) in the general population to be 0.18 μg/L and 0.27 μg/g creatinine, respectively, whereas the LOAELs were estimated to be >0.18 μg/L and >0.27 μg/g creatinine, respectively. The current levels of environmental exposure to Cd in industrialized countries have resulted in Cd concentrations in the blood and urine of 0.02–4.40 μg/L and 0.04–3.39 μg/g creatinine, respectively, within the range at which an increased risk of damage to the kidney tubules and glomeruli has been observed (above 0.18 μg/L and 0.27 μg/g creatinine, respectively). Thus, this level of exposure may pose a substantial risk of damage to the kidneys, potentially causing, or at least contributing to, the development of CKD. Moreover, β2-MG and NAG in the urine appear to be sensitive biomarkers, allowing for the detection of disturbances in the proximal tubules, whereas albuminuria and the glomerular filtration rate (evaluated as either the GFR or eGFR) are early markers of glomerular damage during low-level exposure to this heavy metal.

This paper shed new light on the possible causative factors of CKD, one of the main worldwide health problems. Even low levels of environmental exposure to Cd may increase the risk of not only tubular damage, but also clinically relevant disorders such as glomerular dysfunction in the general population. Thus, this xenobiotic should be considered as a possible causative factor of kidney diseases with unknown etiologies. Environmental exposure to Cd may be responsible for some etiologically unspecified cases of CKD among the inhabitants of industrialized countries worldwide. Hence, the clinical histories of patients presenting with CKD of an unknown etiology should include their histories of exposure to Cd; furthermore, in such cases, the Cd concentration in the blood and/or urine should be determined, as this may be helpful. Considering the credibility of the evidence that the current levels of environmental exposure to Cd may pose a risk of kidney damage in the general population, re-establishing lower "normal Cd concentrations" in the blood and urine should be considered. The overview presented herein showed that exposure to Cd in industrialized countries should be carefully monitored, and further studies should be conducted to more accurately assess the global risk of kidney damage due to environmental Cd exposure. The evidence that Cd poses a risk of kidney damage at the current levels of exposure in numerous countries suggests that environmental exposure to this toxic metal is an important public health problem. Thus, protective strategies, such as decreasing the Cd pollution in the environment, increasing the efficiency of Cd absorption from the gastrointestinal tract, and enhancing the body's ability to defend against the toxic effects of this element, should be sought out and implemented.

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Abbreviations

α1-MG, α1-microglobulin; AM, arithmetic mean; β2-MG, β2-microglobulin; b.w., body weight; CC16, Clara cell protein 16; Cd, cadmium; Cd^{2+}, cadmium ions; Cd-MT, cadmium–metallothionein complex; CKD, chronic kidney disease; Cu^{+}, copper (I) ions; DNA, deoxyribonucleic acid; eGFR, estimated glomerular filtration rate; Fe^{2+}, iron (II) ions; GFR, glomerular filtration rate; GM, geometric mean; GSH, reduced glutathione; KIM-1, kidney injury molecule-1; LOAEL, lowest observed adverse effect level; MT, metallothionein; NAG, N-acetyl-β-D-glucosaminidase; NAG-A, N-acetyl-β-D-glucosaminidase isoenzyme A; NAG-B, N-acetyl-β-D-glucosaminidase isoenzyme B; NF-κB, nuclear factor kappa-B; NOAEL, no observed adverse effect level; $O_2^{\bullet -}$, superoxide radical; OR, odds risk; $^{\bullet}OH$, hydroxyl radical; PTMI, provisional tolerable monthly intake; PTWI, provisional tolerable weekly intake; RBP, retinol binding protein; ROS, reactive oxygen species; SD, standard deviation; -SH group, thiol group (sulfhydryl group); w.w., wet weight.

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Supplementary Materials

Current Levels of Environmental Exposure to Cadmium in Industrialized Countries as a Risk Factor for Kidney Damage in the General Population: A Comprehensive Review of Available Data

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Table S1. Etiological factors for kidney damage.

Etiological Factors of Kidney Damage			
Illnesses	Medicines		Other factors
· hypertension	· polymixin A	· tobramycin	· xenobiotics other than medicines: heavy metals (cadmium, lead, mercury, arsenic, chromium, bismuth, nickel), alcohols and glycols (ethyl alcohol, methyl alcohol, ethylene glycol)
· diabetes	· bacitracin	· colistin	
· sepsis	· phenacetin	· amphotericin B	
· liver failure	· acetaminophen (paracetamol)	· foscarnet	
· obesity		· adefovir	
· glomerular disease	· cisplatin	· cidofovir	· toxins (amanitin)
· polycystic kidney disease	· cyclosporine	· tenofovir	· components of tobacco smoke
	· ifosfamide	· iopromide	
	· pemetrexed	· tacrolimus	
	· gentamycin	· pamidronate	
	· kanamycin	· zoledronic acid	
	· streptomycin		

Table S2. The accumulation of cadmium (Cd) in the kidney in an experimental rat model of human environmental exposure to this heavy metal[a].

Cd Content (μg) or Concentration (μg/g w.w.)	Exposure Duration				Time-related Changes
	3 Months	10 Months	17 Months	24 Months	
			Control		
Content	0.0682 ± 0.0060	0.0912 ± 0.0056	0.1078 ± 0.0059	0.2271 ± 0.0297	3–10*, 3–17‡, 3–24‡ 10–24‡, 17–24*
Concentration	0.0375 ± 0.0102	0.0497 ± 0.0079	0.0467 ± 0.0085	0.0844 ± 0.0357	3–24†, 10–24†, 17–24†
			1 mg Cd/kg of feed (daily Cd intake: 37.50–84.88 μg/kg b.w.)		
Content	0.6340± 0.0369***	2.1794± 0.1202***	2.8677± 0.3347***	5.247± 0.4784***	3–10‡, 3–17‡, 3–24‡ 10–24‡, 17–24†
Concentration	0.3495± 0.0601***	1.103± 0.1968***	1.213± 0.3763***	1.981± 0.5089***	3–17‡, 3–24‡, 10–24†
			5 mg Cd/kg of feed (daily Cd intake: 196.69–404.76 μg/kg b.w.)		
Content	2.562± 0.1454***	10.16 ± 0.427***	25.23 ± 1.678***	22.18 ± 0.971***	3–10‡, 3–17‡, 3–24‡ 10–17‡, 10–24‡
Concentration	1.362± 0.2254***	4.788± 0.5586***	10.77± 1.9360***	8.009± 0.8918***	3–10†, 3–17‡, 3–24† 10–17†, 10–24†

Data is mean ± standard error (SE) for 8 rats, except for 7 animals in the group maintained on the diet containing 1 mg Cd/kg for 24 months. *** $p < 0.001$ compared to the control group at the same time point; time-related changes: * $p < 0.05$, † $p < 0.01$, ‡$p < 0.001$; w.w., wet weight, b.w., body weight. [a] prepared based on Brzóska et al. [1].

Table S3. The effect of oral exposure to cadmium (Cd) on the kidneys of experimental animals[b].

Dosage, Form, and Time of Exposure to Cd	Experimental Model	Changes in the Morphological Structure of the Renal Tissue	Changes in Various Parameters in the Renal Tissue or Serum	Reference
100 mg Cd/L of drinking water, 2 weeks	Male Wistar rats	– intertubular congestion – loss of the brush border – dilatation of convoluted tubules		[2]
200 mg $CdCl_2$/kg b.w. /day in drinking water, 12 weeks	Male rabbits		– ↑ renal expression of the apoptotic (Caspase3), proliferation (MKI67), proto-oncogene (C–fos), and antioxidant (GST) genes –↓ renal expression of anti-apoptotic (Bcl2) genes	[3]
5 mg $CdCl_2$/kg of feed, 30 days	Male Wistar rats	– congestion of the cortical blood vessels –focal replacement of the renal parenchyma with numerous lymphocytes infiltrates –dilation of glomeruli		[4]
15 mg $CdCl_2$/kg b.w./day, 5 weeks	Male Wistar rats	– disruption in the organization of the renal glomeruli and tubules	– ↑ DNA damage in the kidney	[5]
6.3 mg $Cd(NO_3)_2$/kg b.w./day, single dose	Male Wistar rats	– swelling with thickened blood vessel – fatty vacuole – fatty infiltrate – lymphocyte aggregate and infiltration	– ↓ SOD, ↓ CAT, and ↓ GPx activities in the kidney	[6]
5 mg $CdCl_2$/kg b.w./day, 5 weeks	Male Sprague–Dawley rats	– tubular necrosis	– ↓ SOD, ↓ CAT, and ↓ GPx activities in the kidney – ↑ creatinine and ↑ LDH concentrations in the serum – ↓ total thiol concentration in the kidney	[7]
8.8 mg $CdCl_2$/kg b.w./day, 20 days	Pregnant female Sprague–Dawley rats	– hydropic degeneration of the cytoplasm – deterioration of the nuclei of the lining cells of PT and DT in both maternal and fetal kidney		[8]
0.685 mg $CdCl_2$/L of drinking water, 90 days	8 weeks old C57BL mice	– severe vascular degeneration and necrosis of renal tubules with glomerular deterioration		[9]

CAT, catalase; $CdCl_2$, cadmium chloride; $Cd(NO_3)_2$, cadmium nitrate(V); DT, distant tubule; GPx, glutathione peroxidase; GSH, reduced glutathione; GST, glutathione S-transferase; LDH, lactate dehydrogenase; MKI67, a marker of proliferation KI67; PT, proximal tubule; SOD, superoxide dismutase. [b] based on the studies published within the last 10 years.

Table S4. The effect of exposure to cadmium (Cd) via routes other than oral on the kidneys of experimental animals[b].

Dosage, Form, Route, and Time of Exposure to Cd	Experimental Model	Changes in the Morphological Structure of the Renal Tissue	Changes in the Various Parameters in the Renal Tissue, Blood/serum, or Urine	Reference
10 mg $CdCl_2$/kg b.w./day, s.c., 15 and 30 days	Male Wistar rats	mononuclear cell infiltration, interstitial congestion around the glomeruli with wide lumen, pyknotic nuclei, high dilatation in intertubular blood vessels impacted with hemolysed blood and cellular infiltration, congestion and inflammation around glomeruli, degenerated glomeruli with wide space and detached basement membrane, DT with wide lumen, deformed PT with detached brush border, degeneration and hyalinization of glomerular tuft, tubular degeneration, tubular and tubulointerstitial necrosis	– ↑ LOOH, ↑ MDA, and ↑ PC concentrations in the kidney, ↑ TOS, ↑ OSI – ↑ expression of inflammatory markers: Hsp70, COX2, and TNFα in the kidney – ↓ TAS, ↓ activities of SOD and CAT, ↓ GSH concentration in the kidney	[10]
0.6 mg $CdCl_2$/kg b.w., s.c., 5 days/week, 12 weeks	Male Sprague–Dawley rats	fibrosis of the tubules	– ↑ KIM–1 and ↑ β2-MG concentrations and ↑ NAG activity in the urine	[11]
6.5 mg $CdCl_2$/kg b.w./day, i.p., 5 days	Male Wistar rats	moderate to severe inflammation and widespread degeneration of cells, cytoplasmic vacuolization, congested glomeruli, severe apoptosis, karyomegaly, and hyperchromatic nuclei in the tubular epithelial cells	– ↑ MDA concentration in the kidney – ↓ SOD, ↓ CAT, and ↓ GPx, activities in the kidney	[12]
2 mg $CdCl_2$/kg b.w./day, i.p., 4 weeks	Male Wistar rats	severe tubular necrosis and apoptosis, vacuolization, degeneration	– ↑ urea nitrogen concentration in the blood – ↑ creatinine concentration in the serum – ↓ creatinine clearance – ↓ NO, ↓ protein thiols, ↓ free thiols, and ↓ total thiols concentrations and ↓ CAT and ↓ SOD activities in the kidney	[13]
3 mg $CdCl_2$/kg b.w./day, i.p., 7 days	BALB/c mice	cloudy swelling of tubular cells, narrow renal tubules, and fibrosis	– ↓ SOD activity and ↓ GSH concentration in the kidney – ↑ ROS generation and ↑MDA concentration in the kidney	[14]
1 mg $CdCl_2$/kg b.w./day, i.p., 2 weeks	Male Sprague–Dawley rats		– ↑ creatinine concentration in the urine – ↑ ADA, ↑ TNFα, ↑ IL–6, and ↑ IL–10 concentrations in the kidney	[15]
1 mg $CdCl_2$/kg b.w./day, i.p., 5 weeks	Wistar rats	vacuolization of epithelial lining renal tubules, congestion and atrophy of glomerular tufts, and distension of Bowman's space	– ↓ SOD, ↓ CAT, and ↓ GPx, activities in the kidney	[16]
4 mg $CdCl_2$/kg b.w./day, i.v., 2 weeks	Male Wistar rats	tubular degeneration, necrosis and severe renal cortical congestion	– ↑ lipid peroxidation in the kidney – ↓ GSH concentration, ↓ AST, ↓ ALT, ↓ SOD, ↓ CAT and ↓ GPx activities in the kidney	[17]

ADA, renal adenosine deaminase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; β_2-MG, β2-macroglobulin; CAT, catalase; $CdCl_2$, cadmium chloride; COX2, cyclooxygenase 2; DT, distant tubule; GPx, glutathione peroxidase; GSH, reduced glutathione; GST, glutathione S-transferase; Hsp70, heat shock protein 70; IL-6, interleukin 6; IL-10, interleukin 10; i.p., intraperitoneally; i.v., intravenously; KIM-1, kidney

injury molecule-1; LOOH, lipid peroxides; MDA, malondialdehyde; NAG, N-acetyl-β-D-glucosaminidase; NO, nitrogen(II) oxide; OSI, oxidative stress index; PC, protein carbonyls; PT, proximal tubule; ROS, reactive oxygen species; s.c., subcutaneously; SOD, superoxide dismutase; TAS, total antioxidative status; TNFα, tumor necrosis factor α; TOS, total oxidative status. [b] based on the studies published within the last 10 years.

Table S5. The odds risk (OR) of decreased estimated glomerular filtration rate (eGFR) and albuminuria due to low-level environmental exposure to cadmium (Cd).

Country	n	Cd in the Blood (µg/L)	OR[c] (95% Confidence Interval)		Cd in the Urine (µg/L or *µg/g Creatinine*)	OR (95% Confidence Interval)		Reference
			Decrease in eGFR	Albuminuria		Decrease in eGFR	Albuminuria	
China	884	Men:			Men:			[18]
S and NS		< 0.5	1.00		< 0.29	1.00		
		0.5–2.3	1.88		0.29–0.54	0.89		
			(1.10–3.21)			(0.71–1.77)		
		> 2.3	1.17		> 0.54	0.91		
			(0.67–2.05)			(0.54–1.54)		
		Women:			Women			
		< 0.35	1.00		< 0.21	1.00		
		0.35–0.69	1.38		0.21–0.48	0.82		
			(0.82–2.32)			(0.67–1.54)		
		> 0.69	0.97		> 0.48	0.86		
			(0.56–1.66)			(0.52–1.42)		
USA	3502	< 0.2	1.00	1.00				[19]
S and NS		0.2–0.4	0.91	1.10				
			(0.73–1.13)	(0.89–1.36)				
		0.4–0.6	1.05	1.32				
			(0.82–1.34)	(1.07–1.64)				
		> 0.6	1.32	1.92				
			(1.04–1.68)	(1.53–2.43)				
USA	12577	0.22–0.34	0.85	0.93				[20]
S and NS			(0.60–1.22)	(0.78–1.11)				
		0.35–0.60	1.39	1.12				
			(1.03–1.89)	(0.92–1.36)				
		> 0.60	1.80	1.61				
			(1.23–2.65)	(1.25–2.07)				
USA	5426	< 1	1.00	1.00	*< 1*	1.00	1.00	[21]
S and NS		1 <	0.96	1.28	*> 1*	1.61	2.23	
			(0.70–1.32)	(1.02– 1.61)		(1.14–2.28)	(1.72–2.89)	
USA	1545	< 0.18	1.00	1.00				[22]
S and NS		0.18–0.53	1.09	1.10				
			(0.50–2.40)	(0.62–1.93)				
		> 0.53	2.21	2.04				
			(1.09–4.50)	(1.13–3.69)				
USA	6243	> 0.62	1.663 (1.38–2.01)	1.329				[23]
S and NS				(1.123 – 1.573)				
Spain	1397				*< 0.27*		1.00	[24]
S and NS					*0.27–0.54*		1.58	
					> 0.54		(0.83–3.02)	
							4.54	
							(2.58–8.00)	
South	1797	1.17±0.68	1.57					[25]
Korea			(1.26–1.96)					
S and NS								
Taiwan	658	< 0.8	1.00					[26]
S and NS		0.8–1.3	2.40					
			(1.14–5.07)					
		> 1.3	6.37					
			(3.05–13.29)					
South	1907				< 0.87	1.00 (Ref.)		[27]
Korea					0.87–1.55	1.46		
S and NS					> 1.55	(0.62–3.43)		
						1.80		
						(0.79–4.10)		

Albuminuria, the albumin/creatinine ratio in the urine > 30; decrease in eGFR, eGFR < 60 mL/min/1.73 m^2. NS, non-smokers; S, smokers; OR, odds risk. [c] The OR represents the odds of an outcome during a particular level of exposure, compared to the odds of the outcome occurring in the absence of this exposure. The OR > 1 indicates an increased risk of the appearance of an effect.

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10.2. Publikacja II

The protective potential of *Aronia melanocarpa* L. berry extract against cadmium-induced kidney damage: A study in an animal model of human environmental exposure to this toxic element.

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The Protective Potential of *Aronia melanocarpa* L. Berry Extract against Cadmium-Induced Kidney Damage: A Study in an Animal Model of Human Environmental Exposure to This Toxic Element

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Abstract: The impact of cadmium (Cd) on the function and structure of the kidney and the potential protective effect of an extract from *Aronia melanocarpa* L. berries were investigated in a rat model of low- and moderate-level environmental exposure to this heavy metal (1 and 5 mg Cd/kg feed for up to 24 months). The sensitive biomarkers of Cd-induced damage to the kidney tubules (N-acetyl-β-D-glucosaminidase (NAG), alkaline phosphatase (ALP), β2-microglobulin (β2-MG), and kidney injury molecule-1 (KIM-1) in the urine), clinically relevant early markers of glomerular damage (albumin in the urine and creatinine clearance), and other markers of the general functional status of this organ (urea, uric acid, and total protein in the serum and/or urine) and Cd concentration in the urine, were evaluated. The morphological structure of the kidney and inflammatory markers (chemerin, macrophage inflammatory protein 1 alpha (MIP1a), and Bcl2-associated X protein (Bax)) were also estimated. Low-level and moderate exposure to Cd led to damage to the function and structure of the kidney tubules and glomeruli. The co-administration of *A. melanocarpa* berry extract significantly protected against the injurious impact of this toxic element. In conclusion, even low-level, long-term exposure to Cd poses a risk of kidney damage, whereas an intake of Aronia berry products may effectively protect from this outcome.

Keywords: cadmium; kidney; nephrotoxicity; tubular damage; glomerular damage; biomarkers; histopathology; *Aronia melanocarpa* berry extract; protection

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1. Introduction

A growing body of epidemiological data shows that environmental exposure to heavy metals, including cadmium (Cd), is now a real threat to public health in industrialized countries around the world and that the exposure is increasing [1,2]. Cd was ranked by the Agency for Toxic Substances and Disease Registry as the 7th most dangerous xenobiotic that endangers the health of the general population [3]. This results from the fact that environmental exposure to this heavy metal can lead to damage to numerous organs and systems, including the kidney [2,4–6] and liver [7,8], as well as the skeletal system [8,9], nervous system [8,10], and cardiovascular system [11].

The kidney is the main organ of Cd accumulation in the body and the first organ to be damaged (i.e., the target organ) under chronic intoxication with this heavy metal [1,2,8]. This xenobiotic has a damaging impact on the structure and function of the renal

tubules and glomeruli [2,4,8,12–14]. Current global environmental exposure to Cd is generally low to moderate (except for areas considered excessively polluted). However, the results of epidemiological studies show that even such exposure may negatively affect the kidney [2,13–16]. The lowest observed adverse effect levels (LOAELs) of this element concentration for clinically relevant kidney damage (glomerular dysfunction) have been estimated to be >0.18 μg/L in the blood and >0.27 μg/g creatinine in the urine and are within the lower range of concentrations noted in inhabitants of industrialized countries [2,4–7,9,11,13,14]. These indicate that current levels of exposure of the general population to this heavy metal may pose a real risk of organ injury, but the risk is not well known so far (for a review, see [2]). That is why it is of high importance not only to recognize the risk but also to find an effective protective strategy. However, due to the probability of the coexistence of numerous factors that may influence kidney status in the general population, it is very difficult to evaluate the involvement of this xenobiotic in the development of organ damage under low-level exposure [2]. The impact may be well estimated in experimental models that allow for the exclusion of confounding factors. Such models are also appropriate for looking for effective protective strategies against Cd toxicity.

Thus, we have undertaken the study to investigate both the damaging impact of Cd on the kidney and the possibility of protection against it in a rat model (1 and 5 mg Cd/kg feed for 3–24 months) that well reflects the environmental exposure of the general population to this heavy metal in industrialized countries [17]. It has already been reported in the model that even low-level exposure (1 mg Cd/kg feed) injured the liver [18–20] and skeletal system [21–23], as well as destroyed the oxidative/reductive balance in the sublingual salivary glands [24] and the body status of zinc (Zn) [25], copper (Cu) [25], and manganese (Mn) [26]. In contrast, the co-administration of a 0.1% aqueous extract from the berries of *Aronia melanocarpa* L. (AM) had a protective impact [18–26]. The fruits of *A. melanocarpa* ((Michx.) Elliott, Rosaceae; chokeberry) are one of the richest sources of polyphenols in nature [27–29]. Chokeberries are also a rich source of vitamins (vitamins from group B and vitamins A, C, E, and K), macro- and microelements (e.g., calcium, magnesium, and iron), carotenoids (β-carotene), phytosterols, tannins, carbohydrates, triterpenes, pectins, sugar and sugar alcohols, organic acids, dietary fiber, and proteins [27–29]. Polyphenol-rich products are of particular interest among the possible agents that could be useful in counteracting the toxic action of Cd because of the multidirectional beneficial properties of these compounds, including antioxidative, anti-inflammatory, and anti-carcinogenic effects and their ability to bind the ions of divalent metals [27,29–32]. We have already revealed that the administration of AM during low-level (1 mg Cd/kg feed) and moderate (5 mg Cd/kg feed) intoxication with Cd significantly improved the antioxidative barrier (enzymatic and nonenzymatic), decreased concentrations of pro-oxidants, and protected from oxidative stress development and its consequences, such as oxidative modifications of lipids, proteins, and deoxyribonucleic acid (DNA) in the liver and changes in its morphological structure, as well as normalized the serum activities of liver enzyme markers [18,19]. Moreover, the intake of AM ameliorated Cd-mediated changes in the expression of collagen types I and III at the messenger ribonucleic acid (mRNA) and protein levels, as well as a rise in the concentrations of matrix metalloproteinases (MMP-1 and MMP-2) and their tissue inhibitors (TIMP-1 and TIMP-2) in the liver, indicating that it may be effective in preventing this heavy metal-caused disturbance in collagen homeostasis in this organ [20]. The intake of the extract at both levels of exposure to Cd provided important protection from this xenobiotic-induced oxidative stress, lipid peroxidation, and oxidative damage to the protein and DNA in the bone tissue, as well as from disturbances in bone turnover, and changes in bone mineral status [21,22]. Moreover, it improved bone collagen biosynthesis and femur biomechanical properties. The administration of the extract counteracted the development of oxidative stress and oxidative modifications of macromolecules not only in the liver [18,19] and bone tissue [22] but also in the sublingual salivary glands [24]. The supplementation with AM prevented or at least partially protected from most of the Cd-induced changes in the metabolism of Zn [25], Cu [25], and

Mn [26], as well as ameliorated changes in the activity of Mn-dependent superoxide dismutase (Mn-SOD) and the concentration of Mn, and protected from Cd accumulation in the mitochondria, mainly in the liver [26].

The research so far carried out in the above-described experimental model of the general population's exposure to Cd [17–26] allowed us to hypothesize that the exposure would also result in kidney damage. Taking into account the strong antioxidative potential of chokeberries [28–30] and prooxidative Cd effects [2,18,19,22,24], as well as the finding that the administration of AM during the treatment with this heavy metal decreased the body burden of this element, including its accumulation in the kidney [17], and counteracted numerous outcomes of the toxic action of this xenobiotic [18–26], it was hypothesized that the extract can also protect the kidney from damage. To investigate these hypotheses, we conducted a comprehensive study using a model of human environmental exposure to this heavy metal that we created. The present paper is the first report from this research, and it aimed to investigate whether low-level and moderate (1 and 5 mg Cd/kg feed, respectively) chronic exposure to Cd can result in damage to the function and structure of the kidney and if the administration of AM during intoxication can prevent these consequences. For this purpose, the sensitive biomarkers of damage to the kidney tubules (kidney injury molecule-1 (KIM-1), β2-microglobulin (β2-MG), N-acetyl-β-D-glucosaminidase (NAG), and alkaline phosphatase (ALP) in the urine) and clinically relevant early markers of glomerular injury (albumin concentration in the urine and creatinine clearance reflecting the glomerular filtration rate (GFR)), as well as other markers of the overall kidney function such as the concentrations of uric acid and urea in the serum and urine, and total protein concentration in the urine, were estimated. The morphological structure of the kidney and markers of inflammatory processes in this organ (chemerin, macrophage inflammatory protein 1 alpha (MIP1a), and Bcl2-associated X protein (Bax)) were evaluated as well. A similar study has not been conducted before. The present study is the first to not only assess Cd nephrotoxicity during intoxication, well reflecting the current levels of exposure of the general population worldwide, but also focus on the possibility of protection against this outcome via the administration of chokeberry extract.

2. Results

2.1. The Impact of Cd and AM Alone and Their Co-Administration on the Values of Biomarkers of Damage to the Kidney Tubules

The administration of AM alone for up to 24 months had no effect on any of the biomarkers of damage to the kidney tubules (KIM-1, β2-MG, NAG, and ALP) determined in the urine (Figures 1–4, Tables S1–S5). The exposure to Cd in fodder at concentrations of 1 and 5 mg/kg affected the levels of all biomarkers of tubular damage, and the occurrence of these effects was dependent on the level and duration of exposure; however, at any of the time points, there were no differences in the values of specific parameters between the Cd_1 and Cd_5 groups (Figures 1–4, Tables S1–S5).

In the rats maintained on the feed containing 1 and 5 mg Cd/kg for 3, 10, 17, and 24 months, the concentration of KIM-1 in the urine was higher (2.3–9.7 times) compared to the control animals, except for the Cd_1 group after 24 months. Although the Kruskal-Wallis post hoc test showed a lack of difference ($p > 0.05$) in KIM-1 concentration between the Cd_1 group (range 279.0–752.6 pg/mg creatinine) and the control group (range 131.2–239.3 pg/mg creatinine), this parameter in particular animals in the group exposed to Cd reached higher numerical values than in the control group (Figure 1, Table S1).

The concentration of β2-MG in the urine was unchanged after the 3- and 10-month feeding with the 1 and 5 mg Cd/kg diet compared to the control group; however, after 17 and 24 months, it was increased (1.6–3.6-fold) (Figure 1, Table S1). The measurements of β2-MG concentration, carried out every other month in the animals maintained in the experiment for 24 months, revealed that at the exposure to 1 mg Cd/kg feed, the concentration of this low-molecular-weight protein increased compared to the control group after

14 months (2-fold), while in the case of the higher treatment, it was elevated (2.2-fold) after 12 months, and at both levels of exposure, it remained at the increased level (2–2.9 times in the Cd_1 group and 2–3.6 times in the Cd_5 group, compared to the control group) until the end of the study (Figure 2, Table S2).

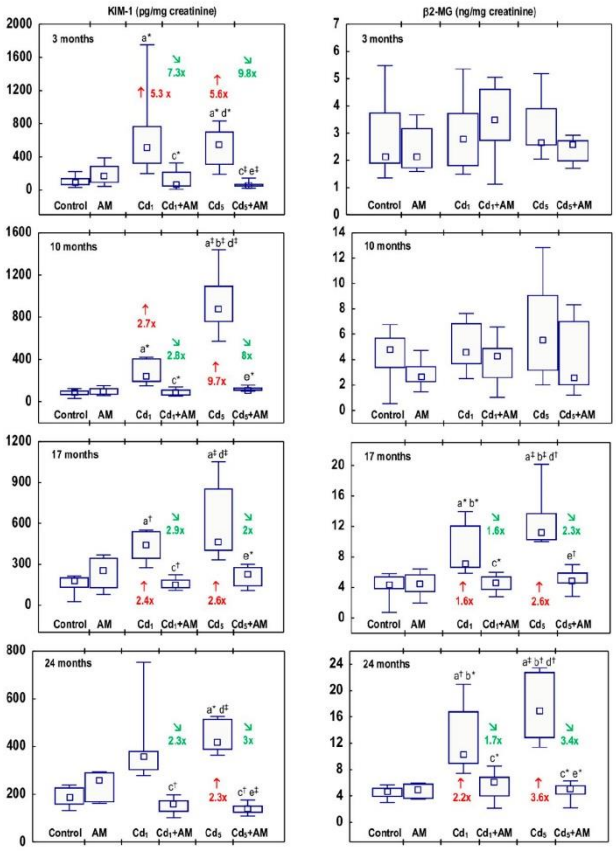

Figure 1. The concentrations of kidney injury molecule-1 (KIM-1) and β2-microglobulin (β2-MG) in the urine of female rats. The animals were treated with cadmium (Cd) in the feed at a concentration of 0, 1, or 5 mg/kg (Control, Cd_1, and Cd_5 groups) and/or 0.1% extract from the berries of *Aronia melanocarpa* L. (AM, Cd_1+AM, and Cd_5+AM groups) for 3, 10, 17, and 24 months. Data are shown as a median, 25–75% confidence interval, and minimum and maximum values for eight animals (except for seven females in the AM, Cd_1, and Cd_5 groups after 24 months). Statistically significant differences (Kruskal-Wallis test) compared to: a—Control group; b—AM group; c—Cd_1 group; d—Cd_1+AM group; and e—Cd_5 group, where * $p < 0.05$, † $p < 0.01$, and ‡ $p < 0.001$ are marked. The factors of change compared to the control group (↑, increase) or the adequate group treated with Cd alone (↘, decrease) are indicated by the numerical values below or above the bars. Detailed data are presented in Table S1.

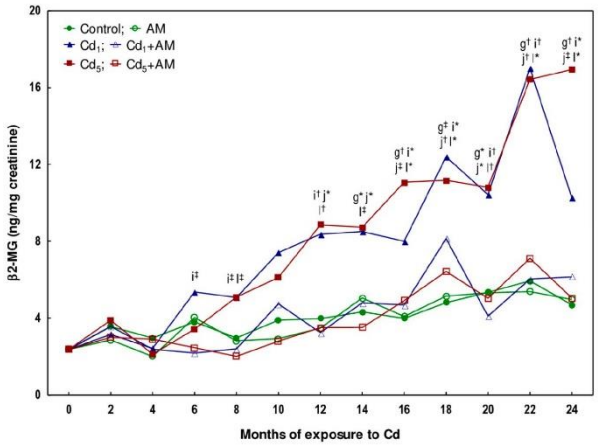

Figure 2. The concentration of β2-microglobulin (β2-MG) in the urine of female rats evaluated every other month during the 24-month study. The animals were treated with cadmium (Cd) in the feed at a concentration of 0, 1, or 5 mg/kg (Control, Cd_1, and Cd_5 groups) and/or 0.1% extract from the berries of *Aronia melanocarpa* L.. (AM, Cd_1+AM, and Cd_5+AM groups). Data are shown as a median value for eight animals (except for seven females in the AM, Cd_1, and Cd_5 groups after 20, 22, and 24 months). An occurrence of statistically significant differences (Kruskal-Wallis test; * $p < 0.05$, † $p < 0.01$, and † $p < 0.001$) between every two experimental groups at each time point was evaluated and marked as follows: f—AM and Control groups; g—Cd_1 and Control groups; h—Cd_1+AM and Control groups; i—Cd_1 and Cd_1+AM groups; j—Cd_5 and Control groups; k—Cd_5+AM and Control groups; l—Cd_5 and Cd_5+AM groups; m—Cd_1 and Cd_5 groups; and n—Cd_1+AM and Cd_5+AM groups. A lack of the particular letter symbol means a lack of statistically significant differences between appropriate groups. Detailed data are presented in Table S2.

The activity of NAG in the urine of rats fed with fodder containing 1 mg Cd/kg for 3 and 10 months was within the range of values noted in the control group, while after 17 and 24 months, it was increased (4.6- and 22-fold, respectively) (Figure 3, Table S3). In the animals exposed to the 5 mg Cd/kg feed for 10, 17, and 24 months, this enzyme activity was elevated 5.2–25-fold (Figure 3, Table S3). The measurements of NAG taken every two months during the 24-month study disclosed that at low-level exposure to Cd, the urinary activity of this enzyme began to increase after 12 months (was 3.1-fold higher than in the control group) and confirmed the rise in this enzyme activity after 10 months of the higher exposure noted in the animals sacrificed at this time point (Figures 3 and 4). At both levels of exposure to Cd, the activity of NAG, once increased, remained at an elevated (3.2–5.5-fold compared to the control group) but relatively stable level during the following months; however, between the 20th and 22nd months, a marked increase (4.8- and 5.8-fold in the Cd_1 and Cd_5 groups, respectively) in this enzyme activity (up to values 18 and 25 times higher in the Cd_1 and Cd_5 groups, respectively, than in the control group) was noted (Figure 4, Table S4).

In the animals maintained on the feed containing 1 and 5 mg Cd/kg for 10, 17, and 24 months, the activity of ALP in the urine was increased (2.6–7.3-fold) (Figure 3; Table S3). Bimonthly monitoring of ALP activity in the animals maintained in the experiment for 24 months revealed that at moderate exposure to Cd, the activity of this enzyme increased as early as after 4 months (4.8-fold), whereas in the low-level treatment, it rose (2.8-fold) after 10 months, analogously as in the animals sectioned after this time of exposure duration (Figure 4, Table S5). Once the activity of ALP increased, it remained elevated (2.5–3.9 times in the Cd_1 group and 3.2–7.3 times in the Cd_5 group) until the end of the experiment

(Figure 4, Table S5). In the Cd_5 group, between the 22nd and 24th months, a marked increase (2.3-fold) in ALP activity was noted (Figure 4, Table S5).

The administration of AM during the exposure to the 1 and 5 mg Cd/kg feeds entirely prevented all the changes described above in the values of KIM-1, β2-MG, NAG, and ALP except for the rise in ALP activity after 6 months of the treatment with the 5 mg Cd/kg feed (Figures 1–4, Tables S1–S5). The activity of ALP in the Cd_5+AM group after 6 months was 2.9-fold higher compared to the control group and did not differ compared to the Cd_5 group (Figure 4, Table S5). Apart from this one exception, the values of all biomarkers of damage to the kidney tubules in the Cd_1+AM group and the Cd_5+AM group were within the ranges of values determined in the control group (Figures 1–4, Tables S1–S5).

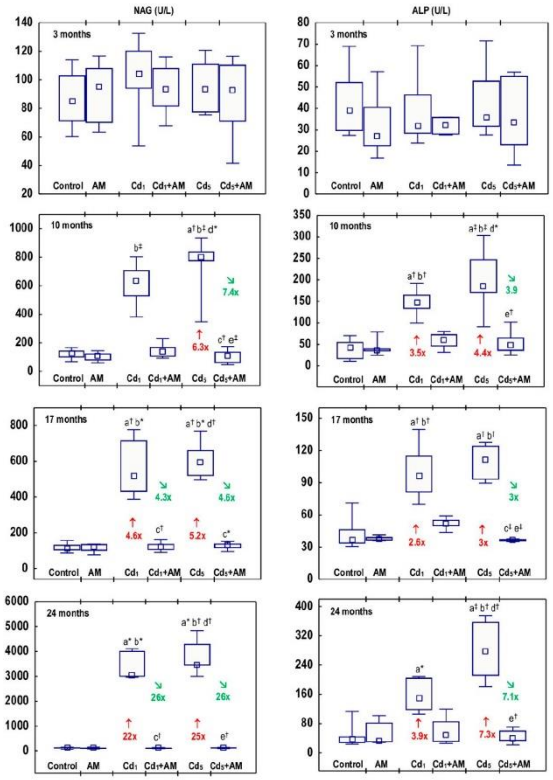

Figure 3. The activities of N-acetyl-β-D-glucosaminidase (NAG) and alkaline phosphatase (ALP) in the urine of female rats. The animals were treated with cadmium (Cd) in the feed at a concentration of 0, 1, or 5 mg/kg (Control, Cd_1, and Cd_5 groups) and/or 0.1% extract from the berries of *Aronia melanocarpa* L. (AM, Cd_1+AM, and Cd_5+AM groups) for 3, 10, 17, and 24 months. Data are shown as a median, 25–75% confidence interval, and minimum and maximum values for eight animals (except for seven females in the AM, Cd_1, and Cd_5 groups after 24 months). Statistically significant differences (Kruskal-Wallis test) compared to: a—Control group; b—AM group; c—Cd_1 group; d—Cd_1+AM group; and e—Cd_5 group, where * $p < 0.05$, † $p < 0.01$, and ‡ $p < 0.001$ are marked. The factors of change compared to the control group (↑, increase) or the adequate group treated with Cd alone (↘, decrease) are indicated by the numerical values below or above the bars. Detailed data are presented in Table S3.

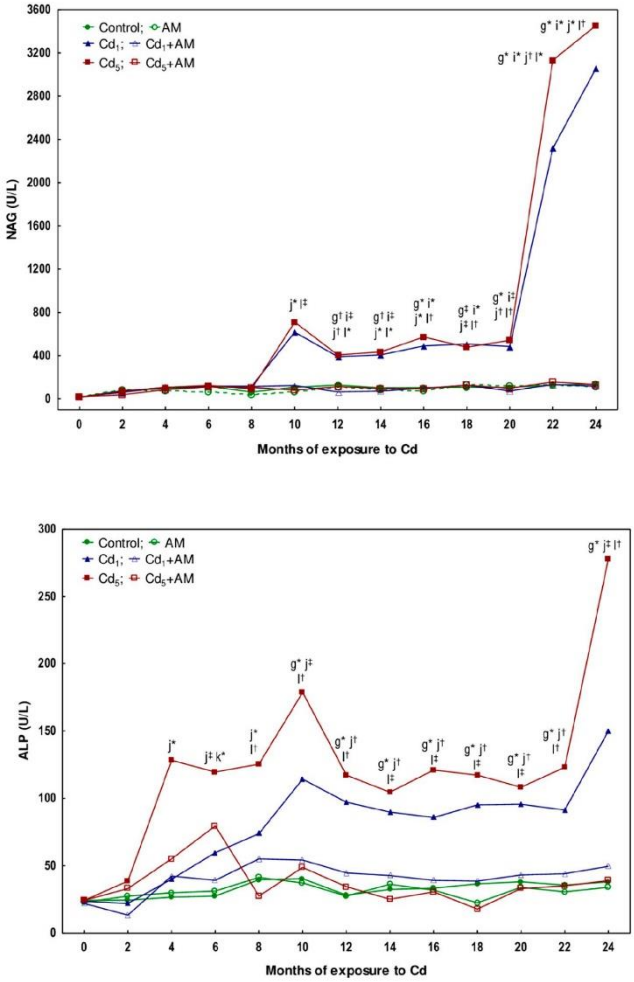

Figure 4. The activities of N-acetyl-β-D-glucosaminidase (NAG) and alkaline phosphatase (ALP) in the urine of female rats evaluated every other month during the 24-month study. The animals were treated with cadmium (Cd) in the feed at a concentration of 0, 1, or 5 mg/kg (Control, Cd_1, and Cd_5 groups) and/or 0.1% extract from the berries of *Aronia melanocarpa* L.. (AM, Cd_1+AM, and Cd_5+AM groups). Data are shown as a median value for eight animals (except for seven females in the AM, Cd_1, and Cd_5 groups after 20, 22, and 24 months). An occurrence of statistically significant differences (Kruskal-Wallis test; * $p < 0.05$, † $p < 0.01$, and † $p < 0.001$) between every two experimental groups at each time point was evaluated and marked as follows: f—AM and Control groups; g—Cd_1 and Control groups; h—Cd_1+AM and Control groups; i—Cd_1 and Cd_1+AM groups; j—Cd_5 and Control groups; k—Cd_5+AM and Control groups; l—Cd_5 and Cd_5+AM groups; m—Cd_1 and Cd_5 groups; and n—Cd_1+AM and Cd_5+AM groups. A lack of the particular letter symbol means a lack of statistically significant differences between appropriate groups. Detailed data are presented in Tables S4 (NAG) and S5 (ALP).

2.2. The Impact of Cd and AM Alone and Their Co-Administration on the Values of Biomarkers of Damage to the Kidney Glomeruli

In the rats administered AM alone for up to 24 months, the concentrations of albumin and total protein in the urine adjusted for creatinine concentration (ACR and PCR, respectively), creatinine concentration in the serum and urine, creatinine clearance, as well as the concentrations of uric acid and urea in the serum and their content in the 24-h urine, were within the ranges of the control group (Figures 5–8, Tables S6–S11).

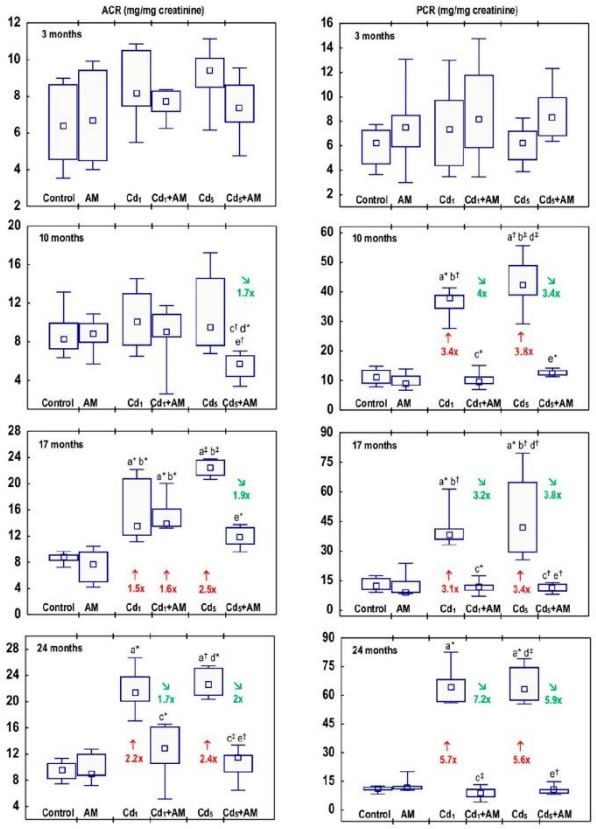

Figure 5. The concentrations of albumin and total protein in the urine of female rats adjusted for creatinine concentration (ACR and PCR, respectively). The animals were treated with cadmium (Cd) in the feed at a concentration of 0, 1, or 5 mg/kg (Control, Cd_1, and Cd_5 groups) and/or 0.1% extract from the berries of *Aronia melanocarpa* L. (AM, Cd_1+AM, and Cd_5+AM groups) for 3, 10, 17, and 24 months. Data are shown as a median, 25–75% confidence interval, and minimum and maximum values for eight animals (except for seven females in the AM, Cd_1, and Cd_5 groups after 24 months). Statistically significant differences (Kruskal-Wallis test) compared to: a—Control group; b—AM group; c—Cd_1 group; d—Cd_1+AM group; and e—Cd_5 group, where * $p < 0.05$, † $p < 0.01$, and ‡ $p < 0.001$ are marked. The factors of change compared to the control group (↑, increase) or the adequate group treated with Cd alone (↘, decrease) are indicated by the numerical values below or above the bars. Detailed data are presented in Table S6.

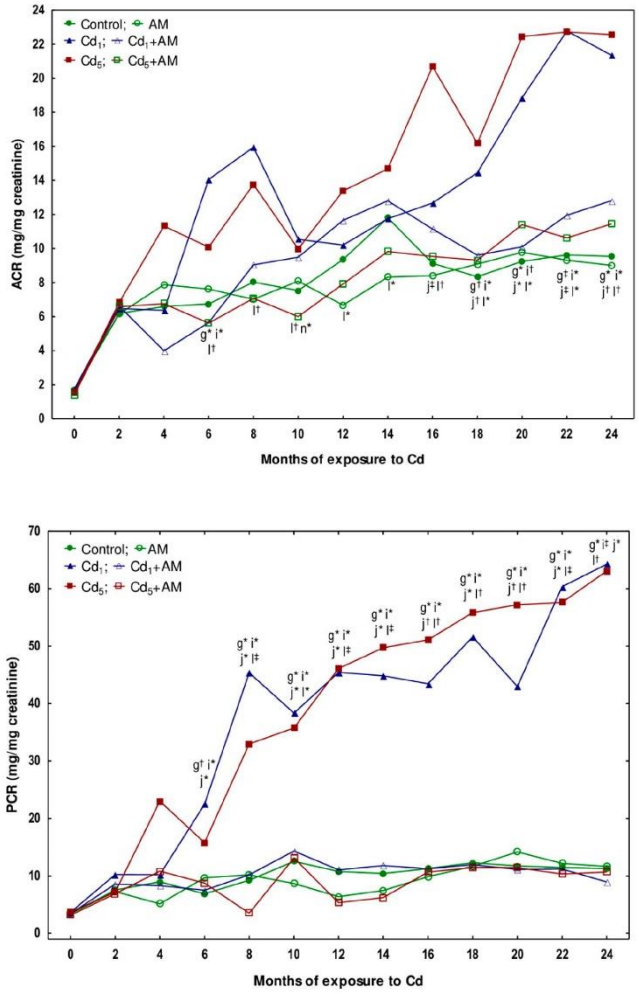

Figure 6. The concentrations of albumin and total protein in the urine of female rats adjusted for creatinine concentration (ACR and PCR, respectively) evaluated every other month during the 24-month study. The animals were treated with cadmium (Cd) in the feed at s concentration of 0, 1, or 5 mg/kg (Control, Cd_1, and Cd_5 groups) and/or 0.1% extract from the berries of *Aronia melanocarpa* L. (AM, Cd_1+AM, and Cd_5+AM groups). Data are shown as a median values for eight animals (except for seven females in the AM, Cd_1, and Cd_5 groups after 20, 22, and 24 months). An occurrence of statistically significant differences (Kruskal-Wallis test; $^{*}p < 0.05$, $^{\dagger}p < 0.01$, and $^{\ddagger}p < 0.001$) between every two experimental groups at each time point was evaluated and marked as follows: f—AM and Control groups; g—Cd_1 and Control groups; h—Cd_1+AM and Control groups; i—Cd_1 and Cd_1+AM groups; j—Cd_5 and Control groups; k—Cd_5+AM and Control groups; l—Cd_5 and Cd_5+AM groups; m—Cd_1 and Cd_5 groups; and n—Cd_1+AM and Cd_5+AM groups. A lack of the particular letter symbol means a lack of statistically significant differences between appropriate groups. Detailed data are presented in Tables S7 (ACR) and S8 (PCR).

Low-level and moderate exposure to Cd for 17 and 24 months resulted in an increase in ACR (1.5–2.5-fold) (Figure 5, Table S6). The estimation of ACR every other month in the animals maintained in the experiment for 24 months revealed that in the Cd_1 group, a temporary growth (2.1-fold) in this parameter was noted after 6 months. Next, for several months, ACR remained proper, while after 18 months, it increased again and was enhanced (1.7–2.4-fold) until the end of the experiment (Figure 6, Table S7). In the females treated with the 5 mg Cd/kg feed, ACR increased (2.3-fold) after 16 months and remained at the elevated (1.9–2.4 times) level until the end of the study (Figure 6, Table S7).

In the animals sectioned after 10, 17, and 24 months, PCR was higher (3.1–5.7 times) than in the control group (Figure 5, Table S6). The measurements carried out every other month at both levels of exposure to Cd disclosed that PCR increased for the first time after 6 months (3.2 and 2.3 times in the Cd_1 and Cd_5 groups, respectively) and remained enhanced (3.0–5.7 times and 2.8–5.6 times in the Cd_1 and Cd_5 groups, respectively) until the end of the 24-month study (Figure 6, Table S8).

The exposure to Cd at the concentrations of 1 and 5 mg Cd/kg feed had no impact on creatinine concentrations in the serum or urine (data presented only in the Supplementary Materials; Table S9). The creatinine clearance was not affected by the low-level exposure to Cd for 3–24 months, but it decreased after 17 and 24 months of the moderate treatment (by 35% and 22%, respectively) (Figure 7, Table S9).

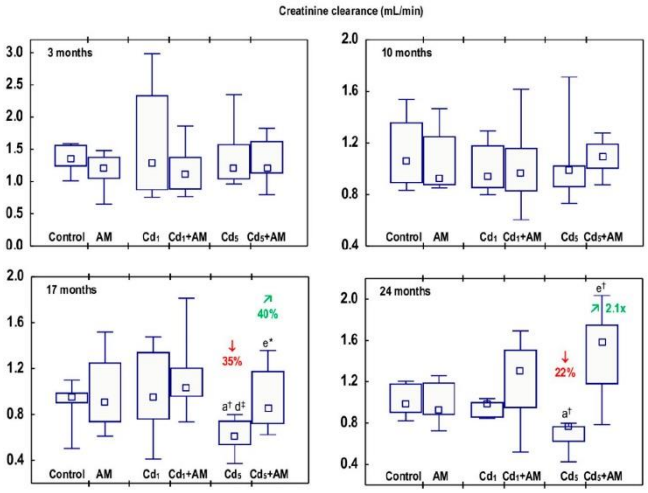

Figure 7. Creatinine clearance in female rats. The animals were treated with cadmium (Cd) in the feed at a concentration of 0, 1, or 5 mg/kg (Control, Cd_1, and Cd_5 groups) and/or 0.1% extract from the berries of *Aronia melanocarpa* L. (AM, Cd_1+AM, and Cd_5+AM groups) for 3, 10, 17, and 24 months. Data are shown as a median, 25–75% confidence interval, and minimum and maximum values for eight animals (except for seven females in the AM, Cd_1, and Cd_5 groups after 24 months). Statistically significant differences (Kruskal-Wallis test) compared to: a—Control group; d—Cd_1+AM group; and e—Cd_5 group, where * $p < 0.05$, † $p < 0.01$, and ‡ $p < 0.001$ are marked. The percentage changes or a factor of change compared to the control group (↓, decrease) or the adequate group treated with Cd alone (↗, increase) are indicated by the numerical values above the bars. Detailed data are presented in Table S9.

The concentrations of uric acid and urea in the serum in the Cd_1 group were unaffected, except for an increase (by 40%) in uric acid concentration after 3 months (Figure 8, Table S10). In the Cd_5 group, urea concentration was enhanced after 17 and 24 months (by

34% and 2.2-fold, respectively), and uric acid concentration was elevated (by 30%) after 24 months (Figure 8, Table S10). Intoxication with Cd in fodder at concentrations of 1 and 5 mg/kg had no impact on the content of uric acid and urea in the 24-h urine (data presented only in the Supplementary Materials; Table S11).

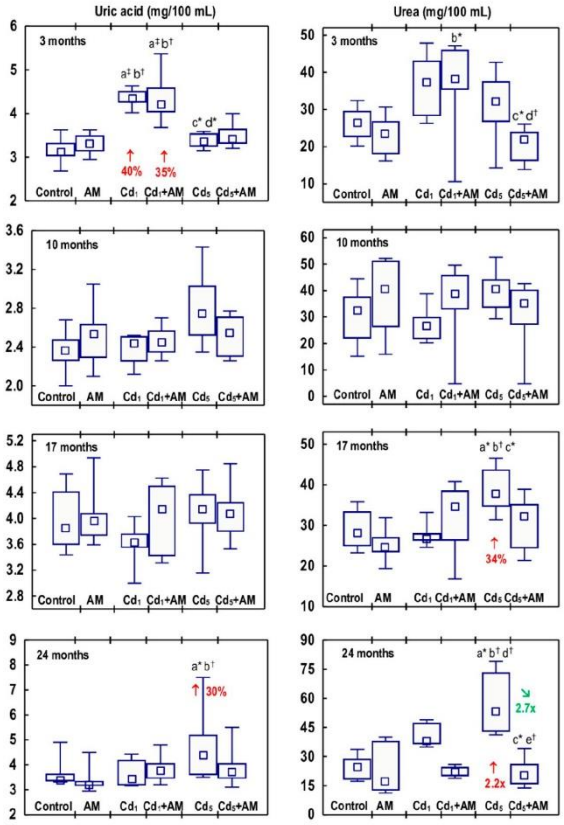

Figure 8. The concentrations of uric acid and urea in the serum of female rats. The animals were treated with cadmium (Cd) in the feed at a concentration of 0, 1, or 5 mg/kg (Control, Cd_1, and Cd_5 groups) and/or 0.1% extract from the berries of *Aronia melanocarpa* L. (AM, Cd_1+AM, and Cd_5+AM groups) for 3, 10, 17, and 24 months. Data are shown as a median, 25–75% confidence interval, and minimum and maximum values for eight animals (except for seven females in the AM, Cd_1, and Cd_5 groups after 24 months). Statistically significant differences (Kruskal-Wallis test) compared to: a—Control group; b—AM group; c—Cd_1 group; d—Cd_1+AM group; and e—Cd_5 group—where * $p < 0.05$, † $p < 0.01$, and ‡ $p < 0.001$ are marked. The percentage changes or factors of changes compared to the control group (↑, increase) or the adequate group treated with Cd alone (↘, decrease) are indicated by the numerical values below or above the bars. Detailed data are presented in Table S10.

There were no differences in the values of particular markers of glomerular function between the Cd_1 group and the Cd_5 group, except for a lower (by 22%) concentration of uric acid in the serum after 3 months and a higher (by 41%) concentration of urea after 17 months in the Cd_5 group (Figures 5–8, Tables S6–S11).

All of the evaluated biomarkers of damage to the kidney glomeruli (ACR, PCR, creatinine concentration in the serum and urine, and creatinine clearance, as well as the concentrations of uric acid and urea in the serum and their content in the 24-h urine) in the animals co-administered with Cd and AM were within the ranges of values determined in the control group, except for ACR in the Cd_1+AM group after 17 months and the serum concentration of uric acid after 3 months that were increased (1.6-fold and by 35%, respectively) compared to the control group but did not differ versus the Cd_1 group (Figures 5–8, Tables S6–S11). Moreover, creatinine concentration in the serum in the Cd_5+AM group after 3, 17, and 24 months was lower (by 26–39%) than in the Cd_5 group (Table S9).

2.3. The Impact of Cd and AM Alone and Their Co-Administration on the Concentration of Cd in the Urine

The concentration of Cd in the urine of the female rats maintained on the feed containing 1 mg Cd/kg, evaluated every other month throughout the 24-month study, did not differ compared to the control group, except for its higher (by 86%) value after 6 months, and ranged from 0.1114 to 0.6386 µg Cd/g creatinine (Figure 9, Table S12). In the animals fed with the 5 mg Cd/kg diet, the concentration of this toxic element in the urine starting from the 2nd month until the end of the experiment ranged from 0.1664 to 0.9785 µg Cd/g creatinine and was higher (2.1–3.3-fold) than in the control group (0.0620–0.2894 µg Cd/g creatinine) but did not differ statistically significantly compared to the Cd_1 group; however, the median concentration of this heavy metal reached clearly higher numerical values (Figure 9, Table S14).

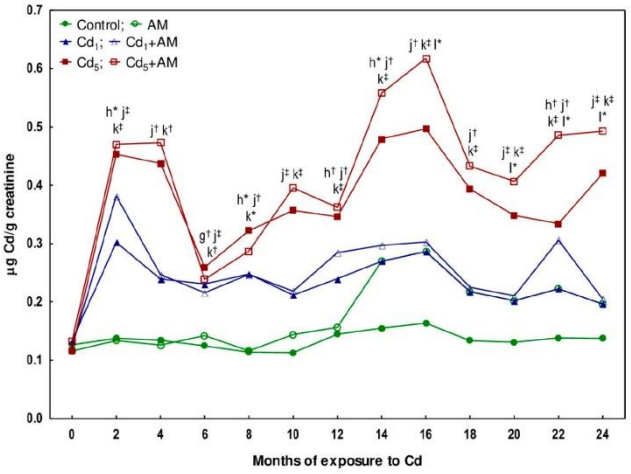

Figure 9. The concentration of cadmium (Cd) in the urine of female rats evaluated every other month during the 24-month study. The animals were treated with Cd in the feed at a concentration of 0, 1, or 5 mg/kg (Control, Cd_1, and Cd_5 groups) and/or 0.1% extract from the berries of *Aronia melanocarpa* L. (AM, Cd_1+AM, and Cd_5+AM groups). Data are shown as a median value for eight animals (except for seven females in the AM, Cd_1, and Cd_5 groups after 20, 22, and 24 months). An occurrence of statistically significant differences (Kruskal-Wallis test; * $p < 0.05$, † $p < 0.01$, and ‡ $p < 0.001$) between every two experimental groups at each time point was evaluated and marked as follows: f—AM and Control groups; g—Cd_1 and Control groups; h—Cd_1+AM and Control groups; i—Cd_1 and Cd_1+AM groups; j—Cd_5 and Control groups; k—Cd_5+AM and Control groups; l—Cd_5 and Cd_5+AM groups; m—Cd_1 and Cd_5 groups; and n—Cd_1+AM and Cd_5+AM groups. A lack of the particular letter symbol means a lack of statistically significant differences between appropriate groups. Detailed data are presented in Table S12.

The administration of AM to the animals maintained on the standard diet (containing 0.0584 ± 0.0049 mg/kg) and the 1 mg Cd/kg diet had no impact on Cd concentration in 24-h urine samples (Figure 9, Table S14). In the Cd_5+AM group, the Cd concentration in the urine after 16 months and between the 20th and 24th months was higher by 17–45% compared to the Cd_5 group (Figure 9, Table S14).

2.4. The Impact of Cd and AM Alone and Their Co-Administration on the Morphological Structure of the Kidney

The macroscopic picture of both kidneys in the control group was normal. The kidney was a bean-shaped organ of brick-red color and had a soft consistency. In the animals that received Cd and AM alone and together, the macroscopic picture of the kidneys did not differ from that in the control group.

Both kidneys in each rat had the same weight. The median absolute weight of the left kidney of control females reached 0.9420 g (0.8595–1.0100 g) after 3 months and 1.3065 g (1.1163–2.0292 g) after 24 months, whereas the relative weight of this organ was 0.3032 g/100 g body weight (b.w.) (0.2732–0.3222 g/100 g b.w.) and 0.1937 g/100 g b.w. (0.1786–0.3624 g/100 g b.w.), respectively (Table S13). There were no differences in the absolute and relative weight of the kidney between the experimental groups throughout the study (Table S13).

The morphological microscopic image of the kidney in the control group was almost proper, except for slight glomerulonephritis (found in 50% of the animals) and perivascular oedema (occurring in 25% of the animals) (Table 1, Figures 10 and 11). In the AM group, slight intensity changes such as tubular vacuolization, an extension of the tubular lumen, hyperplasia of the epithelium of the convoluted tubules, or glomerulonephritis were observed, and each of these changes occurred only in one female (25%) (Table 1, Figures 10 and 11).

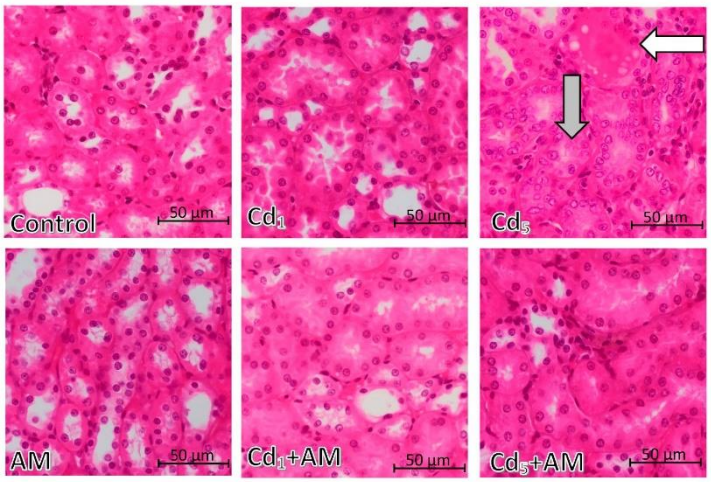

Figure 10. Histopathological image of the renal tubules of female rats in hematoxylin–eosin (H+E) staining. The animals were treated with cadmium (Cd) in the feed at a concentration of 0, 1, or 5 mg/kg (Control, Cd_1, and Cd_5 groups) and/or 0.1% extract from the berries of *Aronia melanocarpa* L. (AM, Cd_1+AM, and Cd_5+AM groups) for 24 months. Tubular changes were the most advanced in the Cd_5 group, in which hyalinization (white arrow), hyperplasia, and hypertrophy (grey arrow) of the tubular epithelium and pronounced proliferation of the interstitial tissue of the kidney were found.

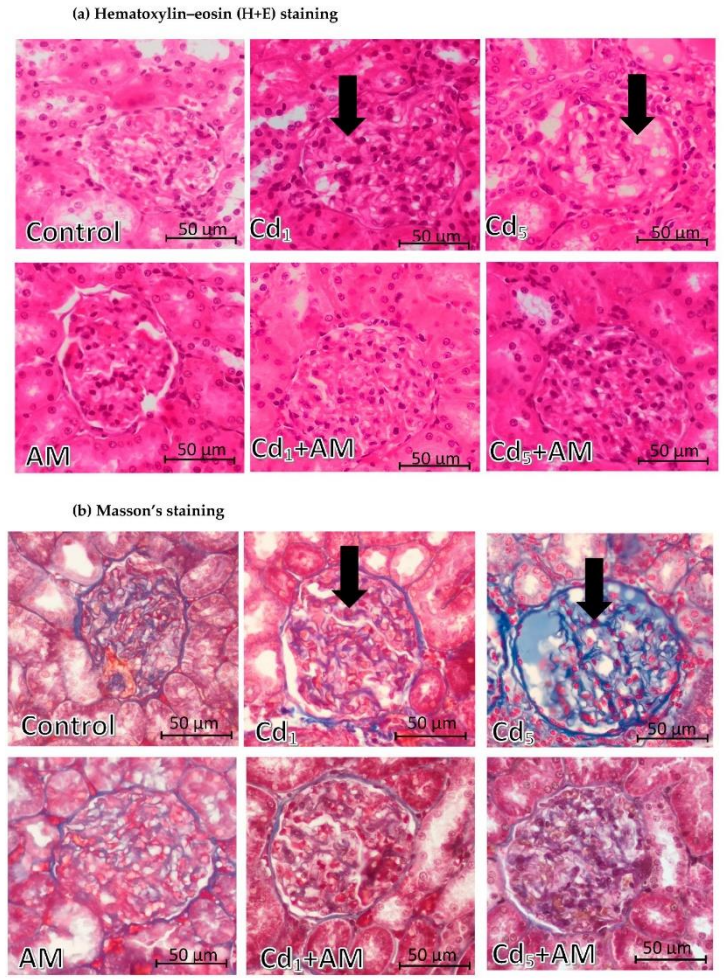

Figure 11. Histopathological image of the renal glomeruli of female rats. The animals were treated with cadmium (Cd) in the feed at a concentration of 0, 1, or 5 mg/kg (Control, Cd_1, and Cd_5 groups) and/or 0.1% extract from the berries of *Aronia melanocarpa* L.. (AM, Cd_1+AM, and Cd_5+AM groups) for 24 months. In the Cd_1 and Cd_5 groups, moderate and severe, respectively, glomerulonephritis is evident. There is a thickening of the basement membranes (blue in Masson's staining) of the glomerulus and progressive atrophy of the mesangium (decrease in cellularity) (black arrow). In the Cd_1+AM and Cd_5+AM groups, minimal or slight, respectively, glomerulonephritis is observed.

Table 1. Changes in the morphology of the kidneys of female rats in particular experimental groups.

Group and the Extent of Changes [1]	Tubular Vacuolization	Hyalinization	Extension of the Tubular Lumen	Hyperplasia of the Epithelium of the Convoluted Tubules	Hypertrophy of the Epithelium of the Convoluted Tubules	Tubular Necrosis	Interstitial Proliferation	Congestion at the Cortex/Medullary Interface	Perivascular Oedema	Glomerulonephritis	Glomerular Congestion
	% of rats with the same severity of the lesion										
Control											
0	100	100	100	100	100	100	100	100	75	50	100
1									25	50	
2											
3											
AM											
0	75	100	75	75	100	100	100	100		75	100
1	25		25	25					100	25	
2											
3											
Cd_1											
0	75	25	50	75	75	100		25	100		100
1	25	25		25	25		25	25		25	
2			25				50	50		75	
3		50	25				25				
Cd_1+AM											
0	100	100	100	100	100	100	75	25	75	75	100
1							25	75	25	25	
2											
3											
Cd_5											
0	100	25	100			100		25	50		100
1				50	50				50		
2				50	50		100	25		25	
3		75						50		75	
Cd_5+AM											
0	100	25	50	100	50	100	25	25	25	50	100
1		75	50		50		75	75	75	50	
2											
3											

The animals were treated with cadmium (Cd) in the diet at a concentration of 0, 1, or 5 mg/kg (Control, Cd_1, and Cd_5 groups) and/or 0.1% extract from the berries of *Aronia melanocarpa* L. (AM, Cd_1+AM, and Cd_5+AM groups) for 24 months. The data are shown as a percentage of four rats in each experimental group with the same severity of the lesion or its absence. [1] The following criteria were used to score the presence or absence of the changes and their intensity: 0—without a change; 1—a slight change; 2—a moderate change; and 3—a severe change.

In the animals maintained for 24 months on the feed containing 1 and 5 mg Cd/kg (Cd_1 and Cd_5 groups), pathological changes in the tubules such as vacuolization (only in the Cd_1 group), hyalinization, an extension of the tubular lumen (only in the Cd_1 group), hyperplasia and hypertrophy of the epithelium of the convoluted tubules, and proliferation of the interstitial tissue of the kidney, as well as glomerulonephritis and congestion at the cortex/medullary interface and perivascular oedema (only in the Cd_5 group), were observed (Table 1, Figures 10 and 11). There was no tubular necrosis or glomerular congestion (Table 1). The Cd-induced changes in the morphological structure of the kidney in the Cd_5 group were more advanced compared to those noted in the Cd_1 group, except for tubular vacuolization, which was not observed in the Cd_5 group (Table 1, Figures 10 and 11).

In the animals administered with AM during the treatment with Cd (Cd_1+AM and Cd_5+AM groups), the intensity of pathological changes in the morphological structure of the kidney was milder compared to the respective groups that did not receive the extract under the exposure to Cd (Cd_1 and Cd_5 groups) (Table 1, Figures 10 and 11). The administration of AM at the exposure to the 1 mg Cd/kg feed completely protected the tubular vacuolization and hyalinization, an extension of the tubular lumen, and hyperplasia and hypertrophy of the epithelium of the convoluted tubules, as well as a weakened proliferation of interstitial tissue of the kidney (Table 1, Figure 10). The application of the extract at the time of intoxication with 5 mg Cd/kg feed prevented hyperplasia of the epithelium of the convoluted tubules and attenuated the other Cd-induced changes in the histological structure of tubules, such as hyalinization, hypertrophy of the epithelium of the convoluted tubules, and interstitial proliferation (Table 1, Figure 10). However, a slight extension of the tubular lumen was observed in 50% of animals in the Cd_5+AM group, whereas no such change occurred in the Cd_5 group (Table 1). In the Cd_1 group, glomerulonephritis developed in all animals, and in 25% of the females, it was slight and in 75%, moderate, while in the Cd_1+AM group, it was noted only in 25% of the animals and was slight. Similarly, in the Cd_5 group, glomerulonephritis was severe in 75% of the animals and moderate in 25% of the females, whereas in the Cd_5+AM group, it was noted only in 50% of the animals and was slight in intensity (Table 1, Figure 11). The administration of AM during the exposure to 1 and 5 mg Cd/kg feed also weakened congestion at the cortex/medullary interface. In addition, AM administration under higher exposure to Cd resulted in a weakening of perivascular oedema; however, in the Cd_1+AM group, a slight intensity change of this kind was noted in 25% of animals, while in the Cd_1 group, it was absent (Table 1).

2.5. The Impact of Cd and AM Alone and Their Co-Administration on the Markers of Inflammation in the Kidney

The administration of AM alone for up to 24 months had no impact on the concentrations of chemerin, MIP1a, and Bax in the kidney (Figure 12, Table S14).

In the rats treated with the 1 mg Cd/kg feed, the concentration of chemerin in the kidney tissue was increased (2.4-fold) after 24 months, whereas the concentrations of MIP1a and Bax throughout the whole experiment were unchanged compared to the control group (Figure 12, Table S14). In the animals fed with fodder containing 5 mg Cd/kg, chemerin concentration was elevated after 17 and 24 months (1.4- and 2.3-fold, respectively), and Bax concentration was enhanced after 24 months (1.4-fold), whereas the concentration of MIP1a was decreased (2.3-fold) after 3 months (Figure 12, Table S14). The kidney concentrations of chemerin, MIP1a, and Bax did not differ

between the Cd_1 and Cd_5 groups, except for the lower (by 53%) concentration of MIP1a in the Cd_5 group after 3 months (Figure 12, Table S14).

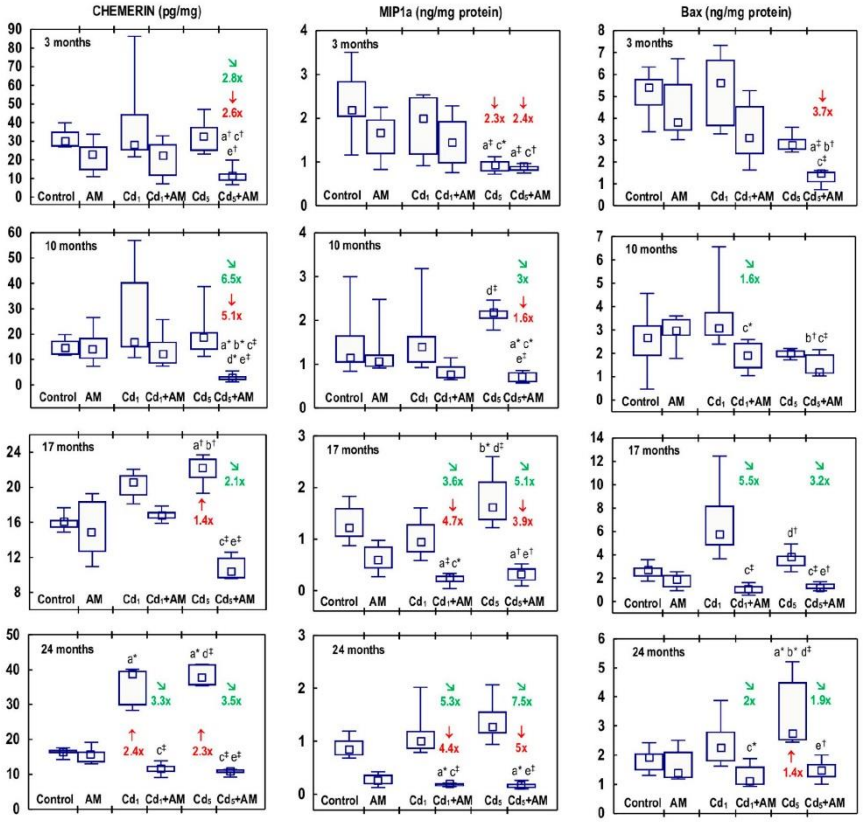

Figure 12. The concentrations of chemerin, macrophage inflammatory protein 1 alpha (MIP1a), and Bcl2-associated X protein (Bax) in the kidneys of female rats. The animals were treated with cadmium (Cd) in the diet at a concentration of 0, 1, or 5 mg/kg (Control, Cd_1, and Cd_5 groups) and/or 0.1% extract from the berries of *Aronia melanocarpa* L. (AM, Cd_1+AM, and Cd_5+AM groups) for 3, 10, 17, and 24 months. Data are shown as a median, 25–75% confidence interval, and minimum and maximum values for eight animals (except for seven females in the AM, Cd_1, and Cd_5 groups after 24 months). Statistically significant differences (Kruskal-Wallis test) compared to: a—Control group; b—AM group; c—Cd_1 group; d—Cd_1+AM group; and e—Cd_5 group—where * $p < 0.05$, † $p < 0.01$, and ‡ $p < 0.001$ are marked. The factors of change compared to the control group (↓, decrease; ↑, increase) or the adequate group treated with Cd alone (↘, decrease) are indicated by the numerical values below or above the bars. Detailed data are presented in Table S14.

The administration of AM during the exposure to Cd prevented these heavy-metal-induced changes in the concentration of chemerin, but it decreased the exposure to the 5 mg Cd/kg feed-unchanged concentration of this parameter after 3 and 10 months compared to the control and Cd_5 groups (2.6–6.5-fold) (Figure 12, Table S14). The concentration of MIP1a in the Cd_1+AM

group after 17 and 24 months and in the Cd_5+AM group at all time points was lower compared to the control group, except for a lack of difference in the concentration of MIP1a between the Cd_5 group and the Cd_5+AM group after 3 months (Figure 12, Table S14). In the animals administered with AM during the 10-, 17-, and 24-month feeding with fodder containing 1 mg Cd/kg, the concentration of Bax was lower (1.6–5.5-fold) compared to the Cd_1 group; however, it did not differ compared to the control group (Figure 12, Table S14). The 3-month administration of AM to the females maintained on the 5 mg Cd/kg feed resulted in a decrease (3.7-fold) in the concentration of Bax compared to the control group. After 10, 17, and 24 months, Bax concentration in the Cd_5+AM group did not differ compared to the control, and after 17 and 24 months, it was lower (3.2 and 1.9 times, respectively) than in the Cd_5 group (Figure 12, Table S14).

2.6. Relationships between the Investigated Biomarkers of Kidney Status and the Body Burden of Cd

In the female rats that were not administered with AM (the control group and the Cd_1 and Cd_5 groups), positive dependencies were noted between the indices of the body burden of Cd, such as this heavy metal concentration in the blood, urine, and kidney, and all evaluated markers of renal tubular damage (KIM-1, β2-MG, NAG, and ALP), as well as indices of glomerular damage such as ACR, PCR, and the serum concentrations of uric acid and urea, except for a lack of dependence between NAG activity and Cd concentration in the urine (Table 2). The creatinine clearance was negatively correlated with the Cd concentration in the blood and kidney (Table 2). Moreover, the kidney concentration of chemerin was positively correlated with the Cd concentration in the blood (Table 2).

In the rats that were administered AM alone and during the exposure to Cd (the AM, Cd_1+AM, and Cd_5+AM groups), there were no dependencies between the Cd concentration in the blood, urine, and kidney and the indices of tubular and glomerular damage, except for positive correlations between the kidney Cd concentration and β2-MG, NAG, and ACR (Table 2). Moreover, negative dependencies occurred in these animals between the kidney concentrations of chemerin, MIP1a, and Bax and the concentrations of Cd in the blood, urine, and kidney (Table 2).

2.7. Mutual Relationships between the Investigated Markers of the Kidney Status

In the female rats that were not administered with AM (the control group and the Cd_1 and Cd_5 groups), mutually positive relationships were noted between each of the investigated markers of renal tubular damage (KIM-1, β2-MG, NAG, and ALP), ACR, and PCR (Table 3). The creatinine clearance was negatively correlated with the urinary β2-MG, NAG, ALP, ACR, and PCR, as well as urea the concentration in the serum, and positively correlated with the urinary concentrations of uric acid and urea (Table 3). Positive relationships also occurred between the markers of kidney damage determined in the urine (KIM-1, β2-MG, NAG, ALP, ACR, and PCR) and the serum concentrations of uric acid and urea, except for a lack of relationship between the serum concentration of uric acid and ALP activity and PCR (Table 3). There was no relationship between the serum and urinary concentrations of uric acid and urea (Table 3). The urinary activities of NAG and ALP, ACR, creatinine clearance, and the concentrations of uric acid and urea in the serum positively correlated with the kidney concentration of chemerin (Table 3).

Table 2. Relationships between the investigated biomarkers of kidney status and cadmium (Cd) concentration in the blood, urine, and kidney of female rats administered or not with a 0.1% extract from the berries of *Aronia melanocarpa* L.. (AM) [1,2,3].

Parameter	Regression Analysis [4]	Cd in the Blood of Rats		Cd in the Urine of Rats		Cd in the Kidney of Rats	
		Not Administered with AM [2]	Administered with AM [3]	Not Administered with AM	Administered with AM	Not Administered with AM	Administered with AM
KIM-1 in the Urine	β^p	0.339 ‡	NS	0.309 †	NS	0.509 ‡	NS
	R^2	0.105		0.086		0.251	
β2-MG in the Urine	β^p	0.425 ‡	NS	0.380 ‡	NS	0.638 ‡	0.284 †
	R^2	0.172		0.135		0.401	0.071
NAG in the Urine	β^p	0.316 †	NS	NS	NS	0.434 ‡	0.206 *
	R^2	0.090				0.179	0.032
ALP in the Urine	β^p	0.489 ‡	NS	0.420 †	NS	0.553 ‡	NS
	R^2	0.231		0.092		0.298	
ACR	β^p	0.450 ‡	NS	0.418 ‡	NS	0.694 ‡	0.224 *
	R^2	0.194		0.166		0.476	0.040
PCR	β^p	0.409 ‡	NS	0.279 †	NS	0.624 ‡	NS
	R^2	0.159		0.068		0.383	
Creatinine Clearance	β^p	0.232 *	NS	NS	NS	−0.414 †	NS
	R^2	0.043				0.162	
Uric Acid in the Serum	β^p	0.225 *	NS	0.231 *	NS	0.308‡	NS
	R^2	0.040		0.043		0.085	
Uric acid in the Urine	β^p	NS	NS	NS	NS	NS	NS
	R^2						
Urea in the Serum	β^p	0.525 ‡	NS	0.372 ‡	NS	0.532 ‡	NS
	R^2	0.268		0.129		0.276	
Urea in the Urine	β^p	NS	NS	NS	NS	NS	NS
	R^2						
Chemerin in the Kidney	β^p	0.289 †	−0.498 ‡	NS	−0.431 ‡	NS	−0.429 ‡
	R^2	0.074	0.240		0.177		0.175
MIP1a in the Kidney	β^p	NS	0.315 †	NS	−0.256 *	NS	0.444 ‡
	R^2		0.090		0.056		0.188
Bax in the Kidney	β^p	NS	0.394 ‡	NS	0.375 ‡	NS	0.370 ‡
	R^2		0.146		0.131		0.127

[1] Cd concentrations in the blood, urine, and kidneys of the rats subjected to necropsy after 3, 10, 17, and 24 months have already been published [17]. [2] The results of the analysis of regression are presented as the β coefficient, R^2, and the level of statistical significance (p; where * $p < 0.05$, † $p < 0.01$, and ‡ $p < 0.001$). NS, a lack of relationship ($p > 0.05$). [3] All groups not administered with AM were included in the analysis (the control group that received fodder containing 0.0584 ± 0.0049 mg Cd/kg and the Cd_1 and Cd_5 groups maintained on feed containing 1 and 5 mg Cd/kg, respectively). [4] All groups administered with AM were included in the analysis (the AM, Cd_1+AM, and Cd_5+AM groups). β2-MG, beta2-microglobulin; ACR, albumin concentration in the urine adjusted for creatinine concentration; ALP, alkaline phosphatase; Bax, Bcl2-associated X protein; KIM-1, kidney injury molecule 1; MIP1a, macrophage inflammatory protein 1 alpha; NAG, N-acetyl-β-D-glucosaminidase; PCR, total protein concentration in the urine adjusted for creatinine concentration.

Table 3. Mutual relationships between the investigated parameters describing the kidney status in the female rats administered (*italic*) or not administered with a 0.1% extract from the berries of *Aronia melanocarpa* L. (AM) [1,2].

Parameter	Regression Analysis [3]	KIM 1 in the Urine	β2-MG in the Urine	NAG in the Urine	ALP in the Urine	ACR	PCR	Creatinine Clearance	Uric Acid in the Serum	Uric Acid in the Urine	Urea in the Serum	Urea in the Urine	Chemerin in the Kidney	MIP1a in the Kidney	Bax in the Kidney
β2-MG in the Urine	β/R^2	0.447 ‡ / 0.191	-	*0.375* ‡ / *0.132*	*NS*	*0.543* ‡ / *0.287*	*NS*	*NS*	*NS*	*0.300* † / *0.080*	*NS*	*NS*	*NS*	*−0.420* ‡ / *0.168*	*−0.309* † / *0.086*
NAG in the Urine	β/R^2	0.235 * / 0.045	0.733 ‡ / 0.532	-	*NS*	*0.204* * / *0.031*	*NS*	*NS*	*NS*	*NS*	*NS*	*NS*	*NS*	*−0.250* * / *0.053*	*NS*
ALP in the Urine	β/R^2	0.273 ‡ / 0.064	0.594 ‡ / 0.346	0.741 ‡ / 0.544	-	*NS*	*0.269* † / *0.063*	*−0.260* † / *0.060*	*NS*	*−0.220* * / *0.039*	*NS*	*NS*	*−0.203* * / *0.031*	*−0.270* † / *0.064*	*−0.250* * / *0.050*
ACR	β/R^2	0.563 † / 0.309	0.862 † / 0.739	0.704 † / 0.490	0.548 † / 0.239	-	*NS*	*NS*	*0.312* † / *0.088*	*0.341* † / *0.107*	*NS*	*NS*	*NS*	*−0.460* † / *0.207*	*−0.360* † / *0.120*
PCR	β/R^2	0.488 ‡ / 0.230	0.755 ‡ / 0.566	*0.777* ‡ / 0.600	*0.767* ‡ / 0.585	0.803 ‡ / 0.641	-	*−0.320* † / *0.090*	*NS*	*NS*	*NS*	*NS*	*NS*	*−0.321* † / *0.094*	*−0.320* † / *0.092*
Creatinine Clearance	β/R^2	NS	−0.418 ‡ / 0.165	−0.310 † / 0.088	−0.360 ‡ / 0.122	−0.460 ‡ / 0.199	−0.464 ‡ / 0.207	-	*NS*	*NS*	*NS*	*0.220* * / *0.038*	*NS*	*NS*	*NS*
Uric Acid in the Serum	β/R^2	0.345 † / 0.109	0.356 † / 0.113	0.251 * / 0.053	NS	0.390 † / 0.143	NS	NS	-	*NS*	*NS*	*0.217* * / *0.037*	*0.209* * / *0.033*	*NS*	*NS*
Uric Acid in the Urine	β/R^2	NS	NS	NS	NS	NS	NS	0.248 * / 0.052	NS	-	*NS*	*0.421* / *0.168*	*NS*	*NS*	*NS*
Urea in the Serum	β/R^2	0.259 * / 0.057	0.520 ‡ / 0.262	0.591 ‡ / 0.342	0.577 ‡ / 0.325	0.499 ‡ / 0.241	0.512 ‡ / 0.254	−0.305 † / 0.083	0.474 ‡ / 0.216	NS	-	*NS*	*NS*	*NS*	*NS*
Urea in the Urine	β/R^2	0.398 ‡ / 0.149	0.273 † / 0.065	NS	NS	0.289 † / 0.073	NS	0.241 * / 0.048	0.394 ‡ / 0.146	0.628 ‡ / 0.387	NS	-	*NS*	*NS*	*NS*
Chemerin in the Kidney	β/R^2	NS	NS	0.360 † / 0.120	0.240 * / 0.047	0.223 * / 0.040	NS	0.279 † / 0.068	0.232 * / 0.043	NS	0.239 * / 0.047	NS	-	*0.445* ‡ / *0.189*	*0.481* ‡ / *0.223*
MIP1a in the Kidney	β/R^2	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	0.258 * / 0.057	-	*0.794* ‡ / *0.626*
Bax in the Kidney	β/R^2	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	0.219 * / 0.037	0.273 † / 0.065	0.265 † / 0.060	-

[1] The groups not administered with AM included in the analysis were the control group that received fodder containing 0.0584 ± 0.0049 mg Cd/kg, and the Cd_1 and Cd_5 groups maintained on feed containing 1 and 5 mg Cd/kg, respectively. [2] The groups administered with AM included in the analysis were the AM, Cd_1+AM, and Cd_5+AM groups. [3] The results of the analysis of regression are presented as the β coefficient, R^2, and the level of statistical significance (p, where * $p < 0.05$, † $p < 0.01$, and ‡ $p < 0.001$). NS, a lack of relationship ($p > 0.05$). β2-MG, beta2-microglobulin; ACR, albumin concentration in the urine adjusted for creatinine concentration; ALP, alkaline phosphatase; Bax, Bcl2-associated X protein; KIM-1, kidney injury molecule 1; MIP1a, macrophage inflammatory protein 1 alpha; NAG, N-acetyl-β-D-glucosaminidase; PCR, total protein concentration in the urine adjusted for creatinine concentration.

Unlike the female rats that were not administered with AM, in the animals that received this extract (alone and during the exposure to Cd), only a few mutual dependencies were noted between the evaluated markers of tubular and glomerular damage (Table 3). Moreover, negative dependencies occurred between β2-MG, NAG, ALP, ACR, and PCR and the kidney concentrations of MIP1a and/or Bax (Table 3).

In both animals administered or not with AM, positive relationships occurred between the kidney concentrations of chemerin, MIP1a, and Bax (Table 3).

3. Discussion

The present article is the first report, from studies carried out in an experimental animal model, that even low-level, long-term exposure to Cd poses a substantial risk of damage to the kidney, whereas the intake of an extract from the berries of *A. melanocarpa* considerably protects against this outcome. Since the study was conducted in an in vivo model that well reflects the current levels of Cd exposure in the worldwide general population, it can be concluded that lifetime environmental exposure to this heavy metal in industrialized countries is a risk factor for kidney damage. Moreover, the article presents a proposal for an effective protective strategy against this organ injury due to long-term low-to-moderate intoxication with this xenobiotic.

The kidney, as the main organ of Cd accumulation in the body and the organ responsible for its detoxification and elimination, is especially vulnerable to damage caused by this xenobiotic [2,4–6,8,12,14]. Because Cd is very slowly eliminated from the body, it gradually accumulates in the kidney during chronic intoxication, mainly in the form of complexes with a low-molecular-weight protein—metallothionein (MT) [2,13,25]. The binding of Cd ions (Cd^{2+}) in the kidney cells by MT results in the formation of non-toxic (in the intracellular space) complexes of this element with MT (Cd-MT complexes), which is the process of detoxification of this heavy metal. However, Cd-MT complexes are characterized by a short lifespan (about 3 days) and are decomposed via the release of Cd^{2+} ions, which further induce the synthesis of MT and bind to this protein. Nevertheless, the ability of the kidney to biosynthesize MT and accumulate Cd in the form of non-toxic Cd-MT complexes is limited [2,13,25]. Thus, repeated exposure to this toxic heavy metal creates a threat to kidney health [1,2,4,12,14], and as shown in the present study, even low-level intoxication with this xenobiotic can result in organ damage.

As performed in the present investigation, measurements of the sensitive biomarkers of kidney status together with histological evaluation of this organ revealed that repeated, low-level, and moderate exposure to Cd resulted in abnormalities in the function and morphological structure of kidney tubules and glomeruli. The injury to tubules exceeded damage to the glomeruli, and the first signs of the damaging impact of this xenobiotic on the kidneys were found as early as after 3 months of low-level exposure. Compared to the proper values determined in the control animals, the elevated concentrations of KIM-1 and β2-MG and the activities of NAG and ALP in the urine reflect tubular damage, which gradually progressed with the duration of exposure, especially at the higher investigated levels of intoxication. It is important to emphasize that although there were no differences in the values of the biomarkers of tubular damage between the Cd_1 and Cd_5 groups at particular time points, the changes in the values of these parameters occurred earlier at the higher exposure, except for KIM-1. The positive relationships found between almost all determined indices of renal tubular damage and Cd concentration in the blood, urine, and kidneys of the animals exposed to this element in trace-to-moderate amounts show that the extent of tubular damage progressed with the increasing body burden of this xenobiotic. Moreover, histopathological studies also revealed that the changes in the morphological structure of the tubules were more advanced at a higher level of exposure to Cd. Similar to those noted in the Cd_1 and Cd_5 groups, pathological changes in the histological structure of the kidney were also reported by other authors, however, at higher levels of exposure to this xenobiotic [33–38].

The finding that, at both levels of exposure to Cd, the first biomarker whose values were enhanced already after 3 months was KIM-1 and that this effect occurred from a few to several months earlier than changes in the values of β2-MG and NAG, which are commonly considered sensitive markers of this heavy metal nephrotoxicity [2,8,13–15], shows that this parameter appears to be the earliest biomarker of this xenobiotic-induced tubular damage. KIM-1 is a transmembrane glycoprotein localized on the epithelial cells of the proximal tubules. Cd undergoes accumulation in the cells of the tubular epithelium, and once damage to these cells occurs due to the toxic action of Cd^{2+} ions, KIM-1 is shed into the urine [39]. Although an increase in the concentration of KIM-1 in the urine as a result of exposure to Cd has been reported in numerous studies in humans and experimental animals [2,13,39–41], according to some authors, the usefulness of this marker during low-level exposure to this toxic element may be limited [13,40]. However, our study provides credible evidence that KIM-1 can be an early marker of tubular damage during such exposure, and thus, it is worth determining the impact of Cd on kidney status. In humans environmentally exposed to Cd, the higher prevalence rate of increased urinary concentration of KIM-1 has been shown to occur at this heavy metal concentration in the urine higher than 1 μg/g creatinine [39]. Our study shows that an increased concentration of this biomarker can be noted already after a relatively short exposure (3 months in the experimental model) and at markedly lower levels (several times) than in human Cd concentrations in the urine (0.0852–0.2820 μg/g creatinine) (Table S15) [17].

Like KIM-1, NAG is another biomarker of Cd-induced cytotoxic damage to the renal tubules. It is a lysosomal enzyme abundantly present in the epithelial cells of the proximal tubules. Its activity is low under physiological conditions, but it increases due to Cd-induced injury of the tubular cells [2,8,13,37,39,42,43]. Thus, the increased activity of NAG noted in the animals fed with fodder containing 1 and 5 mg Cd/kg indicates proximal tubular damage. The Cd concentration in the urine of female rats at which the increase in the activity of NAG was first detected (median 0.2403 μg/g creatinine; range 0.1912–0.4959 μg/g creatinine in the Cd_1 group and median 0.3570 μg/g creatinine; range 0.2120–0.5084 μg/g creatinine in the Cd_5 group) (Table S12) is similar to the heavy metal concentration at which an increased activity of this enzyme was reported in humans (0.38 μg Cd/g creatinine in men and 0.42 μg Cd/g creatinine in women) [4]. Apart from KIM-1 and NAG, the urinary activity of ALP is also a biomarker of cytotoxic damage to the proximal tubules. This enzyme is present in the cells of the brush border of the epithelium of the proximal tubules, and thus, damage to these cells is reflected in the increased activity of this enzyme in the urine [13]. Increased activity of ALP in the urine has been found in workers occupationally exposed to Cd who have high concentrations of this xenobiotic in the urine [37] and in experimental animals [43]; however, this biomarker is not commonly used in epidemiological studies. The fact that in the present study, at both levels of treatment with Cd, the increase in the activity of ALP in the urine occurred earlier (after 4 and 10 months of low-level and moderate exposure, respectively) than the changes of commonly used sensitive markers, such as β2-MG and NAG, indicates that the determination of ALP may be useful in monitoring the impact of low-to-moderate exposure to Cd on kidney status.

At both levels of exposure to Cd, the concentration of β2-MG in the urine increased for the first time two months later than when the increase in the activity of NAG occurred, confirming that NAG is a more sensitive biomarker for Cd-induced renal tubular damage than β2-MG [14]. β2-MG is a low-molecular-weight protein present on the surface of almost all nucleated cells and routinely shed by these cells into the blood. Under physiological conditions, this protein passes by the glomeruli and is reabsorbed by the proximal tubules. Thus, it is present in the urine only in low concentrations; however, Cd-induced damage to the reabsorptive function of tubules results in an increase in its amount in the urine [4,14]. The increase in the concentration of β2-MG in the urine of female rats exposed to Cd reflects the destroyed reabsorptive function of the renal proximal tubules. Based on our results, it can be concluded that the reabsorptive function of the renal tubules may

start to weaken at such low Cd concentrations in the urine as 0.1850–0.3110 μg/g creatinine (Table S12). In humans, β2-MG was found in the urine in enhanced amounts at Cd concentrations, reaching 0.38 μg/g creatinine in men and 0.42 μg/g creatinine in women [4].

The Cd-induced increase in ACR, PCR, and the serum concentrations of uric acid and urea, as well as the decrease in creatinine clearance, reflect the impairment of glomerular filtration. Our recently performed overview of the available literature data allowed for the conclusion that albuminuria and GFR (estimated in the present study based on ACR and creatinine clearance, respectively) are sensitive markers of Cd-induced glomerular damage and that LOAELs of the Cd concentration in the blood and urine for glomerular dysfunction (albuminuria and decreased GFR) in the general population are >0.18 μg/L and >0.27 μg/g creatinine, respectively [2,4,5]. In the present study, the first obvious signs of glomerular damage (increased PCR that occurred after 6 months and remained until the end of the 24-month study) were observed at the urinary concentration of the toxic element, ranging from 0.1485 to 0.3397 μg/g creatinine (median 0.2311 μg/g creatinine). The 22–35% reduction in creatinine clearance (reflecting the amount of creatinine filtered in the glomeruli per unit of time) and the increased concentration of uric acid and/or urea in the serum after the 17–24-month exposure to the 5 mg Cd/kg feed indicate significant deterioration in the GFR. Although there were no differences in the values of particular markers of glomerular damage between the Cd_1 and Cd_5 groups, the fact that at the exposure to the 1 mg Cd/kg feed, the creatinine clearance as well as the serum concentration of uric acid and urea were unaffected shows that the damaging impact of this heavy metal on the kidney was more serious at the exposure to the 5 mg Cd/kg feed. Moreover, the histopathological study revealed more advanced glomerulonephritis at the higher of the investigated levels of exposure to Cd. Low-level exposure to this xenobiotic would be expected to not affect creatinine clearance and the serum concentrations of uric acid and urea; however, the finding that the increase in the urinary excretion of total protein (increased PCR) at both levels of exposure to Cd occurred after 6 months and exceeded (for 10–12 months) an occurrence of the enhancement in the excretion of albumin (increased ACR) was unexpected, especially at low-level exposure. The elevation in the values of KIM-1 and β2-MG and enzymatic proteins such as NAG and ALP noted in Cd-exposed rats was not high enough to explain the increase in PCR; however, the presence in the urine of other proteins (e.g., α1-microglobulin, Clara cell protein 16, and MT) might also be enhanced [2,8,13,14]. The positive relationships between Cd concentration in the blood, urine, and kidney and the urinary ACR and PCR in the animals that did not receive AM during trace-to-moderate exposure to Cd indicate that the risk of Cd-induced proteinuria, including albuminuria, increased with the increasing body burden of Cd. Albumin is the main plasma protein physiologically present in the blood. Under proper kidney function, only trace amounts of this protein occur in the urine, but when glomerular damage occurs, its concentration in the urine increases [2].

Detailed analysis of the changes in the values of particular biomarkers of the kidney status during the 24-month exposure to Cd showed that the kidney injury developed and progressed with the duration of the exposure to this xenobiotic, leading to both tubular and glomerular damage at relatively low concentrations of this xenobiotic in the blood and urine and its low accumulation in the kidney (Table S15). Histopathological studies confirmed the damaging impact of Cd on kidney morphology, including both the injury of tubules and the development of glomerulonephritis due to lifelong, even low-level, exposure to this xenobiotic. It is important to emphasize that although the statistical analysis revealed no difference in Cd concentration in the urine or biomarkers of kidney damage between the Cd_1 and Cd_5 groups, the measurements of biochemical parameters and microscopic assessment of the morphological structure of this organ allowed for the conclusion that the damaging impact of Cd on the kidney at the higher exposure occurred earlier and was more advanced at the same exposure duration than at the lower level of exposure. The lack of statistically significant differences in the values of biomarkers of Cd nephrotoxicity between the Cd_1 and Cd_5 groups may be explained by the lack of difference

in this toxic element's concentration in the urine (the most useful biomarker of long-term exposure to Cd) between these two groups. Moreover, the lack of differences in both the values of the estimated biomarkers of kidney status and the Cd concentration in the urine between these groups might also result from a relatively wide range of values in terms of particular parameters.

The fact that in the experimental model of human exposure to Cd that we created, the 24-month exposure to the 1 mg Cd/kg feed resulted in cytotoxic damage to the kidney tubules, weakened their resorptive function, and led to glomerulonephritis, allows for the conclusion that lifelong exposure to this toxic heavy metal may create a risk for kidney damage in the general population. Even under low environmental exposure, Cd gradually accumulates in the kidney during a person's lifetime and reaches a peak concentration in this organ at around 60 years of age [44,45]. In the female rats maintained on fodder containing only trace amounts of Cd (0.098 mg/kg) and those fed with the 1 mg Cd/kg diet, the accumulation of this element in the kidneys increased throughout the experiment and reached its peak after 24 months (0.0844 ± 0.0357 and 1.981 ± 0.5089 μg/g wet weight (w.w.), respectively) [17], when the age of the animals was equivalent to the human age of 60 years [46]. The accumulation of Cd in the kidneys during the exposure to 5 mg Cd/kg reached a peak after 17 months (10.77 ± 1.936 μg/g w.w.), corresponding to approximately 45 human years [46], and a plateau was reached thereafter [17].

Detailed analysis of the results of the present study shows that even under low-level exposure, the first signs of the damaging impact of Cd on the kidney tubules may occur already at a young age (i.e., after 3 months of the experiment, corresponding to the human age of around 18 [46]), while at the stage of adulthood (i.e., after 10–17 months of the study, corresponding to 30–45 years in humans [46]), the damage to both tubules and glomeruli was clearly evident, whereas in the elderly (i.e., after 24 months of the study), tubular damage intensified and glomerulonephritis developed. The finding that even low-level exposure to Cd has a detrimental effect on the kidney that progresses with exposure duration and may lead to destroying kidney function and pathological changes in this organ structure is a very important result of the study. Revealing that these effects occur at this heavy metal concentration in the urine (0.0852–0.2820 μg/g creatinine) within the lower range of values noted in the worldwide general population confirms our recently published [2] conclusion made based on the overview of literature data that even low-level environmental exposure currently occurring in developed countries is a risk factor for kidney damage.

Although the toxic impact of Cd on the kidneys has been known for a long time and has been the subject of many studies, the mechanism of the nephrotoxic action of this xenobiotic, as well as the risk of this organ damage under low-level exposure, have not been sufficiently studied (for a review, see [2]). In the available literature, there are numerous studies on the damaging impact of Cd on the kidney conducted in laboratory animals; however, the used experimental models do not correspond to human exposure in terms of the dose (moderate, high, and even very high doses were administered) of this xenobiotic and the route of intoxication [33–38,40,47–49]. To our knowledge, the experimental model we created is the only in vivo model constructed so far that well reflects human environmental exposure, and, therefore, we discuss our findings in the light of data from epidemiological studies. According to current knowledge, the mechanism of the nephrotoxic action of Cd is multidirectional and mainly involves the induction of oxidative stress and oxidative lesions of cellular macromolecules and cellular organelles, the development of inflammatory processes, the stimulation of cell proliferation, and the induction of epigenetic changes [2,4,8]. As noted in the present study, increased concentrations of chemerin and Bax in the renal tissue and changes in the microscopic image of the kidney show that the mechanism of the toxic impact of Cd at low-to-moderate exposure is related to the induction of inflammatory status and the stimulation of apoptotic and proliferative processes [35,37,40]. The present study was focused primarily on understanding whether

low-level chronic exposure creates a risk for kidney damage and if this effect may be counteracted by Aronia extract. However, our research project also includes an explanation of the mechanisms of both the nephrotoxic action of Cd and the nephroprotective impact of the extract, and the findings will be published soon.

The most important and new finding of the present study is revealing the possibility of protection from the damaging impact of Cd on the kidney via the administration of chokeberry extract and providing further evidence of the beneficial impact of Aronia products to counteract the harmful effect of exposure to this xenobiotic. The finding that the intake of chokeberry extract during low-level and moderate exposure to Cd almost completely protected from changes in the biochemical biomarkers of this xenobiotic-induced tubular and glomerular damage and weakened pathological changes in the histological structure of the renal tissue suggests that Aronia berry products are a good strategy for protecting against kidney damage caused by exposure to this xenobiotic. Since even low-level chronic exposure to Cd, which nowadays is increasingly inevitable in industrialized countries, creates a risk for kidney damage [1,2,4–6,8,12,14], the findings of this study have not only scientific value but are also very important from a public health perspective and have practical implications.

Considering our previous findings taken from the experimental model [17–26] and the chemical composition of the chokeberry extract [21,27,28,30,32], the protective impact of AM regarding Cd nephrotoxicity revealed in the present study may be explained by the direct action of the extract ingredients, especially polyphenolic compounds, as well as their interaction with Cd. It has been revealed that the administration of AM to the animals treated with 5 mg Cd/kg feed decreased the apparent absorption (by 14–17%) and retention in the body (by 14–17%) and increased urinary excretion (by 21–39%) of this toxic element, resulting in lower total accumulation in the internal organs (by 20–29%) (mainly in the kidneys and liver—by 20–29%—but also in the spleen, heart, and brain, by 15–17%) and bone tissue (by 12–25%), as well as a lower concentration in the blood (by 10–19%) (Table S15) [17]. The Cd concentration in the kidneys of the rats co-administered with AM and the 5 mg Cd/kg feed for 3–24 months was lower by 6.5–13% than in the animals that were not supplemented with the extract during the treatment with this toxic element (Table S15). However, at exposure to the 1 mg Cd/kg feed, the beneficial effect of AM was very slight—only a temporary decrease in the apparent absorption and retention of Cd (by 14% and 13%, respectively, after 10 months) and its concentration in the liver (by 29% after 3 months) and kidney (by 33% and 37% after 3 and 24 months, respectively) was noted (Table S15) [17].

The measurements of Cd concentration in the urine carried out in the present study every other month during the 24-month experiment also disclosed that the administration of AM to the animals maintained on fodder containing 5 mg Cd/kg led to an increase in the urinary excretion of Cd. The revealed protective impact of AM on the function and structure of the kidney, especially at moderate exposure, may, at least partially, be explained by the lower concentration of Cd in the blood and kidney and its increased urinary excretion (Table S15) [17]. The finding that the administration of AM during the exposure to the 5 mg Cd/kg feed, similar to the case of low-level exposure, almost entirely protected the kidney from the damaging impact of Cd on the kidney may be explained by the markedly lower body burden of Cd. Moreover, the fact that in the animals that received AM during the trace-to-moderate levels of exposure to Cd, there were almost no dependencies between this heavy metal concentration in the blood, urine, and kidney (unlike the animals that did not receive the extract) may suggest that the decrease in the body burden of Cd was not the only cause of the nephroprotective effect of the extract. The ability of the chokeberry extract to decrease the body burden of Cd resulted, at least to some extent, from the high content of polyphenolic compounds, which, because of the presence of hydroxyl (-OH) groups, are able to form complexes with ions of divalent toxic metals, including Cd^{2+} ions [17,32]. Polyphenols can form complexes with Cd^{2+} ions in the

gastrointestinal tract, preventing their absorption in this way, as well as in the extracellular fluids, facilitating their elimination via the urine, and protecting against their retention in the body and toxic effect [17,30,32].

Taking into account the known health-promoting properties of chokeberries [27,29–31,49,50], the results of the present study, as well as our previous findings in these animals [17–26], the protective impact of AM against Cd nephrotoxicity can also be explained by the antioxidative, anti-inflammatory, antiapoptotic, and antiproliferative properties of the extract ingredients, especially polyphenolic compounds [27,29–31]. Although, at this stage of our study, we are unable to explain these mechanisms, the fact that the administration of AM under both levels of exposure to Cd led to a decrease in the concentration of chemerin, MIP1a, and Bax compared to the animals that did not receive the extract shows the involvement of the anti-inflammatory and antiapoptotic potential of the extract. These, together with the antiproliferative effect of AM revealed in histopathological studies and the lower body burden of Cd, including its lower accumulation in the kidneys, may, to some extent, explain the protective impact of the extract. Further possible mechanisms, especially the strong antioxidative potential of chokeberries [22,29] are also the subject of our research project, and the findings will be published soon. Polyphenolic compounds are the main group of chokeberry extract ingredients characterized by numerous healthful properties [27,29–31]; however, it is important to point out that the nephroprotective effect of AM might also result from the presence of other components that are effective in reducing Cd toxicity, such as β-carotene, triterpenes, fiber, pectin, ascorbic acid (vitamin C), alpha-tocopherol (vitamin E), and essential bioelements, such as Zn or selenium [29–31].

In the available literature, there is no data on the possibility of the protective impact of Aronia products on Cd-induced kidney damage. Only Kowalczyk et al. [49] have revealed that oral administration of anthocyanins from the berries of *A. melanocarpa* at a dose of 10 mg/kg b.w. during a 30-day treatment with cadmium chloride 2.5-hydrate ($CdCl_2 \times 2.5\ H_2O$) at a dose of 4 μg/kg b.w. resulted in a decrease in Cd accumulation in the kidney and protected against this heavy-metal-induced increase in the concentration of urea in the serum. Moreover, there are some reports from experimental studies showing that some other compounds, such as curcumin [43], carvacrol [45], N-acetyl-L-cysteine (NAC) [47], and calcium antagonists such as nimodipine and chlorpromazine [48], can protect from Cd nephrotoxicity; none of these studies, however, was as comprehensive as the present study and did not evaluate the impact of exposure comparable to the exposure of the general population. The present study is the only one so far to indicate that the consumption of Aronia products may be an effective strategy in protecting against kidney damage due to low-to-moderate chronic exposure to Cd. Moreover, this article is our next report, providing evidence for the multidirectional protective impact of chokeberry ingredients in counteracting the negative health outcomes of exposure to Cd.

It is important to underline that the fact that in the animals that received AM alone, the values of all biomarkers of kidney status were within the proper values noted in the control group shows that the enhanced daily intake of polyphenols in the absence of exposure to Cd has no negative impact on the kidney. The slight changes in the morphological picture of the kidney noted in single animals after 24 months of the study may be related to aging-related changes in this organ. The same might be a cause of the slight changes evident in the morphological picture of the kidney in the control group. In the available literature, we have found no data on the unfavorable impact of the consumption of Aronia products on kidney status. Moreover, it has been reported that supplementation with chokeberry juice in type 2 diabetic patients decreased the concentration of creatinine in the urine after 3 months but not after 6 months and had no impact on the concentration of creatinine and urea in the serum [50].

We are fully aware of the limitations of our research and its accomplishments. Since female rats were used in the trial because they are more prone to Cd toxicity than male rats, our findings apply to female kidneys. That is why further studies on both the toxicity of Cd in the model well reflecting human exposure to this heavy metal and the protective

properties of AM during this exposure on male kidneys are necessary. Moreover, at this stage of our research, we are unable to explain some results (e.g., why the increase in PCR exceeded the increase in ACR) and the mechanisms of the nephrotoxic impact of Cd at low-to-moderate exposure and the nephroprotective effect of AM; however, we are currently conducting studies in this area and will publish the results soon. We are aware that extrapolation of our findings regarding Cd concentration in the blood and urine and its accumulation in the kidneys of rats to humans could be inaccurate and should be approached with caution; however, our study provides the only attempt to explore this process in vivo considering a lifetime exposure level comparable to that currently noted in the worldwide general population in industrialized countries. Despite some limitations, the results of this study indicate that Aronia products appear to be an important strategy in preventing the harmful health effects of environmental exposure to Cd. However, further studies are needed in humans to confirm their effectiveness in protecting the kidneys in the general population.

4. Materials and Methods

4.1. Animals

In total, 192 3–4 week-old female Wistar rats (Hannover Wistar rats, bred according to the Charles River International Genetic Standardization Program—Crl: WI (Han)) from a licensed breeding facility (Laboratory Animal House, Brwinów, Poland) were used in the study. The rats were given 5 days to acclimate to the experimental environment before the study began. Throughout the experiment, the animals were kept under standard conditions (temperature 22 ± 2 °C, relative humidity 50 ± 10%, and 12-h light/dark cycle) with free access to feed and drinking water.

4.2. Feed Containing Cd

The feed containing Cd at concentrations of 1 and 5 mg/kg was provided by Label Food "Morawski" (Kcynia, Poland). It was prepared by adding $CdCl_2 \times 2.5\ H_2O$ (POCh, Gliwice, Poland) to the ingredients of the Labofeed H diet (given as a breeding diet for the first 3 months of the experiment) and the standard Labofeed B diet (used as the maintenance diet from the beginning of the 4th month until the end of the study).

The procedure was carried out to determine the homogeneity of Cd content in the Labofeed diets, and the results showed that the concentration of this element in these diets (1.09 ± 0.13 mg/kg and 4.92 ± 0.53 mg/kg; mean ± standard deviation—SD) was exactly in line with the certified values (1 and 5 mg Cd/kg, respectively) [17]. The mean concentration of Cd in the standard Labofeed fodder (maintenance and breeding diets) without the addition of $CdCl_2 \times 2.5\ H_2O$ reached 0.0584 ± 0.0049 mg/kg [17].

4.3. Aronia melanocarpa L. Extract

The lyophilized chokeberry extract, in powdered form, provided by Adamed Consumer Healthcare (Tuszyn, Poland), was used. According to the manufacturer's (Adamed Consumer Healthcare, Tuszyn, Poland) declaration (Certificate KJ 4/2010; Butch No. M100703), the extract contained 65.74% polyphenolic compounds, including 18.65% anthocyanins. The total content of polyphenols in the powdered extract determined in our laboratory was 612.40 ± 3.33 mg/g (mean ± standard error—SE), and the polyphenolic profile (Figure S1) of the extract was as follows: total anthocyanins (202.28 ± 1.28 mg/g); derivatives of cyanidin (cyanidin 3-O-β-galactoside— 80.07 ± 1.05 mg/g; cyanidin 3-O-α-arabinoside—33.21 ± 0.01 mg/g; cyanidin 3-O-β-glucoside—3.68 ± 0.01 mg/g); total proanthocyanidins (129.87 ± 1.12 mg/g); total phenolic acids (110.92 ± 0.89 mg/g); chlorogenic acid (68.32 ± 0.08 mg/g); and total flavonoids (21.94 ± 0.98 mg/g) [21]. The extract also contained other ingredients such as carotenoids, minerals, pectins, sugar, sugar alcohols, phytosterols, triterpenes, and vitamins, as well as 6.1% water (producer data, [27,28]).

To prepare the 0.1% aqueous solution of the extract (AM) for administration to animals, 1 g of the powdered extract by Adamed was dissolved in 1 L of redistilled water. The solution was prepared daily and was stable for at least 24 h. One mL of the solution contained 0.612 ± 0.003 mg of polyphenols [17].

4.4. Design of the Study

The experimental protocol was approved by the Local Ethics Committee for Animal Experiments in Bialystok (Poland; permission number 60/2009 on 21 September 2009). All procedures using animals were carried out in accordance with institutional guidelines, ethical standards, and the International Guide for the Use of Animals in Biomedical Research.

The rats were randomly divided into the following six experimental groups, each consisting of 32 animals:

- Control group: the rats were maintained on the standard Labofeed diet (0.0584 ± 0.0049 mg Cd/kg) and drinking water (redistilled water containing < 0.05 μg Cd/L) without the addition of the extract from the berries of *A melanocarpa* L.
- AM group: the animals received AM (0.1% aqueous solution of the extract from the berries of *A. melanocarpa*) as the only drinking fluid.
- Cd_1 group: the rats were fed a diet containing 1 mg Cd/kg and received redistilled water as drinking fluid.
- Cd_1+AM group: the animals were fed AM as the only drinking fluid for the entire duration of exposure to Cd at a concentration of 1 mg/kg feed.
- Cd_5 group: the rats were treated with Cd at a concentration of 5 mg/kg feed and received redistilled water as drinking fluid.
- Cd_5+AM group: the animals were exposed to Cd at a concentration of 5 mg/kg feed and received AM as the only drinking fluid.

The experiment lasted up to 24 months. During the 24-month study, no unfavorable health outcomes were observed in all groups; however, one animal from the AM group, Cd_1 group, and Cd_5 group died spontaneously between the 18th and 20th month [17]. Eight females of each group, except for seven animals after 24 months in the AM, Cd_1, and Cd_5 groups, were necropsied after 3, 10, 17, and 24 months. At the beginning of the study (before starting the administration of Cd and/or AM) and at the end of every other month of the 24-month study, the 24-h urine was collected from the same eight animals in each group (except for seven females after 20, 22, and 24 months in the AM, Cd_1, and Cd_5 groups). The urine was also collected from eight rats from each group subjected to necropsy after 3, 10, and 17 months. To perform the 24-h urine collection, the rats were housed individually in metabolic cages. During this time, the animals had free access to feed and drinking fluid (Labofeed diet with or without Cd and redistilled water or AM, depending on the experimental group). Immediately after collection, the urine was centrifuged (MPW-350R centrifuge, Medical Instruments; Warsaw, Poland), and its volume was determined.

The exposure to the 1 or 5 mg Cd/kg feed reflects low or moderate levels of environmental human exposure, respectively, which was confirmed by analyzing the Cd concentration in the blood (0.1030–0.3240 μg Cd/L in the Cd_1 group and 0.7350–1.3320 μg Cd/L in the Cd_5 group) and urine (0.0852–0.2820 and 0.2839–0.6949 μg Cd/g creatinine, respectively) of the rats [17] being within the lower range of values currently noted in the general population of industrialized countries (0.02–4.40 μg/L in the blood and 0.04–3.39 μg/g creatinine in the urine (for a review, see [2]). The concentration of Cd in the blood (0.0330–0.1290 and 0.0330–0.1360 μg Cd/L in the control group and AM group, respectively) and urine (0.0764–0.2013 and 0.0730–0.1712 μg Cd/g creatinine in the control group and AM group, respectively), noted in the groups non-exposed to this heavy metal, was irrelevant compared to the other experimental groups [17]. The selection of female rats over male

specimens was conditioned by the higher susceptibility of females to the toxic action of Cd, which has been confirmed both in animal and human studies [8].

Throughout the study, the daily feed and fluid intakes, as well as body weight gain, did not differ between the experimental groups [17]. Based on the measurements of the daily consumption of feed and AM, the intake of Cd, as well as the extract and polyphenols, were calculated (Table 4). The daily intake of Cd and polyphenols did not differ between the experimental groups regardless of whether Cd and AM were administered separately or together (Table 4). The daily intake of polyphenolic compounds in the animals that received AM as the only drinking fluid (Table 4) was several times higher compared to the average intake of these compounds among the general population worldwide. According to the available literature data [31], the daily intake of polyphenols in humans ranges from about 800 mg to over 1700 mg, reaching an average of 1000 mg (14.29 mg/kg b.w. assuming a mean body weight of 70 kg).

Table 4. The daily intakes of cadmium (Cd) and 0.1% *Aronia melanocapra* berry extract (AM) during the 24-month experiment.

Group	Intake during the 24-Month Study [1]	
	Cd (μg/kg b.w./24 h)	Chokeberry Extract [Polyphenols] (mg/kg b.w./24 h)
Control	2.30–4.98	0 [0]
AM	2.25–4.95	67.4–146.6 [44.3–96.4]
Cd_1	39.2–83.8	0 [0]
Cd_1+AM	37.5–84.9	67.2–154.7 [44.2–101.7]
Cd_5	210.1–403.2	0 [0]
Cd_5+AM	200.2–401.9	63.1–150.3 [41.5–98.8]

[1] Data show the ranges of the daily intakes of Cd, chokeberry extract, and polyphenols throughout the 24-month study (the ranges represent the minimum and maximum intake for 32 rats during the first 3 months; 24 females—from the beginning of the 4th up to the end of the 10th month; 16 animals—from the beginning of the 11th month up to the end of the 17th month and then, for 8 rats, except for 7 animals in the AM, Cd_1, and Cd_5 groups between the 18th and 24th months; detailed data on the intakes of the extract and polyphenols in particular groups during 3, 10, 17, and 24 months have been published [17]). The intake of polyphenolic compounds was calculated assuming, according to the manufacturer, that their content in the chokeberry extract reached 65.74%. The intake of Cd in the Cd_1 and Cd_5 groups was calculated based on the content of this heavy metal in the Labofeed diet specified by the producer (1 or 5 mg/kg), while its intake in the control group and AM group was estimated based on its concentration in the standard diet (0.0584 ± 0.0049 mg/kg) [17].

Following the 24-h urine collection performed after 3, 10, 17, and 24 months of the experiment, the rats were deprived of food overnight and then subjected to intraperitoneal anesthesia with barbiturate (Morbital, 30 mg/kg b.w.; Biowet; Pulawy, Poland), under which the whole blood was taken via cardiac puncture with and without anticoagulant (heparin), and various organs and tissues, including both kidneys, were dissected. The kidneys, immediately after collection, were rinsed multiple times with ice-cold 0.9% sodium chloride (physiological saline) and gently dried on the filter paper. Next, these organs were weighed with an analytical balance (OHAUS®, Nanikon, Switzerland; accuracy to 0.0001 g). The left kidney was sectioned in the longitudinal plane into two parts. One of these parts of four animals from each group after 3, 10, 17, and 24 months was subjected to histopathological examination. The second part of the left organ was used for other measurements, including the determination of Cd concentration [17,25,26]. The biological material that was not used immediately was stored frozen (−70 °C) until assayed.

The experimental model has been reported in detail [17–23,25,26]. A schematic representation of the model and range of measurements performed in the present paper are presented in Figure 13.

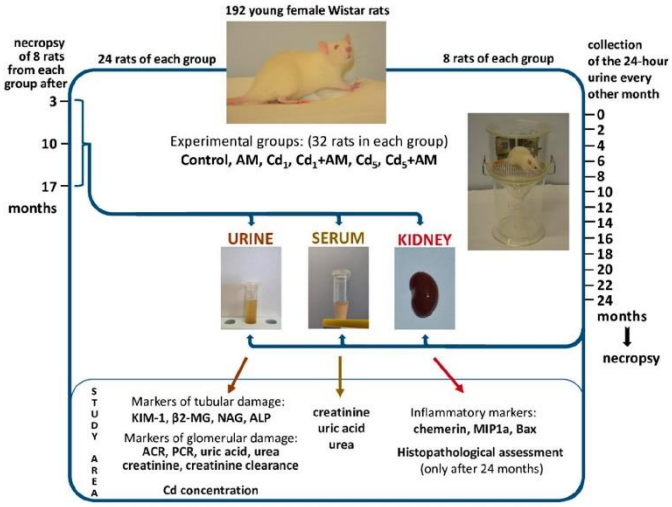

Figure 13. A schematic representation of the experimental model and range of measurements performed in the present study. The animals were treated with cadmium (Cd) in the diet at a concentration of 0, 1, or 5 mg/kg (Control, Cd_1, and Cd_5 groups) and/or 0.1% extract from the berries of *Aronia melanocarpa* L. (AM, Cd_1+AM, and Cd_5+AM groups) for 3, 10, 17, and 24 months. β2-MG, beta2-microglobulin; ACR, albumin concentration in the urine adjusted for creatinine concentration; ALP, alkaline phosphatase; Bax, Bcl-2-associated X protein; KIM-1, kidney injury molecule 1; MIP1a, macrophage inflammatory protein-1 alpha; NAG, N-acetyl-β-D-glucosaminidase; PCR, total protein concentration in the urine adjusted for creatinine concentration. The appropriate parameters, except for Cd in the urine, were determined in the serum, urine, and kidney after 3, 10, 17, and 24 months. Moreover, β2-MG, NAG, ALP, ACR, and PCR, as well as Cd, were evaluated every other month of the 24-month study and before its beginning.

4.5. Analytical Methods

All measurements using commercial kits were performed according to the recommendations of the producers, and the precision of these measurements was expressed as the intra- and/or inter-assay coefficient of variation (CV). The spectrophotometers MULTISCAN GO (Thermo Scientific, Vantaa, Finland), Epoch (Bio Tek Instruments, Inc., Winooski, VT, USA), and Specord 50 Plus (Analityk Jena, Jena, Germany) were used for the quantification of the determined variables.

4.5.1. Measurements of the Markers of Kidney Status in the Urine and Serum

Biomarkers of Tubular Damage

The measurement of the concentration of KIM-1 was performed with the KIM-1 ELISA Kit (Catalog No. MBS355395) produced by MyBioSource, Inc. (San Diego, CA, USA). Sandwich enzyme-linked immunosorbent assay (ELISA) technology served as the foundation for this kit. Purified, horseradish peroxidase (HRP)-conjugated anti-KIM-1 antibody pre-coated on a plate was used as a detecting antibody. Following the addition, mixing, and incubation of the standards and tested samples in the wells, the unbound conjugate was removed from the plate using a wash buffer. The HRP enzymatic reaction was visualized using a chromogenic reagent. The reagent was catalyzed using HRP to create a blue color product that became yellow after the addition of an acidic stop solution. The absorbance of standards and tested samples was measured at 450 nm using a microplate reader, and the concentration of KIM-1 in the tested samples was automatically

quantified from the calibration curve. The intra- and inter-assay CVs for this kit were <3% and 2%, respectively.

The concentration of β2-MG and the activity of NAG in the urine were determined with the use of the B2M ELISA Kit (Catalog No. E0260r) and the Rat N-acetyl-beta-D-glucosaminidase ELISA Kit (Catalog No. E0069r), respectively, by EIAAB Science Inc. (Wuhan, China). Standards and tested samples were added to the wells pre-coated with biotin-conjugated antibodies specific for the target protein (β2-MG or NAG), and after incubation with detection reagents and substrate addition, stop solution was added, producing a yellow-colored product (450 nm). The concentration of β2-MG and the activity of NAG were automatically quantified from their respective calibration curves. The intra- and inter-assay CVs were <4.5% and 3%, respectively, for β2-MG and <5.4% and 2.4%, respectively, for NAG.

The activity of ALP in the urine was assessed using the kit (Catalog No. 1-221-0150) produced by BioMaxima (Lublin, Poland). This method was based on the hydrolysis of colorless p-nitrophenyl phosphate to p-nitrophenol and inorganic phosphate. The rate of an increase in the absorbance of the yellow p-nitrophenol, measured at 405 nm, is proportional to the activity of ALP. The activity of this enzyme assayed by us in the reference BioNorm serum (Catalog No. 1-801-0020; BioMaxima, Lublin, Poland) was 101.7 ± 2.899 U/L (mean ± SD) and agreed with the reference value (86.9–133 U/L).

The concentrations of KIM-1 and β2-MG in the urine were expressed in calculations per creatinine concentration, whereas the activities of NAG and ALP were expressed in international units per litter of the 24-h urine (U/L).

Biomarkers of Glomerular Damage

The concentrations of albumin and total protein in the urine were determined with the use of kits (Catalog No. 1-003-0200 and No. 1-008-0200, respectively) produced by BioMaxima (Lublin, Poland). The measurement of the concentration of albumin was based on the end-point method with bromocresol green. This compound binds to albumin, producing a colored complex. The intensity of the color of the complex, measured photometrically (630 nm), is proportional to the concentration of albumin in a tested sample. The concentration of albumin determined by us in the reference BioNorm serum (Catalog No. 1-801-0020; BioMaxima) was 4.45 ± 0.184 g/100 mL (mean ± SD) and agreed with the reference value (3.89–5.27 g/100 mL). The concentration of total protein was determined by the biuret method. The principle of the method is based on a reaction of peptide bonds of protein with ions of copper(II), leading to the formation of a blue complex whose absorption (546 nm) is proportional to the amount of protein present in a tested sample. The concentration of total protein in the control BioNorm serum (Catalog No. 1-801-0020; BioMaxima) determined in our laboratory (5.89 ± 0.34 g/100 mL; mean ± SD) was within the range of reference values provided by the producer (5.26–6.70 g/100 mL). The concentrations of albumin and total protein in the urine were adjusted for creatinine concentrations (ACR and PCR, respectively).

The determination of creatinine concentration in the serum and urine was performed using a kinetic method (Jaffe's method) with the use of the BioMaxima kit (Catalog No. 1-038-0300; Lublin, Poland) based on the reaction between creatinine and picric acid in alkaline pH, leading to the formation of an orange-red complex, the intensity of which color is proportional to the concentration of creatinine in a tested sample. Based on the concentration of creatinine in the serum and urine and the volume of the 24-h urine, creatinine clearance was calculated. The concentration of creatinine determined by us in the control BioNorm serum (Catalog No. 1-801-0020; BioMaxima) reached 1.270 ± 0.183 mg/100 mL (mean ± SD) and was within the reference range (1.04–1.50 mg/100 mL).

The concentrations of urea and uric acid in the serum and urine were determined with the use of kits (Catalog No. 1-480-0300 and 1-045-0200, respectively) produced by BioMaxima (Lublin, Poland). The measurement of urea concentration was performed us-

ing a kinetic enzymatic method with urease and glutamate dehydrogenase. Urease hydrolyzes urea into ammonia and carbon dioxide. In the presence of glutamate dehydrogenase, glutamate is formed from ammonia and 2-oxoglutarate, and at the same time, nicotinamide adenine dinucleotide (NADH) is oxidized. The rate of decrease in absorbance (340 nm) is proportional to the concentration of urea in the tested sample. The concentration of urea determined by us in the control BioNorm serum (Catalog No. 1-801-0020; BioMaxima) reached 35.02 ± 1.80 mg/100 mL (mean ± SD) and was within the range of reference values provided by the manufacturer (31.0–42.0 mg/100 mL). The assay of uric acid was based on the enzymatic oxidation of this compound to allantoine and hydrogen peroxide. The generated hydrogen peroxide combines with 3,5-dichloro-2-hydroxybenzenesulfonic acid and 4-aminoantipyrine to form a colored complex whose intensity (measured at 546 nm) is proportional to the concentration of uric acid. The concentration of uric acid determined by us in the control BioNorm serum (Catalog No. 1-801-0020; BioMaxima) reached 4.23 ± 0.070 mg/100 mL (mean ± SD) and was within the range of reference values (3.82–5.16 mg/100 mL). The contents of urea and uric acid in the 24 h urine were evaluated.

4.5.2. Determination of Cd Concentration in the Urine

The concentration of Cd in the urine was determined using the flameless atomic absorption spectrometry (AAS) method with electrothermal atomization in a graphite furnace with the use of a Hitachi atomic absorption spectrophotometer model Z-5000 (Tokyo, Japan), as reported [17]. Samples of 24-h urine were diluted appropriately with 0.5% nitric acid (HNO_3) prepared via the dilution of trace-pure 65% HNO_3 (Merck, Darmstadt, Germany) with ultra-pure water (MAXIMA purification system; ELGA, Bucks, UK). The mixture of palladium and magnesium (as nitrates; Merck, Darmstadt, Germany) was used as a matrix modifier in the AAS method. The Cd concentration was automatically read from a calibration curve prepared from a stock of standard solution (Sigma, St. Louis, MO, USA) assigned for the AAS method. The limit of Cd detection was 0.018 µg/L. To check the analytical quality of the analysis, the certified material, Trace Elements Urine level 1 (No. 201305; SeronormTM, Billingstad, Norway), was used. The Cd concentration determined by us (4.75 ± 0.31 µg/L; mean ± SD) in the certified reference urine exactly agreed with the value given by the producer (4.9 ± 0.2 µg/L). The recovery of Cd in the reference sample was 96.9%, whereas the precision of the measurement, expressed as CV, was <8.5%. Cd concentration in the urine was adjusted for creatinine concentration (determined with the use of a commercial kit No. E10051 by EMAPOL; Gdańsk, Poland).

4.5.3. Histopathological Studies

Immediately after dissection and rinsing with ice-cold physiological saline, halves of the left kidneys (section in the longitudinal plane of the organ) were fixed in Bouin's solution (prepared by mixing picric acid (Sigma-Aldrich GmbH, Steinheim, Germany), formalin (CHEMPUR, Piekary Śląskie, Poland), and acetic acid (CHEMPUR)) for 24 h, dehydrated in different concentrations of ethyl alcohol (POCh, Gliwice, Poland), cleared with xylene (POCh), and embedded in paraffin (CHEMPUR). The tissues embedded in paraffin blocks were cut on a Histocore Multicut 1860 histological microtome (Leica Biosystems, Nußloch, Germany). Tissue sections were placed on adhesive basic slides (Mar-four, Konstantynów Łódzki, Poland). Then, manual standard histological topographic staining of the preparations with hematoxylin and eosin was performed with the use of the following reagents: Harris hematoxylin (Mar-four), eosin Y (Mar-four), 99.9% ethanol (pure for analysis) (Alpinus Chemia; Solec Kujawski, Poland), xylene (pure for analysis) (Alpinus Chemia, Poland), acidic ethanol solution with 35–38% hydrochloric acid (pure for analysis) (CHEMPUR) (prepared in-house). Histological slides were also manually stained for Masson trichrome staining using a commercial kit (Catalog No. 04-010802; Bio-Optica, Milano, Italy). Histopathological evaluation and photographs were performed by a veterinary pathologist using Axiolab 5 microscopes, an Axiocam camera, and ZEN 2.0 software

(Zeiss, Halle, Germany). Criteria for the histopathological assessment were based on the scientific literature and recommendations included in the International Harmonization of Nomenclature and Diagnostic Criteria (INHAND), developed and published by the Global Editorial and Steering Committee (GESC) [51]. The structure of renal tissues was evaluated using hematoxylin and eosin topographic staining, while in Masson's staining, the basal membranes of the vessels of the glomeruli (in glomerulopathies) and renal tubules (in tubulopathies), as well as the interstitial connective tissue of the kidneys (in the processes of connective tissue hyperplasia/fibrosis), were assessed. Tissues were evaluated at ×5, ×10, and ×40 objective magnifications. The location, nature, and severity of pathological changes were assessed. Based on the literature review, the experience of the research team, and the initial review of the preparations, the catalog of histopathological changes used in the scoring (scalar) assessment was specified. The occurrence of each lesion was graded on the scale: 0—none; 1—slight; 2—moderate; and 3—severe.

4.5.4. Determination of Proinflammatory Markers in the Kidney

Pre-weighted slices of the kidney (right) were homogenized using a high-performance homogenizer (Ultra-Turrax T25; IKA, Staufen, Germany) in a cold potassium phosphate buffer (50 mM, pH = 7.4; made by combining 50 mM potassium dihydrogen phosphate and 50 mM dipotassium hydrogen phosphate (POCh; Gliwice, Poland)) with the addition of butyl-hydroxytoluene (Sigma-Aldrich GmbH; Steinheim, Germany) as an antioxidant. Then, 0.01 mL of 0.5 M butyl-hydroxytoluene in acetonitrile (Merck, Darmstadt, Germany) were used per 1 mL of 10% homogenate (weight/volume—*w*/*v*). The homogenates were centrifuged at 10,000× *g* for 5 min at 4 °C using an MPW-350R centrifuge (Medical Instruments; Warsaw, Poland) [52].

The concentration of chemerin in the aliquots of the renal tissue homogenates was assayed with the use of the Rat Chemerin ELISA kit (Catalog No. MBS723448) by MyBioSource, Inc. (San Diego, CA, USA) based on the principle of sandwich ELISA technology. In a pre-coated plate, the assay sample and buffer were first incubated with the chemerin-HRP conjugate, followed by the substrate for the HRP enzyme in the wells. The result of the enzyme-substrate reaction was a blue complex. The reaction was stopped by adding a stop solution, causing the solution to turn yellow, and the color intensity was measured spectrophotometrically at 450 nm. The correlation between the color's intensity and chemerin concentration is inverse. The intra- and inter-assay CVs for these measurements were <4% and 2%, respectively.

The concentrations of MIP1a and Bax in the aliquots of the kidney tissue homogenates were assessed with ELISA kits (Catalog No. SEA092Ra and No. SEB343Ra01, respectively) made by Cloud-Clone Corp. (Katy, TX, USA). Standards or samples were added to the wells with a biotin-conjugated specific antibody, and then avidin conjugated to HRP was added to each microplate well and incubated. After the addition of another substrate, the wells that contained the tested proteins changed in color. Following the cessation of the reaction, the change in color was measured spectrophotometrically (450 nm).

The intra- and inter-assay CV for MIP1a measurements were <4.4% and <7.5%, respectively, and <3% and <2% for Bax, respectively.

4.6. Statistical Analysis

All statistical calculations were performed using Statistica 13.3 software (StatSoft, Tulsa, OK, USA), and the data are expressed as a median, 25–75% confidence interval, and minimum and maximum. In the beginning, the normality of the distribution of the data was checked with the Shapiro-Wilk test. Because there was no normal distribution, a nonparametric signed-rank Kruskal-Wallis test with median test was performed to determine whether there were statistically significant ($p < 0.05$) differences between the six groups. If statistically significant differences were found between the six groups, multiple comparisons were carried out to examine between which two groups a statistically significant ($p < 0.05$) difference occurred. A nonparametric Friedman test was performed to test the

results of measurements repeated on the same animals every other month. When the Friedman test showed statistically significant differences ($p < 0.05$) between all time points, the Wilcoxon test was performed to compare paired data.

To calculate the dependence between the values of the measured parameters, a linear regression analysis was performed. The results of this analysis are shown as the β coefficient (this metric represents the percentage of the dependent variable's change for each unit of the independent variable's change), R^2 (shows the percentage of one variable that is responsible for the variability of the other), and the statistical significance (p). A relationship between two variables was acknowledged to be statistically significant at the value of the β coefficient for which $p < 0.05$. Furthermore, dependencies between the parameters measured in the present study and the previously published Cd concentration in the blood, urine, and kidney were analyzed ([17], Table S15).

5. Conclusions

Based on the results of the present study, conducted in an animal model of human lifespan environmental exposure to Cd in industrialized countries, it can be concluded that even low-level repeated intoxication with this toxic heavy metal can result in structural and functional damage to the kidney tubules and glomeruli, creating a risk of this organ injury. The finding in the used experimental model that the unfavorable impact of Cd on kidney function and structure may occur at its concentrations in the urine (0.0852–0.2820 μg/g creatinine) within the lower range of values currently found in the general population worldwide confirms that even low-level environmental exposure to this xenobiotic may pose a risk of damage to the kidney. Moreover, it can be concluded that an intake of polyphenol-rich *A. melanocarpa* berry products during low-to-moderate levels of exposure to Cd may very effectively protect from the nephrotoxic action of this heavy metal. The present study indicates that Aronia products may be an effective strategy for protecting the kidney from damage due to low-to-moderate chronic exposure to Cd.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/ijms241411647/s1.

Author Contributions: conceptualization, N.M.S. and M.M.B.; methodology, N.M.S., M.M.B., J.R. and T.H.; software, N.M.S. and T.H.; formal analysis, N.M.S., M.M.B., J.R. and T.H.; investigation, N.M.S., M.M.B., J.R. and T.H.; resources, M.M.B.; data curation, N.M.S., M.M.B. and T.H.; writing—original draft preparation, N.M.S.; writing—review and editing, M.M.B.; visualization, N.M.S., M.M.B., J.R. and T.H.; supervision, M.M.B.; project administration, M.M.B.; funding acquisition, N.M.S. and M.M.B. All authors have read and agreed to the published version of the manuscript.

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Institutional Review Board Statement: The animal study protocol was approved by the Local Ethics Committee for Animal Experiments in Bialystok (approval no. 60/2009 on 21 September 2009).

Informed Consent Statement: Not applicable.

Data Availability Statement: The data presented in this study are available upon request from the corresponding authors. The data are not publicly available.

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Conflicts of Interest: The authors declare no conflict of interest.

Abbreviations

AAS method	atomic absorption spectrometry method
ACR	albumin concentration in the urine adjusted for creatinine concentration

ALP	alkaline phosphatase
AM	0.1% aqueous extract from the berries of *Aronia melanocarpa* L.
Bax	Bcl2-associated X protein
β2-MG	β2-microglobulin
b.w.	body weight
Cd	cadmium
$CdCl_2 \times 2.5\ H_2O$	cadmium chloride 2.5-hydrate
Cd-MT	cadmium-metallothionein complex
Cd^{2+}	cadmium ions
CV	coefficient of variation
Cu	copper
ELISA	enzyme-linked immunosorbent assay
GFR	glomerular filtration rate
HNO_3	nitric acid
HRP	horseradish peroxidase
KIM-1	kidney injury molecule-1
LOAEL	lowest observed adverse effect level
MIP1a	macrophage inflammatory protein 1 alpha
Mn	manganese
MT	metallothionein
NAC	N-acetyl-L-cysteine
NAG	N-acetyl-β-D-glucosaminidase
PCR	total protein concentration in the urine adjusted for creatinine concentration
SD	standard deviation
w.w.	wet weight
Zn	zinc

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Table S1. The concentration of kidney injury molecule-1 (KIM-1) and β2-microglobulin (β2-MG) in the urine of female rats.

Group	Experiment Duration			
	3 Months	10 Months	17 Months	24 Months
	KIM-1 (pg/mg Creatinine)			
Control	96.71	90.60	179.4	185.7
	32.22 – 222.6	32.04 – 124.6	26.29 – 212.5	131.2 – 239.3
AM	171.9	97.79	254.1	260.2
	44.00 – 389.7	58.20 – 152.2	78.49 – 368.2	161.7 – 293.3
Cd_1	509.8 ^{a*}	240.8 ^{a*}	439.3 ^{a†}	360.5
	200.8 - 1754	149.0 – 419.2	273.6 – 549.9	279.0 – 752.6
Cd_1+AM	69.65 ^{c*}	84.64 ^{c*}	149.7 ^{c†}	159.7 ^{c†}
	9.144 – 327.2	54.10 – 138.3	107.6 – 222.3	101.9 – 197.2
Cd_5	544.0 $^{a*\ d*}$	876.8 $^{a\ddagger\ b\ddagger\ d\ddagger}$	463.3 $^{a\dagger\ d\dagger}$	418.7 $^{a*\ d\dagger}$
	190.4 – 834.1	569.2 - 1441	330.8 – 1050.8	363.4 – 525.5
Cd_5+AM	55.39 $^{c\ddagger\ e\ddagger}$	109.9 ^{e*}	229.2 ^{e*}	141.4 $^{c\dagger\ e\ddagger}$
	25.19 – 143.2	101.1 – 157.7	106.8 – 300.5	109.4 – 176.9
	β2-MG (ng/mg Creatinine)			
Control	2.114	4.803	4.381	4.688
	1.353 – 5.483	0.543 – 6.743	0.740 – 5.816	2.982 – 5.715
AM	2.131	2.663	4.494	4.965
	1.590 – 3.671	1.486 – 4.713	1.958 – 6.402	3.521 – 5.943
Cd_1	2.780	4.545	7.119 $^{a*\ b*}$	10.24 $^{a\dagger\ b*}$
	1.495 – 5.356	2.487 – 7.624	5.876 – 13.95	7.430 – 20.92
Cd_1+AM	3.480	4.309	4.561 ^{c*}	6.145 ^{c*}
	1.121 – 5.045	1.038 – 6.561	2.806 – 6.002	2.164 – 8.531
Cd_5	2.661	5.557	11.23 $^{a\dagger\ b\dagger\ d\ddagger}$	16.94 $^{a\dagger\ b\ddagger\ d\ddagger}$
	2.051 – 5.192	2.012 – 12.84	10.02 – 20.14	11.40 – 23.44
Cd_5+AM	2.580	2.542	4.917 ^{e†}	5.044 $^{c*\ e*}$
	1.702 – 2.929	1.196 – 8.299	2.848 – 7.005	2.209 – 6.278

The animals were treated with cadmium (Cd) in the diet at the concentration of 0, 1, or 5 mg/kg (Control, Cd_1, and Cd_5 groups) and/or 0.1% extract from the berries of *Aronia melanocarpa* L. (AM, Cd_1+AM, and Cd_5+AM groups) for 3, 10, 17, and 24 months. Data are shown as a median and minimum and maximum values for eight animals (except for seven females in the AM, Cd_1, and Cd_5 groups after 24 months). Statistically significant differences (Kruskal–Wallis test) compared to: a—Control group; b—AM group; c—Cd_1 group; d—Cd_1+AM group, and e—Cd_5 group, where * $p < 0.05$, † $p < 0.01$, and ‡ $p < 0.001$, are marked.

Table S2. The concentration of β2-microglobulin (β2-MG) in the urine of female rats evaluated every other month during the 24-month study.
The animals were treated with cadmium (Cd) in the diet at the concentration of 0, 1, or 5 mg/kg (Control, Cd_1, and Cd_5 groups) and/or 0.1% extract from the berries of *Aronia melanocarpa* L. (AM, Cd_1+AM, and Cd_5+AM groups) for 24 months. Data are shown as a median and minimum and maximum values for eight animals (except for seven females in the AE, Cd_1, and Cd_5 groups after 20, 22, and 24 months). Statistically significant differences (Kruskal–Wallis test) compared to: a—Control group; b—AM group; c—Cd_1 group; d—Cd_1+AM group; and e—Cd_5 group. Wilcoxon test was performed to compare data between particular two time points in the same animals during the 24-month study. * $p < 0.05$, † $p < 0.01$, and ‡ $p < 0.001$.

Months	Control	AM	Cd_1	Cd_1+AM	Cd_5	Cd_5+AM
0	2.368	2.319	2.482	2.261	2.221	2.241
	1.606 – 3.064	1.227 – 3.194	1.660 – 3.419	1.736 – 2.964	1.608 – 2.921	1.637 – 2.861
2	3.557	2.862	3.148	3.567	3.886	2.995
	0.076 – 5.407	0.592 – 7.981	2.063 – 5.151	1.338 – 3.968	1.040 – 4.431	1.741 – 4.676
4	2.963	2.006	2.423	2.380	2.123	2.904
	1.180 – 4.587	1.197 – 3.330	1.088 – 5.335	1.657 – 4.680	0.484 – 6.224	0.052 – 8.989
6	3.801	4.025	5.357	2.185 b† c‡	3.422	2.462 c†
	2.607 – 6.724	2.932 – 5.064	3.743 – 8.691	0.795 – 2.392	1.908 – 4.717	1.141 – 4.894
8	2.967	2.811	5.071	2.403 c†	5.095 b* d†	2.009 c‡ e‡
	2.171 – 4.023	1.106 – 4.319	3.948 – 5.886	1.322 – 3.717	4.085 – 6.042	1.433 – 2.368
10	3.889	2.930	7.402 b†	4.740	6.113	2.798 c*
	2.597 – 4.394	1.635 – 5.185	4.402 – 8.386	1.142 – 7.218	3.213 – 8.032	1.316 – 6.469
12	3.970	3.474	8.368 b‡	3.242 c‡	8.842 a* b‡ d‡	3.499 c‡ e‡
	3.288 – 4.802	1.708 – 5.445	7.059 – 11.98	2.621 – 7.370	6.893 – 12.42	1.176 – 5.669
14	4.321	5.047	8.500 a*	4.794	8.722 a*	3.508 c‡ e‡
	2.106 – 6.111	2.446 – 7.757	5.665 – 13.40	3.870 – 7.917	6.301 – 10.72	1.687 – 5.491
16	3.983	4.085	7.992 a† b*	4.677 c*	11.07 a‡ b† d†	4.907 e*
	1.673 – 5.211	1.780 – 5.820	6.389 – 15.41	2.551 – 6.252	9.514 – 20.22	5.879 – 2.589
18	4.802	5.142	12.39 a‡ b†	8.128 c*	11.17 a† b*	6.426 c† e*
	4.128 – 10.11	4.539 – 5.846	10.50 – 20.66	4.518 – 9.939	9.380 – 19.75	5.195 – 7.215
20	5.339	5.318	10.42 a*	4.099 c†	10.81 a*	5.029 e† c*
	3.308 – 7.994	3.893 – 7.017	8.405 – 17.71	2.696 – 7.795	8.956 – 15.18	3.372 – 6.585
22	5.936	5.378	17.01 a†	6.033 c†	16.43 a† d†	7.106 e*
	4.162 – 7.605	3.808 – 6.788	10.11 – 21.99	3.961 – 8.432	11.36 – 31.46	5.040 – 9.443
24	4.688	4.965	10.24 a† b*	6.145 c*	16.94 a‡ b† d†	5.044 c* e*
	2.982 – 5.715	3.521 – 5.943	7.430 – 20.92	2.164 – 8.531	11.40 – 23.44	2.209 – 6.278
Friedman Test	$p < 0.001$	$p < 0.001$	$p < 0.001$	$p < 0.001$	$p < 0.001$	$p < 0.001$
Wilcoxon Test	0 – 6, 10…24 *	0 – 6, 12…24 *	0 – 6…24 *	0 – 2, 10…24 *	0 – 2, 6 *	0 – 2, 12, 16 *
	2 – 22 *	4 – 6, 10…24 *	2 – 6…24 *	2 – 6, 14…18, 22, 24 *	0 – 8…24 †	0 – 16…24 †
	4 – 12, 18…24 *	6 – 10, 18…22 *	4 – 6…24 *	4 – 14… 24 *	2 – 8, 12…24 †	2 – 8, 20, 24 *
	6 – 18, 22 *	8 – 14, 18…24 *	6 – 12, 16…24 *	6 – 10…24 †	2 – 10 *	2 – 18, 22 †
	8 – 10, 12, 18…24 *	10 – 18…24 *	8 – 12…24 *	8 – 10 *	4 – 8, 10 *	6 – 16, 20, 24 *
	10 – 18…22 *	12 – 14, 18…24 *	10 – 12 *	8 – 12…24 †	4 – 12…24 †	6 – 18, 22 †
	12 – 18…22 *	16 – 20, 22 *	10 – 18…24 *	10 – 18 *	6 – 10 *	8 – 10, 12, 14 *
	16 – 20, 22 *		12 – 18…24 *	12 – 18 †	6 – 8, 12…24 †	8 – 16…24 †
	22 – 24 *		14 – 18, 22 *	12 – 22 *	8 – 12…24 †	10 – 18 †
			16 – 18, 22 *	14 – 18 *	10 – 12, 16…24 †	10 -22 *
			20 – 22	16 – 18, 22 †	10 – 14 *	12 – 18, 22 †
			20 – 22 *	18 – 20 †	12 – 16, 22, 24 *	12 – 20, 24 *
				20 – 22 †	14 – 16, 22, 24 †	14 – 16, 20 *
					14 – 18, 20 *	14 – 18, 22 †
					16 – 24 *	16 – 18, 22 †
					18 – 22, 24 *	18 – 24 *
					20 – 22, 24 *	20 – 22 †
						22 – 24*

Table S3. The activities of N-acetyl-β-D-glucosaminidase (NAG) and alkaline phosphatase (ALP) in the urine of female rats.

Group	Experiment Duration			
	3 Months	10 Months	17 Months	24 Months
		NAG (U/L)		
Control	85.25	126.6	113.9	137.6
	60.38 – 114.1	68.55 – 166.7	87.03 – 156.6	68.78 – 154.8
AM	95.34	111.0	123.9	116.6
	63.27 – 116.6	61.20 – 145.4	77.49 – 137.1	76.75 – 160.8
Cd_1	104.5	636.0 ^{b‡}	520.09 $^{a‡ b*}$	3056 $^{a* b*}$
	53.87 – 132.5	380.7 – 801.9	387.9 – 776.9	2971 – 4112
Cd_1+AM	93.65	141.7	121.7 ^{c†}	117.1 ^{c†}
	67.78 – 116.0	92.99 – 230.1	90.41 – 162.7	93.61 – 141.5
Cd_5	93.46	800.4 $^{a† b‡ d*}$	596.9 $^{a† b* d†}$	3453 $^{a* b† d†}$
	75.54 – 120.8	347.7 – 935.5	496.0 – 769.4	2999 – 4836
Cd_5+AM	92.83	108.1 $^{c† e‡}$	129.7 ^{c*}	131.9 ^{e†}
	41.47 – 116.5	46.10 – 173.3	94.98 – 152.2	96.65 – 148.9
		ALP (U/L)		
Control	39.22	42.45	36.82	38.08
	27.41 – 68.93	10.90 – 70.19	30.52 – 71.00	24.11 – 113.0
AM	27.16	36.70	37.81	34.00
	16.80 – 57.10	24.81 – 79.00	35.84 – 41.36	28.71 -102.0
Cd_1	31.99	146.9 $^{a‡ b‡}$	96.05 $^{a‡ b‡}$	149.9 ^{a*}
	23.80 – 69.30	99.75 – 192.0	69.84 – 139.7	105.7 – 208.8
Cd_1+AM	32.08	60.66	51.46	49.60
	27.57 – 35.84	31.46 - 79.95	43.75 – 59.18	26.10 – 120.0
Cd_5	35.84	185.1 $^{a‡ b‡ d*}$	111.7 $^{a† b†}$	278.0 $^{a‡ b† d†}$
	27.57 – 71.50	90.98 – 303.3	89.60 – 127.6	181.6 – 375.2
Cd_5+AM	33.36	47.60 ^{e†}	36.76 $^{c‡ e‡}$	39.06 ^{e†}
	13.51 – 56.90	25.38 – 102.0	34.92 – 37.68	22.10 – 70.36

The animals were treated with cadmium (Cd) in the diet at the concentration of 0, 1, or 5 mg/kg (Control, Cd_1, and Cd_5 groups) and/or 0.1% extract from the berries of *Aronia melanocarpa* L. (AM, Cd_1+AM, and Cd_5+AM groups) for 3, 10, 17, and 24 months. Data are shown as a median and minimum and maximum values for eight animals (except for seven females in the AM, Cd_1, and Cd_5 groups after 24 months). Statistically significant differences (Kruskal–Wallis test) compared to: a—Control group; b—AM group; c—Cd_1 group; d—Cd_1+AM group; and e—Cd_5 group, where * $p < 0.05$, † $p < 0.01$, and ‡ $p < 0.001$, are marked.

Table S4. The activity of N-acetyl-β-D-glucosaminidase (NAG) in the urine of female rats evaluated every other month during the 24-month study.

Months	Control	AM	Cd_1	Cd_1+AM	Cd_5	Cd_5+AM
0	16.75 8.988 – 19.91	16.75 9.913 – 24.13	17.14 14.31 – 21.34	16.68 11.99 – 19.90	16.43 7.280 – 19.91	16.75 9.781 – 20.14
2	78.08 22.65 – 104.9	89.21 47.80 – 103.3	61.01 14.57 – 85.23	69.08 42.79 – 85.69	72.73 65.20 – 93.12	39.17 27.11 – 71.12
4	95.50 7.445 – 151,9	74.08 3.537 – 169.0	108.0 92.17 – 143.5	106.4 49.72 – 169.1	106.5 36.47 – 180.6	86.92 30.03 – 132.6
6	115.3 89.10 – 123.9	63.15 31.55 – 120.5	117.1 72.58 – 150.7	114.5 62.79 – 128.8	124.6 84.56 – 144.5	110.0 75.24 – 133.7
8	64.63 11.74 – 150.8	36.21 10.57 – 103.3	116.6 b† 89.28 – 189.4	92.63 22.08 – 193.4	92.13 73.36 – 128.4	105.4 29.44 – 142.6
10	106.6 88.43 – 198.2	69.69 39.73 – 121.4	617.1 b† 342.5 – 890.7	123.3 49.66 – 287.0	710.4 a* b† d* 312.8 – 841.6	81.04 c† e† 34.56 – 261.3
12	127.6 63.12 – 173.4	111.5 52.14 – 133.3	391.0 a† b† 345.1 – 479.5	65.10 c‡ 46.72 – 99.45	406.4 a† b† d‡ 354.6 – 521.3	112.3 e* 93.00 – 143.5
14	101.8 66.93 – 128.4	86.71 53.61 – 148.7	406.7 a* b* 321.1 – 487.0	72.89 c‡ 44.41 – 85.14	434.4 a* b† d‡ 397.2 – 553.5	97.64 e* 74.00 – 111.5
16	104.6 69.24 – 127.0	78.09 50.17 – 138.1	490.0 a* b‡ 371.7 – 745.5	99.79 c* 58.30 – 117.3	572.7 a† b‡ d† 475.9 – 738.2	94.84 e† 90.20 – 125.4
18	107.3 59.32 – 141.0	134.1 107.6 – 195.2	507.1 a‡ 341.7 – 579.6	126.1 c* 90.37 – 159.4	477.9 a‡ d* 441.9 – 578.8	126.9 c* e† 93.73 – 180.2
20	105.5	122.7	484.4 a*	74.04 c‡	542.2 a† d‡	97.98 c* e†
22	79.10 – 127.1 127.5 118.4 – 171.9	78.80 – 139.7 129.9 107.7 – 192.3	403.1 – 548.8 2319 a* b* 1990 – 3188	61.08 – 124.8 132.4 c* 97.44 – 150.7	420.9 – 570.5 3132 a† b† d† 2727 - 4396	92.13 – 133.7 156.4 e* 122.5 – 210.8
24	137.6 68.78 – 154.8	116.6 76.75 – 160.8	3056 a* b* 2971 – 4112	117.1 c* 93.61 – 141.5	3453 a* b† d† 2999 – 4836	131.9 e† 96.65 – 148.9
Friedman Test	$p < 0.001$	$p < 0.001$	$p < 0.001$	$p < 0.001$	$p < 0.001$	$p < 0.001$
Wilcoxon Test	0 – 2…24 † 2 – 6, 10, 22 ‡ 2 – 12, 16, 20, 24 * 6 – 22 † 8 – 22 * 14 – 22 * 16 – 22 † 16 – 24 * 18 – 22 † 20 – 22 *	0 – 2, 6, 10…18 ‡ 0 – 4, 8, 20…24 * 2 – 18 ‡ 2 – 20…24 * 4 – 18, 22 * 6 – 18 † 6 – 20, 22 * 8 – 12, 16, 20…24 * 8 – 18 † 10 – 18 † 10 – 20…24 * 12 – 18 * 14 – 18 * 16 – 18…24 *	0 – 2, 20…24 * 0 – 4…18 † 2 – 4…18 † 2 – 20…24 * 4 – 10…18 ‡ 4 – 20…24 * 6 – 10…18 ‡ 6 – 20…24 * 8 – 10…18 † 8 – 20…24 * 10 – 12, 14, 18, 22, 24* 12 – 16…24 * 14 – 20…24 * 16 – 22, 24 * 18 – 22, 24 * 20 – 22, 24 * 22 – 24 *	0 – 2…24 † 2 – 4…10, 16, 20 * 2 – 18, 22, 24 † 4 – 12, 14 ‡ 6 – 12, 14 † 6 – 20 * 10 – 12 ‡ 10 – 14 * 12 – 16, 18, 22, 24 † 14 – 16 * 14 – 18, 22, 24 † 16 – 18, 24 * 16 – 22 ‡ 18 – 20 * 20 – 22 ‡ 20 – 24 *	0 – 2…18 † 0 – 20…24 * 2 – 4, 20…24 * 2 – 6…18 ‡ 4 – 10…18 † 4 – 20…24 * 6 – 10…18 † 6 – 20…24 * 8 – 10…18 † 8 – 20…24 * 10 – 12, 14, 18…24 * 12 – 16, 22, 24 * 14 – 16, 22, 24 * 16 – 22, 24 * 18 – 22, 24 * 20 – 22, 24 * 22 – 24 *	0 – 2…24 † 2 – 4, 8, 10 * 2 – 6, 12…24 † 4 – 12, 18, 22 † 4 - 16, 20, 24 * 6 – 22 † 8 – 22 † 12 – 14, 16 * 12 – 22 † 14 – 18, 24 * 14 – 22 † 16 – 18, 24 * 16 – 22 ‡ 18 – 22 * 20 – 22 ‡ 20 – 24 * 22 – 24*

The animals were treated with cadmium (Cd) in the diet at the concentration of 0, 1, or 5 mg/kg (Control, Cd_1, and Cd_5 groups) and/or 0.1% extract from the berries of *Aronia melanocarpa* L. (AM, Cd_1+AM, and Cd_5+AM groups) for 24 months. Data are shown as a median and minimum and maximum values for eight animals (except for seven females in the AE, Cd_1, and Cd_5 groups after 20, 22, and 24 months). Statistically significant differences (Kruskal–Wallis test) compared to: a—Control group; b—AM group; c—Cd_1 group; d—Cd_1+AM group; and e—Cd_5 group. Wilcoxon test was performed to compare data between particular two time points in the same animals during the 24-month study. * $p < 0.05$, † $p < 0.01$, and ‡ $p < 0.001$.

Table S5. The activity of alkaline phosphatase (ALP) in the urine of female rats evaluated every other month during the 24-month study.

Months	Control	AM	Cd_1	Cd_1+AM	Cd_5	Cd_5+AM
0	24.34	22.96	23.64	22.06	24.34	24.34
	15.93 – 34.90	15.14 – 34.90	13.59 – 33.08	19.53 – 33.84	11.03 – 30.33	19.53 – 33.94
2	24.42	27.57	22.06	13.39	38.59	33.08
	22.06 – 33.08	22.06 – 55.14	11.03 – 33.08	11.03 – 22.06	21.31 – 88.22	22.48 – 55.14
4	26.77	29.97	40.00	42.36	128.2 a* b*	55.14
	19.30 – 46.87	15.16 – 49.63	30.33 – 52.38	11.03 – 68.93	38.59 – 140.6	5.514 – 204
6	27.57	31.25	59.67	39.21	119.5 a‖ b‖	79.55 a*b*
	22.06 – 38.60	24.81 – 43.25	27.57 – 132.3	32.45 – 82.71	66.17 – 187.5	41.36 – 173.7
8	39.61	41.36	73.80	55.14	125.3 a*	27.57 e‡
	13.79 – 81.96	27.57 – 82.71	44.11 – 121.3	13.78 – 132.4	55.14 – 198.5	16.54 – 82.71
10	40.38	37.22	114.2 a* b‡	54.22	178.5 a‡ b‡ d*	48.77 e‡
	13.25 – 56.97	24.81 – 42.42	65.10 – 165.0	34.16 – 77.20	129.0 – 196.0	28.53 – 92.00
12	28.27	27.57	97.13 a* b*	44.72	117.2 a‡ b‡	34.46 c* e‡
	24.81 – 60.65	18.55 – 58.47	42.81 – 139.9	24.81 – 65.42	77.19 – 159.9	5.514 – 57.89
14	32.43	36.18	89.76 a*	42.79	104.5 a‡ b*	25.23 c‡ e‡
	13.78 – 68.93	23.78 – 52.38	44.11 – 170.9	27.57 – 79.95	86.66 – 130.0	19.21 – 44.22
16	33.32	31.89	85.75 a* b*	39.18	121.0 a‡ b‡ d*	30.63 c‖ e‖
	29.87 – 59.17	29.87 – 36.33	58.20 – 116.5	29.10 – 60.33	101.1 – 125.6	29.10 – 31.40
18	36.53	22.52	95.12 a* b*	38.60	117.2 a‡ b‡	17.92 c‖ e‖
	21.14 – 44.06	20.22 – 45.03	50.55 – 133.3	25.73 – 42.81	96.50 – 160.8	8.271 – 20.72
20	38.14	34.00	95.58 a*	43.19	108.2 a‡	33.84 c‡ e‡
	31.25 – 51.46	22.06 – 61.57	70.76 – 136.8	21.14 – 66.17	84.03 – 119.7	20.22 – 44.11
22	35.54	30.65	91.29 a*	44.11	123.0 a‡ b‡ d‡	35.03 e‡
	26.65 – 44.06	22.98 – 55.14	61.57 – 123.2	20.22 – 71.16	92.41 – 124.8	22.06 – 77.20
24	38.08	34.00	149.9 a*	49.60	278.0 a‡ b‡ d‡	39.06 e‡
	24.11 – 113.0	28.71 – 102.0	105.7 – 208.8	26.10 – 120.0	181.6 – 375.2	22.10 – 70.36
Friedman Test	$p < 0.001$	$p = 0.20$	$p < 0.001$	$p < 0.001$	$p < 0.001$	$p < 0.001$
Wilcoxon Test	0 – 10, 12, 22, 24 * 0 – 16, 20 † 2 – 10, 16, 20 * 2 – 22 † 6 – 10, 20 *		0 – 4, 8…18 † 0 – 6, 20…24 * 2 – 4, 8…18 † 2 – 6, 20…24 * 4 – 8, 12, 20…24 * 4 – 10, 14…18 † 6 – 24 * 8 – 10, 24 * 10 – 16 * 12 – 24 * 14 – 24 * 16 – 24 * 18 – 24 * 22 – 24 *	0 – 2, 8, 12, 16…22 * 0 – 6, 10, 14, 24 † 2 – 4, 20 * 2 – 6…18, 22, 24 † 8 – 22 * 10 – 18 *	0 – 4…18 † 0 – 20…24 * 2 – 4, 20…24 * 2 – 6…18 † 4 – 10, 24 * 6 – 24 * 8 – 24 * 10 – 12, 18…24 * 10 – 14, 16 † 12 – 24 * 14 – 24 † 16 – 24 * 18 - 24 * 20 – 24 * 20 – 24 *	0 – 2, 16, 20, 24* 0 – 6, 10, 18 † 2 – 6 * 2 – 18 † 6 – 12, 20…24 * 6 – 14…18 † 8 – 18 * 10 – 14, 16 * 10 – 18 † 14 – 18 † 14 – 24 * 16 – 18 † 18 – 20…24 †

The animals were treated with cadmium (Cd) in the diet at the concentration of 0, 1, or 5 mg/kg (Control, Cd_1, and Cd_5 groups) and/or 0.1% extract from the berries of *Aronia melanocarpa* L. (AM, Cd_1+AM, and Cd_5+AM groups) for 24 months. Data are shown as a median and minimum and maximum values for eight animals (except for seven females in the AE, Cd_1, and Cd_5 groups after 20, 22, and 24 months). Statistically significant differences (Kruskal–Wallis test) compared to: a—Control group; b—AM group; c—Cd_1 group; d—Cd_1+AM group; and e—Cd_5 group. Wilcoxon test was performed to compare data between particular two time points in the same animals during the 24-month study. * $p < 0.05$, † $p < 0.01$, and ‖ $p < 0.001$.

Table S6. The concentrations of albumin (ACR) and total protein (PCR) adjusted for creatinine concentration in the urine of female rats.

Group	Experiment Duration 3 Months	10 Months	17 Months	24 Months
		ACR (mg/mg Creatinine)		
Control	6.369	8.282	8.837	9.537
	3.541 – 8.987	6.359 – 13.17	7.225 – 9.645	7.461 – 11.32
AM	6.658	8.888	7.668	9.006
	4.004 – 9.908	5.702 – 10.91	4.220 – 10.43	7.223 – 12.73
Cd_1	8.167	10.04	13.48 a* b*	21.34 a*
	5.491 – 10.87	6.501 – 14.56	11.14 – 22.13	17.06 – 26.69
Cd_1+AM	7.732	9.038	13.94 a* b*	12.18 c*
	6.244 – 8.377	2.602 – 11.75	13.24 – 20.07	5.142 – 16.59
Cd_5	9.400	9.500	22.45 a‡ b‡	22.56 a† d*
	6.156 – 11.13	6.810 – 17.24	20.65 23.80	20.95 – 25.47
Cd_5+AM	7.355	5.716 c† d* e†	11.79 e*	11.45 c‡ e†
	4.764 – 9.541	3.375 – 7.050	9.600 – 13.74	6.473 – 13.38
		PCR (mg/mg Creatinine)		
Control	6.194	11.12	12.27	11.29
	3.634 – 7.743	7.831 – 14.85	9.063 – 17.44	8.320 – 12.56
AM	7.494	9.020	9.230	11.68
	2.978 – 13.09	6.737 – 13.96	8.375 – 23.80	10.36 – 20.00
Cd_1	7.329	38.00 a* b†	38.10 a* b†	64.28 a*
	3.473 – 12.99	27.61 – 41.28	33.20 – 61.49	56.08 – 82.50
Cd_1+AM	8.175	9.500 c*	12.01 c*	8.986 c‡
	3.454 – 14.75	6.974 – 15.08	7.137 – 17.40	4.179 – 13.28
Cd_5	6.218	42.14 a† b‡ d‡	42.06 a* b† d†	63.09 a* d‡
	3.893 – 8.271	29.20 – 55.61	25.51 – 79.63	55.44 – 79.04
Cd_5+AM	8.289	12.40 e*	11.19 c† e†	10.71 e†
	6.380 – 12.31	11.35 – 14.24	8.120 – 13.98	8.080 – 14.72

The animals were treated with cadmium (Cd) in the diet at the concentration of 0, 1, or 5 mg/kg (Control, Cd_1, and Cd_5 groups) and/or 0.1% extract from the berries of *Aronia melanocarpa* L. (AM, Cd_1+AM, and Cd_5+AM groups) for 3, 10, 17, and 24 months. Data are shown as a median and minimum and maximum values for eight animals (except for seven females in the AM, Cd_1, and Cd_5 groups after 24 months). Statistically significant differences (Kruskal–Wallis test) compared to: a—Control group; b—AM group; c—Cd_1 group; d—Cd_1+AM group; and e—Cd_5 group, where * $p < 0.05$, † $p < 0.01$, and ‡ $p < 0.001$, are marked.

Table S7. The concentration of albumin in the urine adjusted for creatinine concentration (ACR) of female rats evaluated every other month during the 24-month study.

Months	Control	AM	Cd_1	Cd_1+AM	Cd_5	Cd_5+AM
0	1.690	1.677	1.539	1.740	1.543	1.412
	1.152 – 2.939	1.025 – 2.316	0.986 – 2.940	0.982 – 2.316	0.931 – 2.155	0.952 – 2.180
2	6.186	6.156	6.475	6.623	6.818	6.578
	3.591 – 7.423	4.166 – 9.156	3.630 – 9.276	3.926 – 8.001	5.477 – 9.266	3.824 – 8.632
4	6.590	7.872	6.367	3.991	11.32	6.754
	4.300 – 8 833	3.551 – 8.703	3.834 – 9.528	2.715 – 11.20	6.790 – 15.62	3.761 – 9.593
6	6.745	7.616	14.06 a*	5.647 c‡	10.08 d†	5.618 c‡ e†
	5.486 – 8.882	5.491 – 8.236	10.33 – 14.91	4.516 – 6.288	8.112 – 11.41	3.950 – 7.141
8	8.025	7.013	15.95 b†	9.037	13.76 b†	7.073 c† e†
	4.945 – 13.68	4.122 – 9.978	8.400 – 24.11	6.491 – 15.03	10.07 – 16.75	5.664 – 8.014
10	7.529	8.080	10.54	9.490	9.974	6.001 c† d* e†
	5.781 – 11.98	5.183 – 9.920	6.826 – 15.29	2.732 – 12.34	7.151 – 18.10	3.544 – 7.403
12	9.350	6.675	10.20	11.65 b*	13.38 b†	7.914 a*
	5.614 – 14.70	4.185 – 9.656	5.433 – 16.38	9.215 – 14.58	9.778 – 17.77	5.138 – 11.54
14	11.81	8.319	11.75	12.80 b*	14.68 b*	9.824 e*
	8.065 – 17.17	6.114 – 12.50	6.720 – 15.86	11.30 – 16.21	8.864 – 25.45	6.527 – 11.93
16	9.126	8.408	12.67	11.14	20.70 a‡ b†	9.534 e†
	3.253 – 11.46	4.773 – 17.37	7.988 – 24.71	6.519 – 23.65	16.73 – 23.64	4.875 – 13.17
18	8.345	9.085	14.44 a† b*	9.461 c*	16.18 a† b* d*	9.313 e*
	4.715 – 13.55	6.327 – 15.42	12.31 – 21.86	5.692 – 12.60	13.55 – 20.21	7.799 – 13.72
20	9.239	9.781	18.86 a*	10.10 c*	22.45 a† b*	11.40 e*
	5.389 – 13.16	3.432 – 20.66	14.37 – 20.77	8.035 – 20.38	16.84 – 25.87	11.07 – 17.16
22	9.626	9.315	22.77 a‡ b†	11.96 c*	22.72 a‡ b†	10.62 c* e*
	7.147 – 11.48	6.073 – 15.13	16.10 – 25.90	10.68 – 15.92	17.39 – 28.61	9.134 – 17.61
24	9.537	9.006	21.34 a*	12.81 c*	22.56 a† d*	11.45 a‡ e†
	7.461 – 11.32	7.223 – 12.73	17.06 – 26.69	5.142 – 16.59	20.95 – 25.47	6.473 – 13.38
Friedman Test	$p < 0.001$	$p < 0.001$	$p < 0.001$	$p < 0.001$	$p < 0.001$	$p < 0.001$
Wilcoxon Test	0 – 2…24 † 2 – 6…12, 16, 22 * 2 – 14, 18, 24 † 4 – 14, 22 * 4 – 24 † 6 – 14, 22, 24 * 10 – 14 * 12 – 14 * 14 – 16 *	0 – 2…18 † 0 – 20…24 * 2 – 16, 20, 22 * 2 – 18 † 4 – 24 * 6 - 22, 24 * 8 – 22, 24 * 10 – 22, 24 * 12 – 14, 22, 24 *	0 – 2…18 † 0 – 20…24 * 2 – 6, 8, 14…18 † 2 – 10, 20…24 * 4 – 6, 16, 18 † 4 – 8, 10, 14, 20…24 * 6 – 20…24 * 8 – 12 † 10 – 20…24 * 12 – 18…24 * 14 – 18…24 * 16 – 20…24 * 18 – 24 * 20 – 22, 24 *	0 – 2…24 † 2 – 8, 20 * 2 – 12…22 † 4 – 8, 12, 14, 22, 24 † 4 – 10, 16…20 * 6 – 8, 12…22 † 6 – 10, 24 * 8 – 14 * 10 – 14 † 10 – 22 * 12 – 18 * 14 – 18 † 18 – 22 *	0 – 2…18 † 0 – 20…24 * 2 – 4, 10, 20…24 * 2 – 6, 8, 12…18 † 4 – 16 † 4 – 18…24 * 6 – 8, 14, 20…24 * 6 – 12, 16, 18 † 8 – 16 † 8 – 18… 24 * 10 – 16 † 10 – 20…24 * 12 – 16 † 12 – 18…24 * 14 – 16 * 16 – 18 * 18 – 20…24 *	0 – 2…24 † 2 – 14, 18…22 † 2 – 24 * 4 - 14, 18, 24 * 4 – 20, 22 † 6 – 8, 12, 16 * 6 – 14, 18…24 † 8 – 10, 14, 24 * 8 – 18…22 † 10 – 14, 18…24 † 10 – 16 * 12 – 20 † 12 – 22 * 16 – 20 *

The animals were treated with cadmium (Cd) in the diet at the concentration of 0, 1, or 5 mg/kg (Control, Cd_1, and Cd_5 groups) and/or 0.1% extract from the berries of *Aronia melanocarpa* L.. (AM, Cd_1+AM, and Cd_5+AM groups) for 24 months. Data are shown as a median and minimum and maximum values for eight animals (except for seven females in the AE, Cd_1, and Cd_5 groups after 20, 22, and 24 months). Statistically significant differences (Kruskal–Wallis test) compared to: a—Control group; b—AM group; c—Cd_1 group; d—Cd_1+AM group; and e—Cd_5 group. Wilcoxon test was performed to compare data between particular two time points in the same animals during the 24-month study. * $p < 0.05$, † $p < 0.01$, and ‡ $p < 0.001$.

Table S8. The concentration of total protein (PCR) in the urine of female rats evaluated every other month during the 24-month study.

Months	Control	AM	Cd_1	Cd_1+AM	Cd_5	Cd_5+AM
0	3.457	3.464	3.706	3.451	3.755	3.566
	2.996 – 5.897	2.669 – 4.770	2.758 – 4.726	2.990 – 4.726	2.332 – 4.628	2.700 – 4.503
2	7.575	7.323	10.26	8.623	7.220	6.941
	6.200 – 15.96	4.653 – 39.63	8.190 – 22.09	6.308 – 24.71	3.381 – 18.31	1.722 – 15.99
4	9.005	5.190	10.24	8.347	23.01 b*	10.86
	4.391 – 18.96	3.104 – 20.35	3.055 – 26.13	2.687 – 10.90	4.868 – 33.42	5.875 – 27.12
6	6.932	9.714	22.46 a†	7.557 c*	15.79 a*	8.794
	3.107 – 10.56	1.963 – 13.50	8.580 – 27.85	4.416 – 16.33	7.045 – 20.35	3.129 – 26.57
8	9.280	10.21	45.34 a*	10.22 c*	32.94 a*	3.559 c‡ e‡
	4.227 – 15.12	3.034 – 32.08	29.34 – 78.80	4.046 – 20.79	30.01 – 48.38	2.689 – 5.184
10	12.55	8.712	38.32 a* b†	14.34 c*	35.72 a* b†	13.15 c* e*
	6.768 – 16.56	6.096 – 12.14	26.16 – 59.32	8.064 – 18.96	28.80 – 43.36	7.680 – 18.96
12	10.80	6.435	45.42 a* b†	11.07 c*	46.10 a* b†	5.385 c‡ e‡
	6.272 – 12.28	4.206 – 12.68	39.28 – 68.00	8.240 – 15.44	38.44 – 57.68	2.614 – 7.626
14	10.46	7.500	44.82 a* b‡	11.82 c*	49.72 a* b‡	6.280 c‡ e‡
	8.040 – 11.20	4.560 – 11.04	38.96 – 51.52	5.760 – 14.36	37.40 – 64.12	4.080 – 9.200
16	11.30	9.900	43.44 a* b‡	11.27 c*	51.08 a† b‡ d†	10.71 c* e†
	6.660 – 16.16	5.720 – 12.00	37.12 – 52.60	4.730 – 14.69	36.00 – 67.57	6.943 – 14.40
18	12.32	11.68	51.60 a* b†	11.98 c*	55.83 a* b† d*	11.48 c‡ e†
	8.680 – 16.36	6.560 – 15.48	36.96 – 87.12	9.440 – 16.20	43.04 – 67.20	4.320 – 14.80
20	11.76	14.20	42.96 a*	11.14 c*	57.22 a† b* d†	11.46 c* e†
	8.120 – 16.28	8.760 – 16.40	38.05 – 81.64	10.44 – 16.20	42.96 – 66.32	5.880 – 19.56
22	11.48	12.20	60.36 a*	11.22 c*	57.62 a* d†	10.36 c† e‡
	9.560 – 16.56	10.92 – 16.60	36.65 – 82.76	9.160 – 13.92	47.96 – 72.76	6.920 – 14.14
24	11.29	11.68	64.28 a*	8.986 c‡	63.09 a* d‡	10.71 c† e†
	8.320 – 12.56	10.36 – 20.00	56.08 – 82.50	4.179 – 13.28	55.44 – 79.04	8.080 – 14.72
Friedman Test	$p < 0.001$	$p < 0.001$	$p < 0.001$	$p < 0.001$	$p < 0.001$	$p < 0.001$
Wilcoxon Test	0 – 2, 6…24 † 2 – 4 * 2 – 10 † 2 – 18 * 6 – 10…20 * 6 – 22, 24 † 8 – 10 † 14 – 18, 22 * 16 – 18…24 *	0 – 2, 10…18 † 0 – 4, 6, 8, 20…24 * 10 – 22, 24 * 12 – 18, 22, 24 * 14 – 18…24 * 16 – 18 † 16 – 22…24 *	0 – 2, 6…18 † 0 – 4, 20…24 * 2 – 8 † 2 – 20…24 * 4 – 6, 20…24 * 4 – 8…18 † 6 – 20…24 * 10 – 24 * 12 – 24 * 14 – 22, 24 * 16 – 22, 24 * 22 – 24 *	0 – 2, 8…24 † 0 – 4, 6 * 4 – 10, 14 † 4 – 10, 16…20 * 4 – 12, 18…22 * 16 – 18 * 18 – 24 * 20 – 24 * 22 – 24 †	0 – 2, 20…24 * 0 – 4…18 † 2 – 4, 20, 24 * 2 – 8…18 † 4 – 8, 20…24 * 4 – 10…18 † 6 – 8…18 † 6 – 20…24 * 8 – 12…18 † 8 – 14, 16, 20…24 * 10 – 12…18 † 10 – 20…24 * 12 – 18 † 12 – 22, 24 *	0 – 2 * 0 – 4, 6, 10, 14…24 † 2 – 8 * 4 – 8 † 4 - 12, 14 * 6 – 8 * 8 – 10, 14…24 † 10 – 12 † 10 – 14 * 12 – 16…24 † 14 – 16, 18 * 14 – 20…24 †

The animals were treated with cadmium (Cd) in the diet at the concentration of 0, 1, or 5 mg/kg (Control, Cd_1, and Cd_5 groups) and/or 0.1% extract from the berries of *Aronia melanocarpa* L. (AM, Cd_1+AM, and Cd_5+AM groups) for 24 months. Data are shown as a median and minimum and maximum values for eight animals (except for seven females in the AE, Cd_1, and Cd_5 groups after 20, 22, and 24 months). Statistically significant differences (Kruskal–Wallis test) compared to: a—Control group; b—AM group; c—Cd_1 group; d—Cd_1+AM group; and e—Cd_5 group. Wilcoxon test was performed to compare data between particular two time points in the same animals during the 24-month study. * $p < 0.05$, † $p < 0.01$, and ‡ $p < 0.001$.

Table S9. The concentration of creatinine in the serum and urine and creatinine clearance in female rats.

Group	Experiment Duration			
	3 Months	10 Months	17 Months	24 Months
	Creatinine in the Serum (mg/100 mL)			
Control	0.780	0.742	0.648	0.862
	0.678 – 0.847	0.645 – 0.774	0.537 – 0.806	0.646 – 0.985
AM	0.758	0.710	0.537	0.708
	0.678 – 0.885	0.613 – 0.903	0.358 – 0.866	0.554 – 0.923
Cd_1	0.692	0.726	0.761	1.015
	0.615 – 0.769	0.645 – 0.839	0.478 – 1.134	0.646 – 1.108
Cd_1+AM	0.769	0.807	0.552	0.708
	0.731 – 0.846	0.704 – 0.936	0.418 – 0.866	0.677 – 1.262
Cd_5	0.750 $^{b\dagger\,d\dagger}$	0.874	0.885	1.126 $^{b\dagger\,d*}$
	0.654 – 0.885	0.667 – 1.412	0.537 – 1.060	0.973 – 1.277
Cd_5+AM	0.556 $^{a\ddagger\,e\dagger}$	0.784	0.619 ^{e†}	0.687 $^{c*\,e\ddagger}$
	0.540 – 0.667	0.745 – 0.902	0.358 – 0.716	0.523 – 0.800
	Creatinine in the Urine (mg/mL)			
Control	1.491	1.046	0.941	0.703
	0.545 – 2.564	0.662 – 1.339	0.484 – 5.210	0.348 – 2.754
AM	1.391	0.709	0.637	0.596
	0.764 – 2.364	0.554 – 1.077	0.274 – 2.081	0.422 – 1.032
Cd_1	1.121	0.928	1.070	0.816
	0.420 – 1.677	0.652 – 1.464	0.404 – 4.404	0.438 – 1.219
Cd_1+AM	0.779	0.900	0.720	0.891
	0.435 – 1.516	0.371 – 3.757	0.229 – 1.421	0.469 – 2.047
Cd_5	0.976	0.923	0.617	0.737
	0.516 – 1.500	0.592 – 2.197	0.325 – 0.983	0.272 – 0.951
Cd_5+AM	1.260	0.675	0.667	0.726
	0.796 – 1.944	0.282 – 2.169	0.500 – 1.400	0.418 – 1.508
	Creatinine Clearance (mL/min)			
Control	1.346	1.060	0.949	0.984
	1.015 – 1.592	0.832 – 1.538	0.505 – 1.001	0.820 – 1.203
AM	1.208	0.927	0.909	0.931
	0.652 – 1.484	0.852 – 1.464	0.610 – 1.519	0.725 – 1.259
Cd_1	1.289	0.943	0.950	0.982
	0.757 – 2.987	0.799 – 1.292	0.410 – 1.476	0.847 – 1.036
Cd_1+AM	1.117	0.967	1.030	1.306
	0.769 – 1.863	0.604 – 1.616	0.736 – 1.813	0.519 – 1.693
Cd_5	1.203	0.988	0.613 $^{a\dagger\,d\ddagger}$	0.764 ^{a†}
	0.960 – 2.348	0.732 – 1.711	0.374 – 0.799	0.425 – 0.798
Cd_5+AM	1.205	1.095	0.860 ^{e*}	1.582 ^{e†}
	0.798 – 1.829	0.874 – 1.277	0.625 – 1.357	0.785 – 2.034

The animals were treated with cadmium (Cd) in the diet at the concentration of 0, 1, or 5 mg/kg (Control, Cd_1, and Cd_5 groups) and/or 0.1% extract from the berries of *Aronia melanocarpa* L. (AM, Cd_1+AM, and Cd_5+AM groups) for 3, 10, 17, and 24 months. Data are shown as a median and minimum and maximum values for eight animals (except for seven females in the AM, Cd_1, and Cd_5 groups after 24 months). Statistically significant differences (Kruskal–Wallis test) compared to: a—Control group; b—AM group; c—Cd_1 group; d—Cd_1+AM group; and e—Cd_5 group, where * $p < 0.05$, $^{\dagger}p < 0.01$, and $^{\ddagger}p < 0.001$, are marked.

Table S10. The concentration of urea and uric acid in the serum of female rats.

Group	Experiment Duration			
	3 Months	10 Months	17 Months	24 Months
		Uric Acid (mg/100 mL)		
Control	3.110	2.360	3.840	3.380
	2.680 – 3.630	2.000 – 2.680	3.440 – 4.690	3.310 – 4.900
AM	3.315	2.540	3.970	3.200
	2.950 – 3.630	2.100 – 3.050	3.590 – 4.940	2.950 – 4.500
Cd_1	4.340 $^{a\downarrow b\dagger}$	2.440	3.640	3.450
	4.020 – 4.630	2.120 – 2.520	3.000 – 4.030	3.160 – 4.430
Cd_1+AM	4.210 $^{a\downarrow b\dagger}$	2.460	4.414	3.750
	3.680 – 5.370	2.260 – 2.700	3.310 – 4.630	3.200 – 4.800
Cd_5	3.380 $^{c* d*}$	2.750	4.140	4.400 $^{a* b\dagger}$
	3.150 – 3.590	2.350 – 3.430	3.160 – 4.750	3.500 – 7.500
Cd_5+AM	3.420	2.550	4.060	3.700
	3.200 – 4.000	2.260 – 2.770	3.530 – 4.840	3.100 – 5.500
		Urea (mg/100 mL)		
Control	26.30	32.54	28.26	24.57
	20.18 – 32.40	10.09 – 44.40	23.28 – 35.80	17.24 – 33.62
AM	23.47	40.52	24.56	17.24
	16.20 – 30.75	15.95 – 52.16	19.40 – 31.90	11.20 – 40.00
Cd_1	37.22	26.51	26.79	38.02
	26.29 – 47.88	20.26 – 38.79	24.60 – 33.20	34.90 – 48.90
Cd_1+AM	38.38 ^{b*}	38.80	34.50	21.88
	10.61 – 47.18	4.810 – 49.57	16.80 – 40.80	18.75 – 25.86
Cd_5	32.16	40.73	37.87 $^{a* b\dagger c*}$	53.62 $^{a* b\dagger d\dagger}$
	14.32 – 42.72	29.31 – 52.58	31.40 – 46.55	41.14 – 79.16
Cd_5+AM	21.92 $^{c* d\dagger}$	35.35	32.35	20.10 $^{c* e\dagger}$
	13.85 – 26.06	4.810 – 42.67	21.40 – 38.90	17.71 – 34.04

The animals were treated with cadmium (Cd) in the diet at the concentration of 0, 1, or 5 mg/kg (Control, Cd_1, and Cd_5 groups) and/or 0.1% extract from the berries of *Aronia melanocarpa* L. (AM, Cd_1+AM, and Cd_5+AM groups) for 3, 10, 17, and 24 months. Data are shown as a median and minimum and maximum values for eight animals (except for seven females in the AM, Cd_1, and Cd_5 groups after 24 months). Statistically significant differences (Kruskal–Wallis test) compared to: a—Control group; b—AM group; c—Cd_1 group; d—Cd_1+AM group; and e—Cd_5 group, where * $p < 0.05$, $^{\dagger}p < 0.01$, and $^{\downarrow}p < 0.001$, are marked.

Table S11. The concentrations of urea and uric acid in the urine of female rats.

Group	Experiment Duration			
	3 Months	10 Months	17 Months	24 Months
		Uric Acid (mg/24 h)		
Control	21.43	23.85	18.01	33.99
	12.42 – 44.00	14.80 – 32.27	16.52 – 24.60	18.71 – 76.35
AM	20.22	35.77	24.20	39.24
	8.300 – 35.85	28.35 – 47.88	3.860 – 39.16	9.650 – 68.88
Cd_1	31.02	26.25	32.35	21.96
	12.73 – 71.10	17.40 – 35.91	3.200 – 41.50	13.89 – 42.39
Cd_1+AM	29.32	23.52	35.95	28.68
	26.25 – 48.43	12.28 – 41.21	11.77 – 66.41	8.253 – 51.99
Cd_5	35.21	21.88	32.68	27.37
	24.5558.56	14.61 – 48.20	14.18 – 52.44	19.86 – 31.26
Cd_5+AM	26.72	26.38	27.86	34.12
	15.63 – 39.84	10.56 – 77.53	9.510 – 47.76	22.53 – 37.97
		Urea (mg/24 h)		
Control	41.85	35.03	57.52	36.93
	18.60 – 72.00	22.80 – 43.40	29.52 – 68.58	21.82 – 74.82
AM	40.12	47.80	43.55	43.16
	19.25 – 50.25	26.95 – 81.90	24.00 – 69.50	13.29 – 83.44
Cd_1	51.30	31.32	57.37	39.46
	16.75 – 99.00	22.40 – 37.80	22.06 – 86.40	24.27 – 64.94
Cd_1+AM	58.10	34.69	42.17	59.65
	24.60 – 86.40	16.70 – 56.64	22.13 – 72.73	14.76 – 82.61
Cd_5	50.38	36.96	53.96	41.76
	33.50 – 81.60	17.10 – 53.80	38.97 – 92.42	33.58 – 72.85
Cd_5+AM	42.95	43.67	43.67	51.27
	20.00 – 52.20	22.89 – 56.52	23.52 – 70 04	26.52 – 100.1

The animals were treated with cadmium (Cd) in the diet at the concentration of 0, 1, or 5 mg/kg (Control, Cd_1, and Cd_5 groups) and/or 0.1% extract from the berries of *Aronia melanocarpa* L. (AM, Cd_1+AM, and Cd_5+AM group) for 3, 10, 17, and 24 months. Data are shown as a median and minimum and maximum values for eight animals (except for seven females in the AM, Cd_1, and Cd_5 groups after 24 months). There were no statistically significant differences (Kruskal–Wallis test) between the experimental groups.

Table S12. The concentration of cadmium (Cd) in the urine of female rats evaluated every other month during the 24-month study.

Months	Control	AM	Cd_1	Cd_1+AM	Cd_5	Cd_5+AM
0	0.1263	0.1157	0.1333	0.1216	0.1170	0.1323
	0.0300 - 0.1749	0.0886 – 0.1764	0.0600 – 0.1766	0.0393 – 0.1794	0.0600 – 0.1794	0.0812 – 0.1467
2	0.1376	0.1337	0.3024	0.3818 a*	0.4534 a‡ b*	0.4706 a‡ b†
	0.0950 - 0.2752	0.1040 – 0.3917	0.1422 – 0.3462	0.2790 – 0.4942	0.1664 – 0.5727	0.2584 – 0.6948
4	0.1342	0.1259	0.2398	0.2474	0.4378 a† b†	0.4733 a† b*
	0.0677 – 0.2894	0.1029 – 0.1570	0.1187 – 0.4146	0.1418 – 0.4231	0.2094 – 0.6720	0.1610 – 0.7368
6	0.1244	0.1421	0.2311 a†	0.2162	0.2599 a‡ b† d*	0.2392 a† b*
	0.0620 – 0.1876	0.1044 – 0.1908	0.1485 – 0.3397	0.1519 – 0.3184	0.2327 – 0.4169	0.1696 – 0.6054
8	0.1136	0.1161	0.2481 b*	0.2476 a* b†	0.3224 a† b†	0.2876 a* b†
	0.0652 – 0.1738	0.0646 – 0.1690	0.1396 – 0.4467	0.2125 – 0.5917	0.1862 – 0.4342	0.2376 – 0.3512
10	0.1129	0.1439	0.2121	0.2188	0.3570 a‡ b‡	0.3957 a‡ b‡
	0.1015 – 0.1601	0.1127 – 0.1605	0.1114 – 0.3586	0.1609 – 0.3720	0.2120 – 0.5084	0.2879 – 0.6738
12	0.1446	0.1564	0.2403	0.2848 a†	0.3462 a† b*	0.3622 a‡ b*
	0.0938 – 0.1883	0.0833 – 0.2836	0.1912 – 0.4959	0.2256 – 0.4953	0.2024 – 0.6804	0.2053 – 0.4630
14	0.1549	0.1996	0.2700	0.2972 a*	0.4798 a† b†	0.5580 a‡ b†
	0.0870 – 0.2560	0.0893 – 0.2553	0.1850 – 0.3110	0.2790 – 0.6836	0.2032 – 0.7207	0.2338 – 0.9776
16	0.1634	0.1697	0.2867	0.3031	0.4968 a† b*	0.6167 a‡ b† e*
	0.0683 – 0.2176	0.0883 – 0.3840	0.1255 – 0.6386	0.2053 – 0.9170	0.2867 – 0.9785	0.3414 – 0.8911
18	0.1335	0.1472	0.2180	0.2256	0.3936 a† b†	0.4329 a‡ b†
	0.1010 – 0.1829	0.1163 – 0.1841	0.1227 – 0.4043	0.1774 – 0.3824	0.1860 – 0.4897	0.2423 – 0.6143
20	0.1310	0.1515	0.2019	0.2107	0.3485 a‡ b†	0.4068 a‡ b† e*
	0 0906 – 0.1898	0.0768 – 0.2050	0.1704 – 0.2918	0.1579 – 0.3246	0.2523 – 0.4841	0.3272 – 0.5538
22	0.1382	0.1552	0.2230	0.3061 a‡ b*	0.3345 a‡ b*	0.4859 a‡ b‡ e*
	0.1217 – 0.2770	0.1251 – 0.2168	0.1565 – 0.2864	0.2584 – 0.4623	0.2034 – 0.4645	0.3927 – 0.8177
24	0.1374	0.1304	0.1958	0.2050	0.4206 a‡ b‡	0.4927 a‡ b‡ e*
	0.0764 – 0.1937	0.1118 – 0.1618	0.1795 – 0.2451	0.1794 – 0.2531	0.3110 – 0.4695	0.4308 – 0.6616
Friedman Test	$p = 0.790$	$p < 0.05$	$p = 0.063$	$p < 0.001$	$p < 0.001$	$p < 0.001$
Wilcoxon Test		2 – 8 * 4 – 22 * 6 – 8 * 8 – 10, 14…18, 22, 24 * 10 – 22 † 12 – 24 *		0 – 2, 8…18, 22, 24 † 0 – 4, 6, 20 * 2 – 4, 18, 22 * 2 - 6, 10, 20, 24 † 6 – 14 * 6 – 22 † 8 – 24 * 10 – 14 † 10 – 16, 22 * 12 – 20, 24 * 14 – 18 * 14 – 20, 24 † 16 – 20, 24 * 18 – 22 * 20 – 22 * 22 – 24 †	0 – 2…18 † 0 – 20…24 * 2 – 6 * 6 – 14, 16, 20, 24 * 8 – 24 * 20 – 24 *	0 – 2…24 † 2 – 6, 8 * 4 – 6 * 6 – 14, 24 * 6 – 16 † 8 – 10, 14, 18...22 * 8 – 16, 24 † 12 – 14, 16, 24 * 16 – 20 *

The animals were treated with cadmium (Cd) in the diet at the concentration of 0, 1, or 5 mg/kg (Control, Cd_1, and Cd_5 groups) and/or 0.1% extract from the berries of *Aronia melanocarpa* L. (AM, Cd_1+AM, and Cd_5+AM groups) for 24 months. Data are shown as a median and minimum and maximum values for eight animals (except for seven females in the AE, Cd_1, and Cd_5 groups after 20, 22, and 24 months). Statistically significant differences (Kruskal–Wallis test) compared to: a—Control group; b—AM group; c—Cd_1 group; d—Cd_1+AM group; and e—Cd_5 group. Wilcoxon test was performed to compare data between particular two time points in the same animals during the 24-month study. * $p < 0.05$, † $p < 0.01$, and ‡ $p < 0.001$.

Table S13. The absolute and the relative weight of the left kidney of rats.

Group	Experiment Duration			
	3 Months	10 Months	17 Months	24 Months
	Absolute Weight of the Kidney (g)			
Control	0.9420 0.8595 – 1.0100	0.9292 0.8075 – 1.0274	1.1014 0.9994 – 1.4990	1.3065 1.1163 – 2.0292
AM	1.0005 0.8176 – 1.0691	0.9575 0.8781 – 1.0104	1.2544 0.8618 – 1.5903	1.1884 0.9532 – 1.9371
Cd_1	0.8962 0.4648 – 0.9746	0.9794 0.8703 – 1.0617	1.1659 0.9084 – 1.8407	1.3195 1.2592 – 1.4661
Cd_1+AM	0.9098 0.8226 – 1.0242	0.9848 0.8094 – 1.0719	1.1322 0.9154 – 1.2132	1.3463 1.1764 – 1.6897
Cd_5	0.9021 0.8628 – 1.0535	1.0326 0.5309 – 1.1813	1.0986 1.0553 – 1.4936	1.3646 1.2528 – 1.6199
Cd_5+AM	0.8806 0.7716 – 1.0770	0.9223 0.8183 – 1.0479	1.1244 1.0384 – 1.3111	1.1801 1.0834 – 1.2960
	Relative Weight of the Kidney (g/100 g b.w.)			
Control	0.3032 0.2732 – 0.3222	0.2104 0.1963 – 0.2581	0.2209 0.1562 – 0.3407	0.1937 0.1786 – 0.3624
AM	0.3128 0.2937 – 0.3300	0.2170 0.1792 – 0.2536	0.2460 0.1413 – 0.3181	0.2246 0.1467 – 0.3522
Cd_1	0.3054 0.1592 – 0.3387	0.2200 0.2868 – 0.2485	0.2451 0.1748 – 0.4184	0.1884 0.1672 – 0.2865
Cd_1+AM	0.3117 0.2856 – 0.3556	0.2153 0.1655 – 0.2666	0.2246 0.1695 – 0.2639	0.2666 0.2262 – 0.3096
Cd_5	0.3005 0,2818 – 0.4130	0.2501 0.1144 – 0.2774	0.2386 0.1505 – 0.4268	0.2481 0.2085 – 0.,2793
Cd_5+AM	0.2825 0.2660 – 0.3121	0.2272 0.1819 – 0.2501	0.2323 0.1783 – 0.2914	0.2123 0.1708 – 0.2875

The animals were treated with cadmium (Cd) in the diet at the concentration of 0, 1, or 5 mg/kg (Control, Cd_1, and Cd_5 groups) and/or 0.1% extract from the berries of *Aronia melanocarpa* L.. (AM, Cd_1+AM, and Cd_5+AM groups) for 3, 10, 17, and 24 months. Data are shown as a median and minimum and maximum values for eight animals (except for seven females in the AM, Cd_1, and Cd_5 groups after 24 months). There were no statistically significant differences (Kruskal-Wallis test) between the experimental groups.

Table S14. The concentrations of chemerin, macrophage inflammatory protein 1a (MIP1a), and Bcl2-associated X protein (Bax) in the kidney of female rats.

Group	Experiment Duration			
	3 Months	10 Months	17 Months	24 Months
	Chemerin (pg/mg Protein)			
Control	29.82	14.68	16.09	16.41
	26.91 – 39.83	11.78 – 19.82	14.90 – 17.66	14.25 – 17.59
AM	22.84	14.01	14.90	15.62
	10.91 – 33.76	7.282 – 26.61	10.93 – 19.28	12.98 – 19.15
Cd_1	28.12	16.73	20.54	38.85 ^{a*}
	21.67 – 86.31	10.81 – 56.95	18.10 – 22.05	28.27 – 40.15
Cd_1+AM	22.02	12.35	16.81	11.75 $^{c\downarrow}$
	7.063 – 32.88	7.414 – 25.75	15.88 – 17.86	9.142 – 13.92
Cd_5	32.35	18.78	22.22 $^{a\dagger\ b\dagger}$	37.69 $^{a*\ d\downarrow}$
	23.04 – 47.10	11.32 – 38.81	19.32 – 23.71	35.44 – 41.36
Cd_5+AM	11.56 $^{a\dagger\ c\dagger\ e\dagger}$	2.887 $^{a*\ b*\ c\downarrow\ d*\ e\downarrow}$	10.43 $^{c\downarrow\ e\dagger}$	10.86 $^{c\downarrow\ e\dagger}$
	6.634 – 19.81	1.174 – 5.572	9.596 – 12.57	9.288 – 11.47
	MIP1a (ng/mg Protein)			
Control	2.168	1.138	1.223	0.837
	1.162 – 3.504	0.840 – 2.996	0.874 – 1.826	0.683 – 1.196
AM	1.652	1.065	0.587	0.266
	0.829 – 2.250	0.912 – 2.478	0.269 – 0.977	0.128 – 0.425
Cd_1	1.984	1.390	0.941	1.000
	0.919 – 2.536	0.920 – 3.180	0.585 – 1.604	0.792 – 2.020
Cd_1+AM	1.441	0.754	0.260 $^{a\downarrow\ c*}$	0.190 $^{a*\ c\downarrow}$
	0.761 – 2.281	0.647 – 1.150	0.036 – 0.331	0.144 – 0.213
Cd_5	0.930 $^{a\downarrow\ c*}$	2.172 $^{d\downarrow}$	1.618 $^{b*\ d\downarrow}$	1.266
	0.721 – 1.125	1.776 – 2.462	1.218 – 2.604	0.942 – 2.076
Cd_5+AM	0.890 $^{a\dagger\ c\dagger}$	0.717 $^{a*\ c*\ e\downarrow}$	0.316 $^{a\dagger\ e\dagger}$	0.169 $^{a*\ e\downarrow}$
	0.751 – 0.968	0.567 – 0.859	0.094 – 0.520	0.102 – 0.262
	Bax (ng/mg Protein)			
Control	5.412	2.651	2.661	1.938
	3.381 – 6.340	0.465 – 4.571	1.728 – 3.572	1.307 – 2.420
AM	3.830	2.974	1.853	1.387
	3.023 – 6.722	1.785 – 3.605	0.914 – 2.550	1.181 – 2.502
Cd_1	5.607	3.075	5.792	2.231
	3.293 – 7.317	2.393 – 6.568	3.637 – 12.47	1.617 – 3.876
Cd_1+AM	3.120	1.924 ^{c*}	1.052 $^{c\downarrow}$	1.096 ^{c*}
	1.638 – 5.257	1.046 – 2.597	0.532 – 1.607	0.922 – 1.877
Cd_5	2.780	19.990	3.839 ^{d†}	2.732 $^{a*\ b*\ d\downarrow}$
	2.456 – 3.594	1.718 – 2.215	2.552 – 4.915	2.442 – 5.216
Cd_5+AM	1.469 $^{a\downarrow\ b\downarrow\ c\downarrow}$	1.195 $^{b\downarrow\ c\downarrow}$	1.186 $^{c\downarrow\ e\downarrow}$	1.452 $^{e\downarrow}$
	0.736 – 1.614	1.031 – 2.163	0.911 – 1.668	1.004 – 2.002

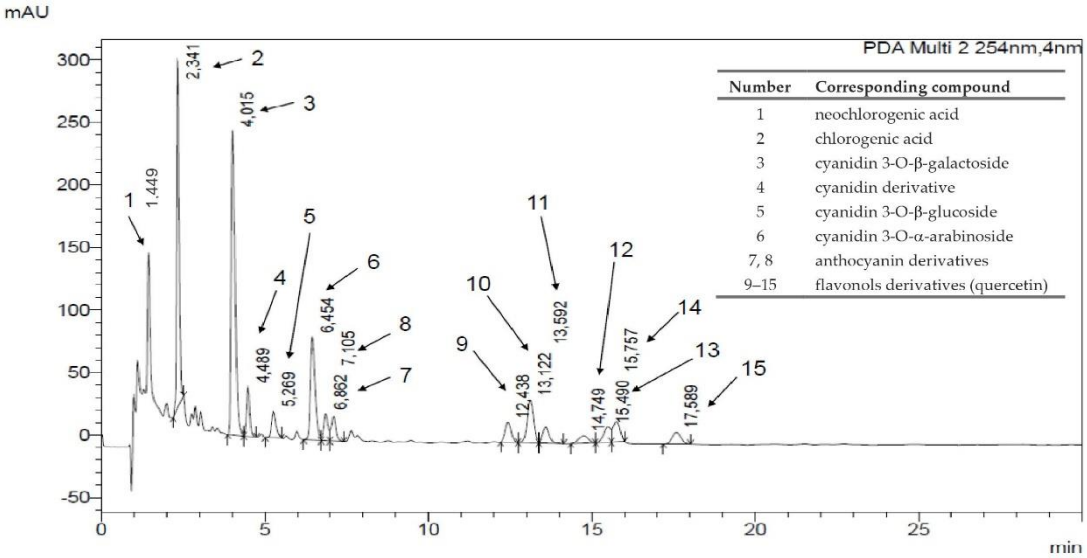
The animals were treated with cadmium (Cd) in the diet at the concentration of 0, 1, or 5 mg/kg (Control, Cd_1, and Cd_5 groups) and/or 0.1% extract from the berries of *Aronia melanocarpa* L. (AM, Cd_1+AM, and Cd_5+AM groups) for 3, 10, 17, and 24 months. Data are shown as a median and minimum and maximum values for eight animals (except for seven females in the AM, Cd_1, and Cd_5 groups after 24 months). Statistically significant differences (Kruskal–Wallis test) compared to: a—Control group; b—AM group; c—Cd_1 group; d—Cd_1+AM group; and e—Cd_5 group, where * $p < 0.05$, $^{\dagger}p < 0.01$, and $^{\downarrow}p < 0.001$, are marked.

Table S15. The concentration of cadmium (Cd) in the blood, urine, and kidney of female rats [1,2].

Group	Experiment Duration			
	3 Months	10 Months	17 Months	24 Months
Blood (µg Cd/L)				
Control	0.0691	0.0860	0.0744	0.0834
	0.0330 – 0.1110	0.0360 – 0.1290	0.0480 – 0.0960	0.0690 – 0.0990
AM	0.0752	0.0802	0.0718	0.0861
	0.0500 – 0.0960	0.0330 – 0.1360	0.0103 – 0.0950	0.0700 – 0.0970
Cd_1	0.1884	0.1792	0.2425	0.2330
	0.1320 – 0.2460	0.1030 – 0.3060	0.1980 – 0.3240	0.1850 – 0.2840
	↑ 2.7x	↑ 2.1x	↑ 3.3x	↑ 2.8x
Cd_1+AM	0.1877	0.1844	0.2375	0.2189
	0.1290 – 0.2480	0.1170 – 0.2760	0.1610 – 0.3050	0.1900 – 0.3000
	↑ 2.7x	↑ 2.1x	↑ 3.2x	↑ 2.6x
Cd_5	1.0236	0.9394	1.0339	1.0467
	0.7390 – 1.3320	0.7350 – 1.1220	0.9400 – 1.1310	0.8250 – 1.2010
	↑ 14.8x	↑ 10.9x	↑ 13.9x	↑ 12.6x
Cd_5+AM	0.8298	0.7948	0.9319	0.8201
	0.6200 – 1.0640	0.5840 – 0.9960	0.7920 – 1.1250	0.6090 – 1.1490
	↑ 12x ↘ 19%	↑ 9.2x ↘ 15%	↑ 12.5x ↘ 10%	↑ 9.8x ↘ 22%
Urine (µg Cd/g Creatinine)				
Control	0.1387	0.1304	0.1491	0.1337
	0.1032 – 0.2013	0.0944 – 0.1722	0.1172 – 0.1889	0.0764 – 0.1937
AM	0.1321	0.1364	0.1445	0.1357
	0.1129 – 0.1507	0.1119 – 0.1558	0.0730 – 0.1712	0.1118 – 0.1618
Cd_1	0.2184	0.1809	0.2096	0.2053
	0.1810 – 0.2509	0.0852 – 0.2558	0.1069 – 0.2820	0.1795 – 0.2451
	↑ 57%	↑ 39%	↑ 41%	↑ 54%
Cd_1+AM	0.2193	0.1913	0.2143	0.2084
	0.1349 – 0.2762	0.1392 – 0.2483	0.1471 – 0.3538	0.1794 – 0.2531
	↑ 58%	↑ 47%	↑ 44%	↑ 56%
Cd_5	0.5008	0.4002	0.4737	0.4104
	0.4183 – 0.6016	0.2839 – 0.6949	0.3369 – 0.6467	0.3110 – 0.4695
	↑ 3.6x	↑ 3.1x	↑ 3.2x	↑ 3.1x
Cd_5+AM	0.6064	0.4997	0.5773	0.4994
	0.4833 – 0.8197	0.3640 – 0.6812	0.4206 – 0.7481	0.4308 – 0.6616
	↑ 4.4x ↗ 21%	↑ 3.8x ↗ 25%	↑ 3.9x ↗ 22%	↑ 3.7x ↗ 22%
Kidney (µg Cd/g)				
Control	0.0385	0.0505	0.0467	0.0844
	0.0283 – 0.0618	0.0385 – 0.0619	0.0364 – 0.0644	0.0452 – 0.1382
AM	0.0355	0.0491	0.0555	0.0930
	0.0282 – 0.0425	0.0308 – 0.0614	0.0454 – 0.0677	0.0599 – 0.1396
Cd_1	0.3482	1.1342	1.2126	1.9807
	0.2626 – 0.4472	0.8769 – 1.4298	0.7714 – 1.8262	1.0909 – 2.8322
	↑ 9x	↑ 22x	↑ 26x	↑ 23x
Cd_1+AM	0.2475	1.0277	1.3048	2.0533
	0.1666 – 0.3018	0.7600 – 1.1772	1.1193 – 1.5348	1.2735 – 2.9219
	↑ 6.4x ↘ 29%	↑ 20x ↘ 9.5%	↑ 28x	↑ 24x
Cd_5	1.3714	4.8425	10.7680	8.0093
	0.9739 – 1.6725	4.2892 – 6.1790	8.4189 – 14.8705	6.5652 – 8.8627
	↑ 36x	↑ 96x	↑ 231x	↑ 95x
Cd_5+AM	1.1805	4.5712	9.6644	7.3042
	0.9976 – 1.4006	3.5350 – 5.1680	8.2900 – 11.7654	5.7523 – 8.6859
	↑ 31x ↘ 14%	↑ 91x ↘ 5.6%	↑ 207x ↘ 10%	↑ 87x ↘ 8.8%

The animals were treated with cadmium (Cd) in the diet at the concentration of 0, 1, or 5 mg/kg (Control, Cd_1, and Cd_5 groups) and/or 0.1% extract from the berries of *Aronia melanocarpa* L. (AM, Cd_1+AM, and Cd_5+AM groups) for 3, 10, 17, and 24 months. Data are shown as a mean and minimum and maximum values for eight animals (with the exception of seven females in the AM, Cd_1,

and Cd5 groups after 24 months). The factors or percentages of changes compared to the control group (↑, increase) or the adequate group treated with Cd alone (↘, decrease; ↗, increase) are indicated if $p < 0.05$. [1] prepared based on Brzóska, M.M.; Gałażyn-Sidorczuk, M.; Jurczuk, M.; Tomczyk, M. Protective effect of *Aronia melanocarpa* polyphenols on cadmium accumulation in the body: A study in a rat model of human exposure to this metal. *Curr. Drug Targets* **2015**, *16*, 1470–1487. https://doi.org/10.2174/1389450116666150102121708. [2] Cd concentration in the blood and urine was also presented in the Supplementary Material for the article Mężyńska, M.; Brzóska, M.M.; Rogalska, J.; Piłat-Marcinkiewicz, B. Extract from *Aronia melanocarpa* L. berries prevents cadmium-induced oxidative stress in the liver: A study in a rat model of low-level and moderate lifetime human exposure to this toxic metal. *Nutrients* **2019**, *11*, 21. https://doi.org/10.3390/nu11010021.



Number	Corresponding compound
1	neochlorogenic acid
2	chlorogenic acid
3	cyanidin 3-O-β-galactoside
4	cyanidin derivative
5	cyanidin 3-O-β-glucoside
6	cyanidin 3-O-α-arabinoside
7, 8	anthocyanin derivatives
9–15	flavonols derivatives (quercetin)

Figure S1. Ultra Performance Liquid Chromatography (UPLC) polyphenolic profile of the *Aronia melanocarpa* L. berry extract. The profile of the extract was investigated and presented in Brzóska, M.M.; Rogalska, J.; Gałażyn-Sidorczuk, M.; Jurczuk, M.; Roszczenko, A.; Tomczyk, M. Protective effect of *Aronia melanocarpa* polyphenols against cadmium-induced disorders in bone metabolism: A study in a rat model of lifetime human exposure to this heavy metal. *Chem. Biol. Interact*. **2015**, *229*, 132–146. https://doi.org/10.1016/j.cbi.2015.01.031.

10.3. Publikacja III

Protective effect of the extract from *Aronia melanocarpa* L. berries against cadmium-induced oxidative stress in the kidney: A study in an *in vivo* experimental model.

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NATURAL DRUGS

PROTECTIVE EFFECT OF THE EXTRACT FROM *ARONIA MELANOCARPA* L. BERRIES AGAINST CADMIUM-INDUCED OXIDATIVE STRESS IN THE KIDNEY: A STUDY IN AN *IN VIVO* EXPERIMENTAL MODEL

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Abstract: The destructive impact of cadmium (Cd) on the oxidative/antioxidative status of the kidney, as well as the possible beneficial effect of co-administration of a 0.1% aqueous extract from *Aronia melanocarpa* L. berries (AM), were studied in a rat model of low-level and moderate general population exposure to this heavy metal (1 and 5 mg Cd/kg feed, respectively, for up to 24 months). Total antioxidative status (TAS) of the kidney and the main indices of the enzymatic (superoxide dismutase – SOD, catalase – CAT, glutathione reductase – GR, and glutathione peroxidase – GPx) and non-enzymatic (reduced glutathione – GSH and thioredoxin – TRx) antioxidative barrier were assessed. The total oxidative status (TOS) and concentrations of hydrogen peroxide (H_2O_2), xanthine oxidase (XOD), myeloperoxidase (MPO), and oxidized glutathione (GSSG) were measured as markers of oxidative status. The oxidative stress index (OSI) was calculated (TOS/TAS) to estimate the intensity of oxidative stress in the kidney. The exposure to Cd, dose- and duration-dependently, weakened the enzymatic and non-enzymatic antioxidative potential of the kidney leading to a decrease in its TAS, as well as enhanced oxidative status of this organ (increased the concentrations of H_2O_2, MPO, and/or XOD, and elevated TOS) resulting in the development of oxidative stress. The administration of AM during the low-level and moderate intoxication with Cd significantly protected from this xenobiotic-induced disruption of the oxidative/antioxidative balance and development of oxidative stress in the kidney. In summary, even low-level long-term exposure to Cd may result in the occurrence of oxidative stress in the kidney, whereas supplementation with chokeberry products may improve the oxidative/antioxidative balance preventing oxidative stress in this organ. Based on the findings it seems possible that the recently noted by us in the experimental model protective effect of the administration of AM against the damaging impact of Cd on the structure and function of the kidney might result, at least partially, from its antioxidative potential and prevention of the development of oxidative stress in the renal tissue.

Keywords: *Aronia melanocarpa* L., cadmium, kidney, oxidative/antioxidative balance, oxidative stress, protection

Abbreviations: ACR - albumin concentration in the urine adjusted for creatinine concentration; ALP - alkaline phosphatase; AM - a 0.1% aqueous extract from *Aronia melanocarpa* L. berries; Bax - Bcl2-associated X protein; b.w. - body weight; CA - catalase; Cd - cadmium; Cd^{2+} - cadmium ions; Cu - copper; CV - coefficient of variation; ELISA - enzyme-linked immunosorbent assay; Fe^{2+} - divalent iron ions; FR - free radicals; GPx - glutathione peroxidase; GR - glutathione reductase; GSH - reduced glutathione; GSSG - oxidized glutathione; HRP - horseradish peroxidase; H_2O_2 - hydrogen peroxide; KIM-1 - kidney injury molecule 1; MIP1a - macrophage inflammatory protein 1 alpha; Mn - manganese; Mn-SOD - manganese-dependent superoxide dismutase; MPO - myeloperoxidase; NADH - nicotinamide adenine dinucleotide; NADPH - nicotinamide adenine dinucleotide phosphate; NAG - N-acetyl-β-D-glucosaminidase; ˙OH - hydroxyl radical, OSI - oxidative stress index; $O_2^{\cdot-}$ - superoxide radical; PCR - total protein concentration in the urine adjusted for creatinine concentration; ROS - reactive oxygen species; SD - standard deviation; –SH group - sulfhydryl group; SOD - superoxide dismutase; TAS - total antioxidative status; TOS - total oxidative status; TRx - thioredoxin; w.w., wet weight; XOD - xanthine oxidase; Zn - zinc; β2-MG - beta2-microglobulin

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For a long time, the interest of not only the medical community and scientists but also of the general population, in the possibility of using biologically active substances naturally occurring in some plants and various products based on these plants in the prevention and treatment of many diseases, including civilization diseases, has been observed (for a review, see 1–3). However, recently more and more attention has been paid to the possibility of using plant-based products to prevent health risks posed by exposure to toxic substances (for a review, see 3–6). One of these plants is chokeberry (*Aronia melanocarpa* L.), a deciduous shrub belonging to the family *Rosaceae*. It is found all over the world and is well-known for the medical and culinary properties of its berries (chokeberries). Aronia fruits are a rich source of numerous substances with health-promoting properties and one of the richest sources of polyphenolic compounds characterized by high antioxidative properties (1–3, 7). Chokeberries are also a source of bioelements (e.g. calcium, magnesium, and iron), vitamins (vitamins A, C, E, and K and vitamins from group B), triterpenes, phytosterols, carotenoids (β-carotene), pectins, sugar, sugar alcohols (parasorboside and sorbitol), tannins, dietary fiber, organic acids (L-malic acid and citric acid), carbohydrates, and proteins (3, 7). The components of aronia berries possess powerful antioxidative, antidiabetic, anti-inflammatory, anti-infective, antimutagenic, and cytotoxic properties (1–4, 7). Thus, consuming products from aronia berries, such as dried fruit, juices, jams, preserves, syrups, teas, tinctures, and powdered fruit in the form of supplements has cardioprotective, gastroprotective, hepatoprotective, radioprotective, and immunomodulatory properties, and may effectively protect from the development of some chronic illnesses, including metabolic disorders, cardiovascular diseases, and cancer, and is used to support the treatment of some diseases (1–4, 7). Because of the many health-promoting properties, the inclusion of aronia products into the daily diet is widely recommended (1–4).

Some time ago, considering the beneficial health properties of aronia berries, including especially their high antioxidative potential (1–3, 7) and the ability of polyphenolic compounds to chelate ions of divalent metals (4, 6), we hypothesized that chokeberry-based products may be effective in preventing from unfavourable effects of exposure to cadmium (Cd). This toxic heavy metal belongs to the main chemical pollutants of the natural environment and food in industrialized countries (for a review, see 5, 8, 9). Numerous epidemiological studies provide evidence that the current exposure of the worldwide population to this heavy metal creates a risk to health, including the risk of kidney damage, which is a target organ for this xenobiotic during long-term exposure (10–13). Moreover, forecasts show that exposure to this heavy metal will grow (11). Thus it is very important from the point of view of public health to find an effective strategy against its toxicity.

To investigate the possibility of a protective effect of chokeberry extract during exposure to Cd, we conducted a widely designed study in a female rat model of low-level and moderate lifespan general population exposure to Cd in industrialized countries (the treatment of animals with Cd in the feed at a concentration of 1 and 5 mg Cd/kg, respectively, for up to 24 months). Previously, it was found in the model that the administration of a 0.1% extract from *A. melanocarpa* berries (AM) protected against Cd accumulation in the body, including mainly the liver and kidneys (14), and provided significant protection against this heavy metal-induced damage to the liver (15–17), bones (18, 19), and salivary glands (20) and that these effects resulted, at least partially, from antioxidative properties of AM.

Our more recent study (21) showed that the administration of AM during the exposure to 1 and 5 mg Cd/kg also protected from changes in the morphological structure of the kidney tubules and glomeruli and deterioration of their function. The low-level and moderate treatment with this xenobiotic resulted in damage to the kidney tubules, detected based on enhanced presence in the urine of sensitive biomarkers such as kidney injury molecule-1 (KIM-1), β2-microglobulin (β2-MG), N-acetyl-β-D-glucosaminidase (NAG), and alkaline phosphatase (ALP), as well as to increase in albumin concentration in the urine (ACR) and decreased glomerular filtration rate (estimated based on creatinine clearance) which are clinically relevant early markers of glomerular injury. Pathological changes in the renal tubules such as hyalinization, hyperplasia and hypertrophy of the epithelium of the convoluted tubules, the proliferation of the interstitial kidney tissue, as well as glomerulonephritis and congestion at the cortex/medullary interface were noted at both levels of the 24-month exposure to Cd. Moreover, vacuolization and an extension of the tubular lumen were observed at the low-level treatment and perivascular oedema occurred at the moderate exposure. Inflammatory processes developed in the kidney as well (increased concentrations of chemerin and/or Bcl2-associated X protein – Bax). The toxic impact of Cd on the kidney occurred at

the concentrations of this xenobiotic in the urine (0.0852–0.2820 μg/g creatinine) which were within the lower range of concentrations currently noted in the worldwide general population (for a review, see 10). The co-administration of AM significantly protected from the above-described nephrotoxic action of Cd (21).

The mechanism of the toxic action of Cd, including its nephrotoxicity, is multidirectional and involves its prooxidative action leading to the development of oxidative stress and oxidative damage to the cellular macromolecules and organelles (10, 13, 22). In the view of our previous findings (15–17, 19, 20), we have hypothesized that the protective impact of AM on the kidney (21) may also be related to its antioxidative potential. To investigate this hypothesis, the present study aimed to evaluate the impact of the administration of AM under low-level and moderate exposure to Cd (1 and 5 mg/kg feed) on the oxidative/antioxidative balance in the kidney. For this purpose, total antioxidative status (TAS), and the main markers of the enzymatic (superoxide dismutase – SOD, catalase – CAT, glutathione peroxidase – GPx, and glutathione reductase – GR) and non-enzymatic antioxidative status (reduced glutathione – GSH and thioredoxin – TRx), as well as total oxidative status (TOS) and some indices of the oxidative status such as the concentrations of hydrogen peroxide (H_2O_2), xanthine oxidase (XOD), myeloperoxidase (MPO), and oxidized glutathione (GSSG), in the renal tissue of the female rats administered with Cd and/or AM were determined. The oxidative stress index (OSI), reflecting the extent of destroying the oxidative/antioxidative balance and the level of oxidative stress, was calculated as well. Moreover, the relationships between the main indices of the oxidative/antioxidative status of the kidney (TAS, TOS, and OSI) and already published Cd concentration in this organ (14), as well as markers of its damage (21) were evaluated. To the best of our knowledge, no such research has ever been carried out.

EXPERIMENTAL

Fodder containing Cd

The feed containing Cd at the concentration of 1 and 5 mg Cd/kg was manufactured by Label Food "Morawski" Keynia by adding cadmium chloride 2.5 hydrate ($CdCl_2$ x 2.5 H_2O; POCh; Gliwice, Poland) to the ingredients of the standard Labofeed H diet (breeding diet ensuring the correct development and growth of young animals) and Labofeed B diet (maintenance diet). Cd content in the fodder was verified in our laboratory by determination of this element concentration (using the atomic absorption spectrometry method). The concentration of Cd in the 1 mg Cd/kg diet was determined at 1.09 ± 0.13 mg/kg (mean ± standard deviation – SD) and 4.92 ± 0.53 mg/kg in the case of the 5 mg Cd/kg diet. Cd concentration in the standard Labofeed diet was 0.0584 ± 0.0049 mg/kg (14).

Extract from the berries of A. melanocarpa

A powdered extract from the berries of *A. melanocarpa*, provided by Adamed Consumer Healthcare (Tuszyn, Poland), was used to prepare AM administered to the animals. According to the manufacturer's certificate (Certificate KJ 4/2010), the extract contained 65.74% polyphenols and 18.65% anthocyanins. Other components found in the extract included carotenoids, pectins, sugar, sugar alcohols, phytosterols, triterpenes, as well as minerals and vitamins (producer's data). To prepare the AM that was administered to the animals, 1 g of the powdered extract was dissolved in 1 L of redistilled water every day. The total concentration of polyphenolic compounds and the polyphenolic profile of the extract were estimated by us (18). One litter of the AM contained (mean ± standard error – SE) 612.40 ± 3.33 μg of total polyphenols, including 202.28 ± 1.28 μg of total anthocyanins (80.07 ± 1.05 μg – cyanidin 3-O-β-galactoside, 33.21 ± 0.01 μg – cyanidin 3-O-α-arabinoside, and 3.68 ± 0.01 μg – cyanidin 3-O-β-glucoside), 129.87 ± 1.12 μg of total proanthocyanidins, 110.92 ± 0.89 μg of total phenolic acids, 68.32 ± 0.08 μg of chlorogenic acid, and 21.94 ± 0.98 μg of total flavonoids (18). Cd concentration in AM did not exceed 0.05 μg/L (14).

Animal model

The experimental protocol was approved by the Bialystok Local Ethics Committee for Animal Experiments (Poland; permit number 60/2009 on September 21, 2009). All animal procedures followed institutional guidelines, ethical standards, and the International Guide for the Use of Animals in Biomedical Research. In the study, 192 female Wistar rats (Hannover Wistar rats, bred according to the Charles River International Genetic Standardization Program - Crl: WI (Han)), aged three to four weeks, from a licensed breeding facility (Laboratory Animal House in Brwinów, Poland) were used. The animals were housed throughout the experiment under standard conditions (temperature 22 ± 2°C, relative humidity 50 ± 10%, and a 12-hour light/dark cycle), with free access to feed and drinking fluids (redistilled water or AM). The rats were kept in stainless steel cages (4 rats in each). After

5-day acclimatization to the experimental environment, the rats were randomly assigned to one of six experimental groups, each of 32 animals. One group was given AM (0.1% aqueous solution of the extract from *A. melanocarpa* berries) as the only drinking fluid (AM group), two groups were intoxicated with Cd via the feed at the concentration of 1 and 5 mg Cd/kg (Cd_1 and Cd_5 groups, respectively), and the next two groups were given Cd (1 or 5 mg Cd/kg feed) and AM simultaneously (Cd_1+AM group and Cd_5+AM group) for up to 24 months. The last group was fed redistilled water (containing < 0.05 μg Cd/L) and a standard Labofeed diet (without the addition of Cd) and served as the control group.

The concentration of Cd in the blood, urine, and kidney of the animals in the Cd_1 group (0.1030–0.3240 μg Cd/L, 0.0852–0.2820 μg/g creatinine, and 0.2626–2.8322 μg/g of wet weight – w.w., respectively) and Cd_5 group (0.7350–1.3320 μg Cd/L, 0.2839–0.6949 μg/g creatinine, and 0.9739–14.8705 μg/g w.w., respectively) (14) was within the range of values noted nowadays in the general population non-exposed occupationally to this heavy metal (10, 11), confirming that the used experimental model well reflects current human environmental exposure to this xenobiotic.

The daily intake of aronia extract and polyphenolic compounds in the female rats that received AM as the only drinking fluid ranged from 63.1 to 154.7 mg/kg body weight (b.w.) and from 41.5 to 101.7 mg/kg b.w., respectively (14). The intake of polyphenols was several times higher than the average consumption of these compounds among the worldwide general population (1000 mg/24 h i.e. 14.29 mg/kg b.w. assuming the body weight of 70 kg) (23).

The model has been reported in detail elsewhere (14–21) and thus in the present study, it is only briefly described and together with the range of performed measurements is presented in Figure 1.

The slices of the right kidneys dissected at necropsy after 3, 10, 17, and 24 months of the experiment (14) were used in the present study. The kidneys, immediately after collection, were rinsed with ice-cold physiological saline (0.9% sodium chloride), dried on the filter paper, and weighed with an analytical balance (OHAUS®, Nanikon, Switzerland; accuracy to 0.0001 g). The organs were stored frozen (−70°C) until assayed.

Determination of the indices of oxidative and antioxidative status of the kidney

Preparation of kidney homogenates

Pre-weighted slices of the right kidney were homogenized in a cold potassium phosphate buffer (50 mM, pH = 7.4; prepared by combining 50 mM potassium dihydrogen phosphate and 50 mM dipotassium hydrogen phosphate (POCh; Gliwice, Poland)) to prepare 10% homogenates (weight/volume), with the addition of butyl-hydroxytoluene

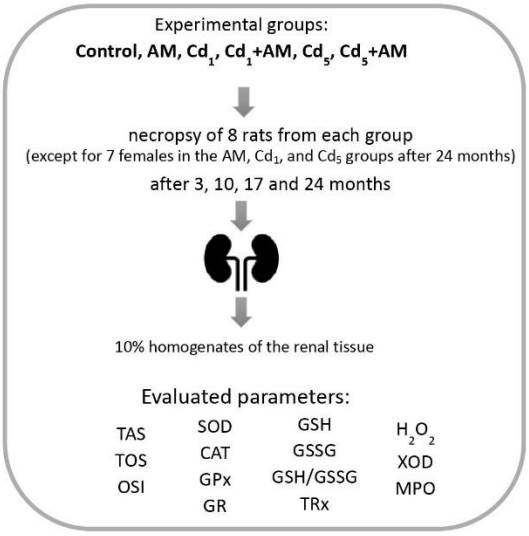

Figure 1. Schematic representation of the experimental protocol.

(Sigma-Aldrich GmbH; Steinheim, Germany) as an antioxidant. Per each 1 mL of the homogenate, 0.01 mL of 0.5 M butyl-hydroxytoluene in acetonitrile (Merck, Darmstadt, Germany) was used. Such prepared homogenates were centrifuged at 10,000 g for 5 minutes at 4°C (24) with a Medical Instruments MPW-350R centrifugator (Warsaw, Poland), and the aliquots were separated and stored frozen (−70°C) until all measurements were performed.

The assessment of the parameters of the oxidative/antioxidative status

The indices of the oxidative/antioxidative status were determined in the aliquots of the kidney homogenates colorimetrically with the use of MULTISCAN GO (Thermo Scientific, Vantaa, Finland) and Epoch (Bio Tek Instruments, Inc, Winooski, USA) spectrophotometers for quantification.

TAS was measured using the Immundiagnostik AG (Bensheim, Germany) ImAnOx (TAS) ELISA kit (Catalogue No. KC 5200). The enzyme-linked immunosorbent assay (ELISA) method is based on the reaction of eliminating a defined amount of H_2O_2 added into the tested sample by antioxidants present in the sample. The remaining H_2O_2 produces compounds that absorb the wavelength of 450 nm. The certified values of TAS in two control samples provided by the manufacturer were within the range of 162–220 μmol/L and 204–276 μmol/L. The values of TAS determined by us were within the reference ranges (184 ± 19 μmol/L and 236 ± 21 μmol/L, respectively, for the first used kit and 183 ± 15 μmol/L and 244 ± 16 μmol/L, respectively, for the second one; mean ± SD). The precision of the measurements of TAS in the aliquots of the kidney homogenates, expressed as the intra-assay coefficient of variation (CV) was < 3%. The inter-assay CV was < 4%.

TOS was evaluated using the Immundiagnostik AG PerOx (TOS) ELISA kit (Catalogue No. KC 5100) based on the determination of total lipid peroxides contained in the studied sample in the reaction with peroxidase at 450 nm. The values of TOS assayed by us in the control samples (190 ± 12 μmol/L and 515 ± 23 μmol/L in the first of the used kits and 204 ± 17 μmol/L and 585 ± 33 μmol/L in the second one; mean ± SD) agreed with the certified values (170–283 μmol/L and 437–728 μmol/L, respectively). The intra-assay CV for TOS measurements in the tested samples was < 6%. The inter-assay CV was < 5%. Based on the determined values of TAS and TOS, OSI was calculated as the ratio of TOS and TAS (OSI = TOS/TAS).

The activity of total SOD was determined using a commercial kit bought from Cayman Chemical Company (Catalogue No. 706002; Ann Arbor, MI, USA). The test detects superoxide radicals ($O_2^{\cdot-}$) produced by xanthine oxidase and hypoxanthine using a tetrazolium salt. The intra-assay CV was < 4% and the inter-assay CV was < 9%. The determination of the activity of CAT was based on the spectrophotometric method by Aebi (25), measuring the quantity of H_2O_2 destroyed by this enzyme. The disappearance of H_2O_2 was seen spectrophotometrically at 240 nm. The intra-assay CV was < 6%. OxisResearch Bioxytech GPx-340 kit (Catalogue No. 21017; Burlingame, CA, USA) was used to measure GPx activity. This enzyme is used in the assay to catalyze the oxidation of GSH to GSSG, which is begun by the addition of tertbutyl hydroperoxide. The intra-assay CV was < 7%. Determination of the activity of GR was made with the use of the commercial Bioxytech GR-340 kit by OxisResearch (Catalogue No. 21018D; Burlingame, CA, USA). This assay is based on the reduction of GSSG with the use of nicotinamide adenine dinucleotide phosphate (NADPH; one particle of NAPDH is consumed for the reduction of one particle of GSSG), which is oxidized (to nicotinamide adenine dinucleotide – NADH) in the reaction catalysed by GR. The amount of GSSG that is reduced to GSH is estimated indirectly by the assessment of consumed NADH reflected in a decrease in the absorbance measured at 340 nm. The intra-assay CV of this measurement was < 5%.

The concentrations of GSH and GSSG were determined colorimetrically using the Cayman Chemical Company Glutathione Assay Kit (Catalogue No. 703002; Ann Arbor, MI, USA). In order to assay the concentration of GSH, the reaction between GSH and 5,5'-dithio-bis-2-nitrobenzoic acid (DTNB) was performed. Then, the absorbance (at 405–412 nm) of the sample containing the product of this reaction (5-thio-2-nitrobenzoic acid – TNB), was measured. The GSSG was quantified by first derivatizing GSH with 2-vinylpyridine. The intra-assay CV for the determination of GSH and GSSG was < 7%. The inter-assay CV was < 7% for GSH and < 3% for GSSG. Furthermore, the GSH/GSSG ratio was calculated.

The concentration of TRx was assayed with the use of commercial Rat (Trx) ELISA KIT (Catalogue No. 201-11-0445; SunRed, Shanghai, China). The principle of this test was based on the incubation of the sample in wells pre-coated with rat TRx monoclonal antibody, and then the addition of horseradish peroxidase (HRP)-conjugated streptavidin (streptavidin-HRP) in order to obtain immune complex, which then is combined with chromogens. The chroma of colour is positively correlated with the

concentration of TRx, hence it was measured calorimetrically (450 nm) in order to determine this parameter. The intra-assay CV was < 4%, whereas the inter-assay CV was < 8%.

The concentration of H_2O_2 was determined with OxisResearch Bioxytech H_2O_2-560 kit (Catalogue No. 21024; Burlingame, CA, USA). The assay is based on the reaction of oxidation of divalent iron ions (Fe^{2+}) by H_2O_2 to trivalent iron ions (Fe^{3+}), which binds with xylenol orange, resulting in the formation of a stable, coloured complex measured spectrometrically at 560 nm. The intra-assay CV was < 9%.

XOD in the kidney was measured with the use of Rat (XOD) ELISA KIT (Catalogue No. SRB-T-88306; SunRed, Shanghai, China). The kit used a double-antibody sandwich ELISA method with wells pre-coated with XOD monoclonal antibody. The coloured product of the reaction with chromogen was measured at 450 nm. The intra-assay CV was < 7% and the inter-assay CV was < 4%.

In order to determine the concentration of MPO, Rat (MPO) ELISA KIT (Catalogue No. 201-11-0575; SunRed, Shanghai, China) was used. The determination is based on the positive correlation between the chroma of the colour of complex being a product of the reaction of chromogen with the complex of MPO, streptavidin-HRP, and rat MPO monoclonal antibody measured at 450 nm with the concentration of MPO in an assayed sample. The intra- and inter-assay CV were < 8% and < 4%, respectively.

Statistical analysis

Statistica 13.3 software (StatSoft, Tulsa, OK, USA) was used for all statistical analyses. The results of the determination of the indices of the oxidative/antioxidative status are presented as a median, 25-75% confidence interval, minimum and maximum. The Shapiro-Wilk test was used to evaluate the normality of the data distribution at first. As no normal distribution was noted, a nonparametric signed-rank Kruskal-Wallis with median test (Kruskal-Wallis test) was performed to check if there were statistically significant ($p < 0.05$) differences between the six groups. If statistically significant differences occurred between the six groups, further comparisons were performed to determine statistically significant ($p < 0.05$) differences between particular two experimental groups.

To calculate the mutual dependences between the values of the measured parameters of the oxidative/antioxidative balance, as well as these parameters and already published Cd concentration in this organ (14) and markers of its damage (21), a linear regression analysis was performed. The results of the analysis of regression are shown as the β coefficient, R^2, and the level of statistical significance (p). A dependence between two variables was statistically significant at the value of the β coefficient for which $p < 0.05$.

RESULTS

H3 TAS, TOS, and OSI

The exposure to the 1 mg Cd/kg fed for 3 and 10 months had no impact on TAS, TOS, and OSI in the renal tissue. After 17 and 24 months of the treatment, TAS was decreased (by 33% and 36%, respectively), whereas TOS (6.5- and 7.9-fold, respectively) and OSI (8.6- and 13.9-fold, respectively) were elevated compared to the proper values noted in the control group (Figure 2). At the higher level of exposure to Cd (5 mg/kg feed), TAS dropped already after 3 months (by 42%) and remained at the decreased level (by 30–49%) until the end of the study. Moreover, TOS and OSI in the Cd_5 group were enhanced after 17 (6.6- and 8.5-fold, respectively) and 24 months (7.9- and 13.2-fold, respectively) (Figure 2). There were no differences in the values of TOS, TAS, and OSI in the renal tissue at particular time points between the Cd_1 group and the Cd_5 group, except for lower (by 44%) value of TAS at the higher level of exposure after 3 months of the study (Figure 2).

The administration of AM alone for 3–24 months had no impact on TOS, TAS, and OSI in the renal tissue, whereas its intake during the low-level and moderate exposure to Cd prevented all the above-described changes in the values of TOS, TAS, and OSI, except for TAS in the Cd_5+AM group after 3 and 10 months, that was decreased compared to the control group (by 45% and 41%, respectively) (Figure 2).

Antioxidative enzymes activity

The exposure to Cd resulted in a decrease in the activities of all determined antioxidative enzymes (SOD, CAT, GR, and GPx) in the renal tissue. The effect of Cd depended on the intensity and duration of the treatment (Figures 3 and 4). The activity of SOD was unaffected by the low-level exposure to Cd but at the moderate intoxication for 3–24 months, it was lower (by 30–56%) than in the control group (Figure 3). The activity of CAT, at the exposure to the 1 mg Cd/kg feed was lower after 3 and 10 months (7- and 2.7-fold respectively) and unaffected due to the longer treatment, whereas at the exposure to

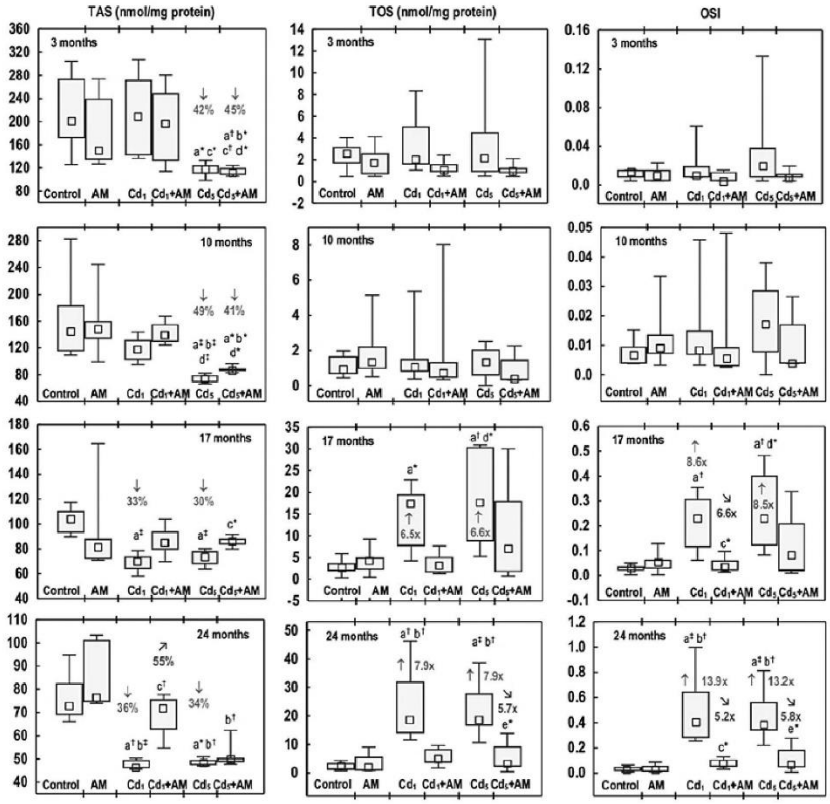

Figure 2. Total antioxidative status (TAS), total oxidative status (TOS), and oxidative stress index (OSI) in the kidney of female rats. The animals were treated with cadmium (Cd) in the feed at the concentration of 0, 1, or 5 mg/kg (Control, Cd_1, and Cd_5 groups) and/or 0.1% extract from the berries of *Aronia melanocarpa* L.. (AM, Cd_1+AM, and Cd_5+AM groups) for 3, 10, 17, and 24 months. Data are shown as median, 25–75% confidence interval, and minimum and maximum values for eight animals (except for seven females in the AM, Cd_1, and Cd_5 groups after 24 months). Statistically significant differences (Kruskal-Wallis test) compared to: a – Control group, b – AM group, c – Cd_1 group, d – Cd_1+AM group, and e – Cd_5 group, where $^*p < 0.05$, $^\dagger p < 0.01$, and $^\ddagger p < 0.001$, are marked. The factors of changes or percentage changes compared to the control group (↓, decrease; ↑, increase) or the adequate group treated with Cd alone (↘, decrease; ↗, increase) are indicated by the numerical values below or above the bars.

5 mg Cd/kg feed, it was decreased (3.7- – 15-fold) throughout the whole experiment (Figure 3). In the Cd_1 group, an increase (6.9 times) in the activity of GR was noted after 24 months, whereas in the Cd_5 group, the activity of this enzyme was enhanced (3 times) after 17 months (Figure 4). Throughout the study, the activity of GPx at both levels of exposure to Cd was lower than in the control group (2–9.2 times in the Cd_1 group and 2.8–8.8 times in the Cd_5 group) (Figure 4). There were no differences in the activities of the measured antioxidative enzymes in the renal tissue at particular time points between the Cd_1 group and the Cd_5 group, except for lower SOD activity after 3 months (by 33%) and GR activity after 24 months (by 71%) and higher (1.8-fold) GR activity after 17 months in the Cd_5 group (Figures 3 and 4).

The supplementation with AM of the animals that were not treated with Cd (AM group) had no impact on the activities of all measured antioxidative enzymes (Figures 3 and 4). The administration of the extract during the low-level and moderate exposure to Cd prevented all changes in the activities of the antioxidative enzymes, induced by this heavy metal, except for CAT activity in the Cd_5+AM group which after 10 months was decreased (3.2-fold) compared to the control group. The administration of AM to the animals treated with the 1 mg Cd/kg feed for

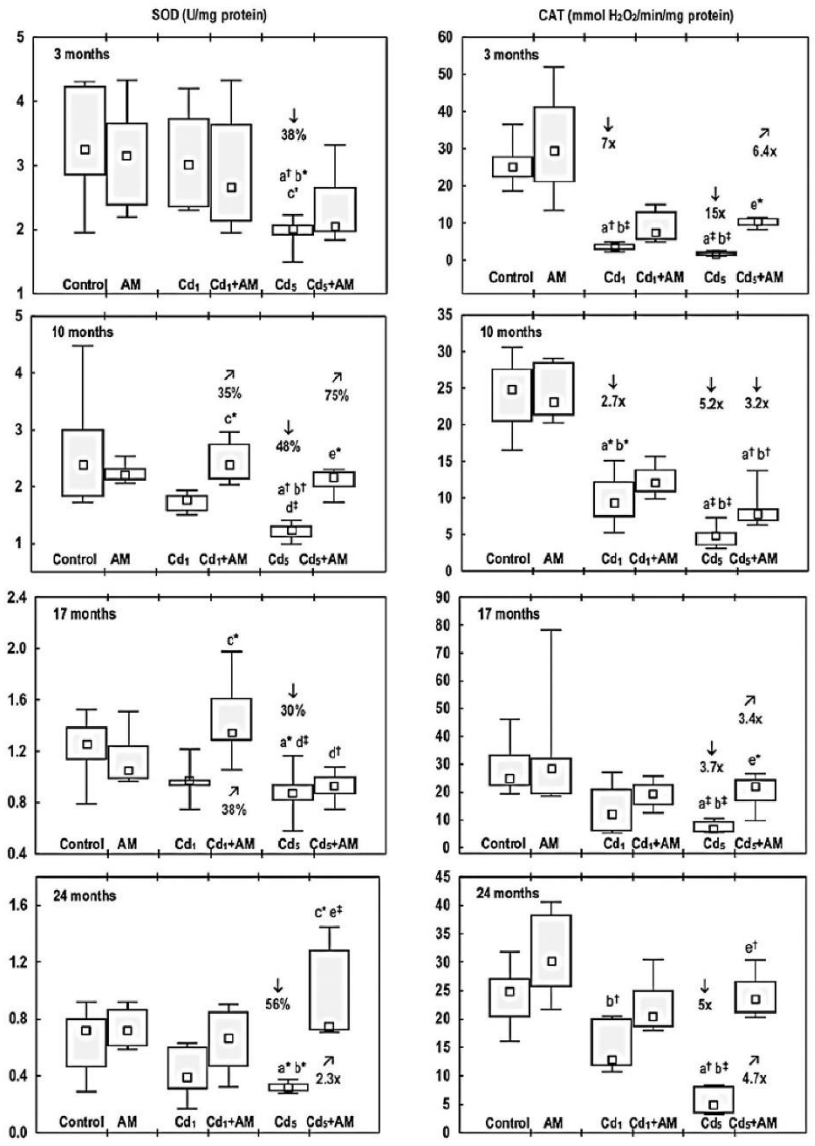

Figure 3. The activity of superoxide dismutase (SOD) and catalase (CAT) in the kidney of female rats. The animals were treated with cadmium (Cd) in the feed at the concentration of 0, 1, or 5 mg/kg (Control, Cd_1, and Cd_5 groups) and/or 0.1% extract from the berries of *Aronia melanocarpa* L. (AM, Cd_1+AM, and Cd_5+AM groups) for 3, 10, 17, and 24 months. Data are shown as median, 25–75% confidence interval, and minimum and maximum values for eight animals (except for seven females in the AM, Cd_1, and Cd_5 groups after 24 months). Statistically significant differences (Kruskal-Wallis test) compared to: a – Control group, b – AM group, c – Cd_1 group, d – Cd_1+AM group, and e – Cd_5 group, where $^{*}p < 0.05$, $^{\dagger}p < 0.01$, and $^{\ddagger}p < 0.001$, are marked. The factors of changes or percentage changes compared to the control group (↓, decrease) or the adequate group treated with Cd alone (↗, increase) are indicated by the numerical values below or above the bars.

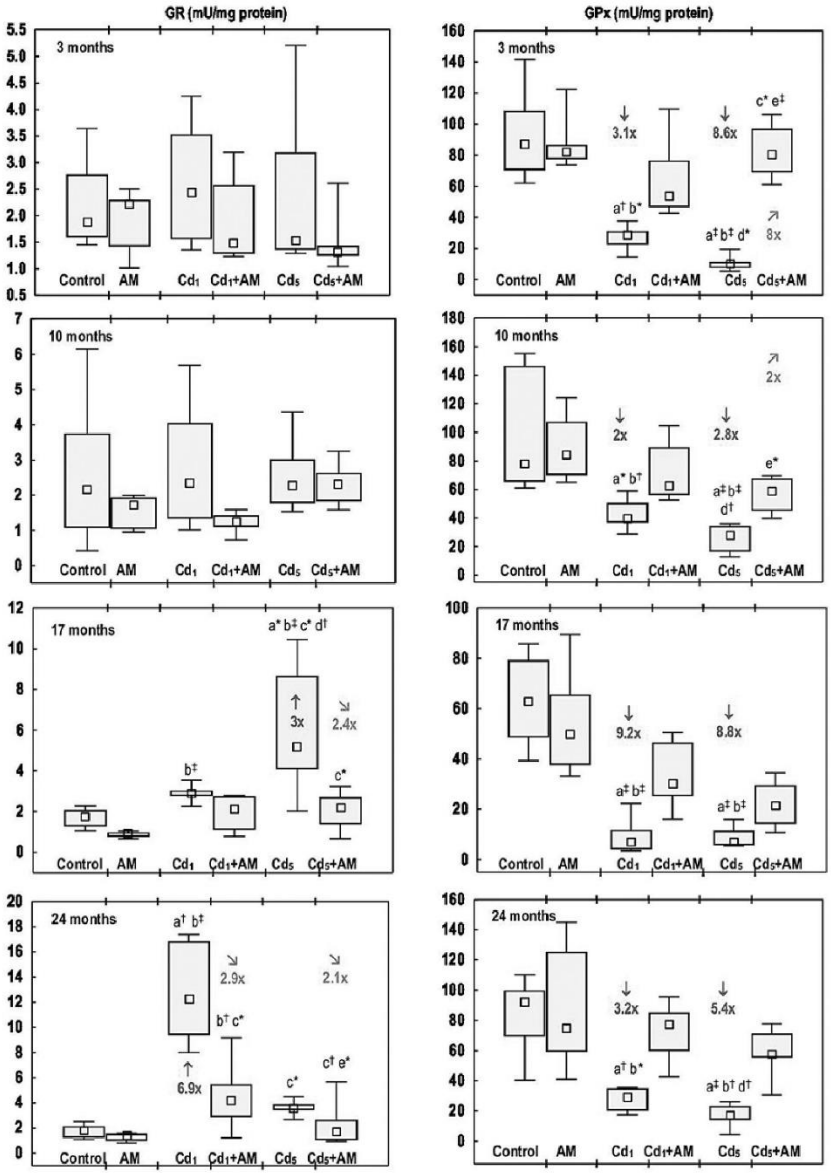

Figure 4. The activity of glutathione reductase (GR) and glutathione peroxidase (GPx) in the kidney of female rats. The animals were treated with cadmium (Cd) in the feed at the concentration of 0, 1, or 5 mg/kg (Control, Cd_1, and Cd_5 groups) and/or 0.1% extract from the berries of *Aronia melanocarpa* L. (AM, Cd_1+AM, and Cd_5+AM groups) for 3, 10, 17, and 24 months. Data are shown as median, 25–75% confidence interval, and minimum and maximum values for eight animals (except for seven females in the AM, Cd_1, and Cd_5 groups after 24 months). Statistically significant differences (Kruskal-Wallis test) compared to: a – Control group, b – AM group, c – Cd_1 group, d – Cd_1+AM group, and e – Cd_5 group, where $^*p < 0.05$, $^\dagger p < 0.01$, and $^\ddagger p < 0.001$, are marked. The factors of changes compared to the control group (↓, decrease; ↑, increase) or the adequate group treated with Cd alone (↘, decrease; ↗, increase) are indicated by the numerical values below or above the bars.

10 and 17 months resulted in an increase in the activity of SOD (by 35% and 38%), respectively, compared to the respective group receiving Cd alone but the activity did not differ compared to the control group (Figure 3). Moreover, the activity of GR in the Cd_5+AM group after 24 months was lower (2.1-fold) compared to the Cd_5 group (Figure 4).

Glutathione homeostasis

The kidney concentration of GSH in the animals fed with the diet containing 1 mg Cd/kg for 17 and 24 months was lower than in the control group (4.7- and 2.4-fold, respectively), whereas at the higher level of exposure, the concentration was decreased, compared to the control group, throughout the whole experiment (2.6–7.3 times) (Figure 5). The concentration of GSSG was increased after 10 months in the Cd_1 group (2.3-fold) and after 24 months in the Cd_1 group and Cd_5 group (3.7- and 3.4-fold, respectively) (Figure 5). At both levels of exposure to Cd, the GSH/GSSG ratio was lower than in the control group (3.8–9.2 times) throughout the study, except for the Cd_1 group after 3 months in which the ratio did not differ compared to the control group (Figure 5). There were no differences in the concentrations of GSII and GSSG, and the GSII/GSSG ratio between the Cd_1 group and the Cd_5 group (Figure 5).

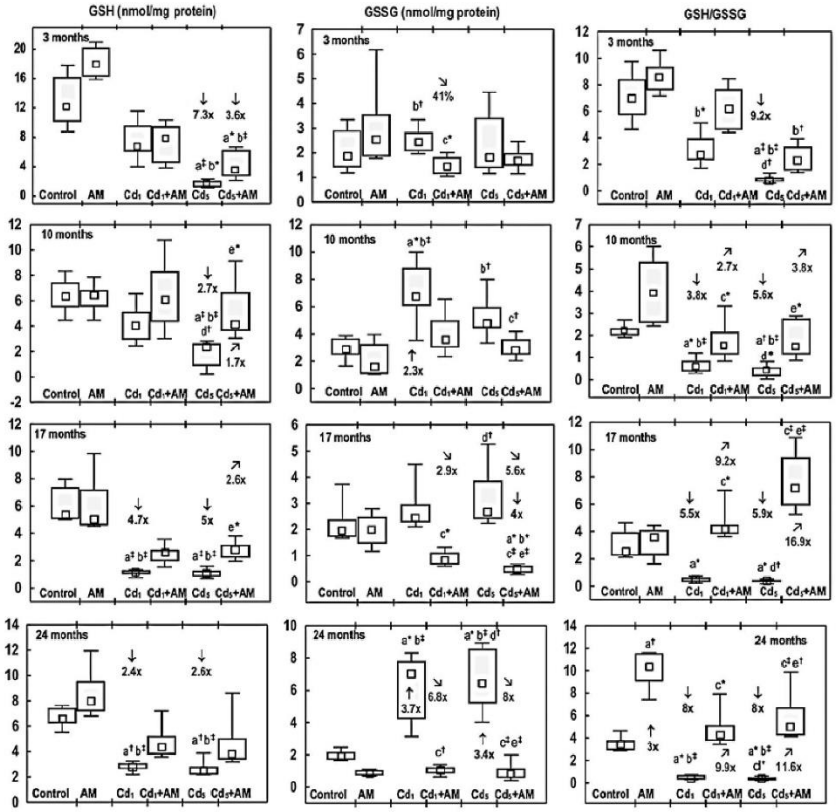

Figure 5. The concentration of reduced glutathione (GSII) and oxidated glutathione (GSSG), as well as their ratio (GSH/GSSG) in the kidney of female rats. The animals were treated with cadmium (Cd) in the feed at the concentration of 0, 1, or 5 mg/kg (Control, Cd_1, and Cd_5 groups) and/or 0.1% extract from the berries of *Aronia melanocarpa* L. (AM, Cd_1+AM, and Cd_5+AM groups) for 3, 10, 17, and 24 months. Data are shown as median, 25–75% confidence interval, and minimum and maximum values for eight animals (except for seven females in the AM, Cd_1, and Cd_5 groups after 24 months). Statistically significant differences (Kruskal-Wallis test) compared to: a – Control group, b – AM group, c – Cd_1 group, d – Cd_1+AM group, and e – Cd_5 group, where $^{*}p < 0.05$, $^{\dagger}p < 0.01$, and $^{\ddagger}p < 0.001$, are marked. The factors of changes or percentage changes compared to the control group (↓, decrease; ↑, increase) or the adequate group treated with Cd alone (↘, decrease(↗, increase) are indicated by the numerical values below or above the bars.

The administration of AM alone had no impact on glutathione homeostasis in the kidney estimated based on the concentrations of GSH and GSSG, and their ratio, except for 3-fold higher GSH/GSSG ratio after 24 months. However, the extract administration during the low-level and moderate exposure to Cd prevented this heavy metal-induced disorder in this homeostasis, except for the decrease in GSH concentration in the Cd_5 group after 3 months (Figure 5). Moreover, the concentration of GSSG in the Cd_5+AM group after 17 months was lower compared to both the Cd_5 group (5.6-fold) and the control group (4-fold) (Figure 5).

TRx concentration

The exposure to the 1 mg Cd/kg feed had no impact on the concentration of TRx in the renal tissue (Figure 6). The treatment with the 5 mg Cd/kg feed resulted in a decrease (by 53%) in TRx concentration after 3 months and an increase (by 41%) after 24 months (Figure 6). The concentration of TRx in the Cd_5 group after 3 months was lower (by 44%) compared to the Cd_1 group but after the longer exposure, there was no difference in the value of this parameter between the two groups (Figure 6).

In the Cd_1+AM group, the concentration of TRx after 10 months was lower (by 39%) compared to the control group. After 24 months, it was lower (by 31%) than in the Cd_1 group but did not differ compared to the control group. The 24-month administration of AM during the moderate exposure to Cd prevented this heavy metal-induced increase in TRx concentration, whereas its 3-month administration did not counteract the impact of Cd (Figure 6).

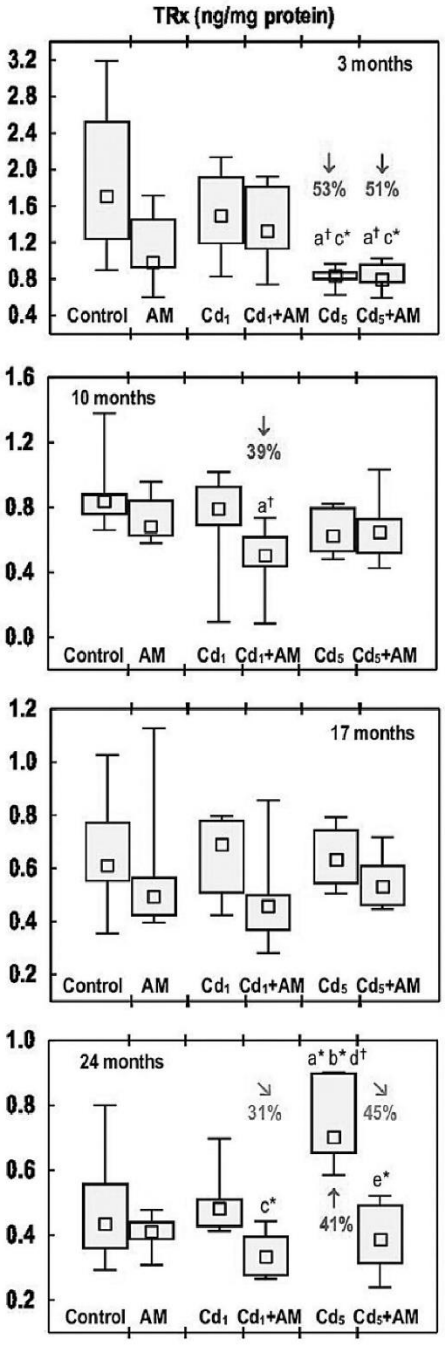

Figure 6. The concentration of thioredoxin (TRx) in the kidney of female rats. The animals were treated with cadmium (Cd) in the feed at the concentration of 0, 1, or 5 mg/kg (Control, Cd_1, and Cd_5 groups) and/or 0.1% extract from the berries of *Aronia melanocarpa* L. (AM, Cd_1+AM, and Cd_5+AM groups) for 3, 10, 17, and 24 months. Data are shown as median, 25–75% confidence interval, and minimum and maximum values for eight animals (except for seven females in the AM, Cd_1, and Cd_5 groups after 24 months). Statistically significant differences (Kruskal-Wallis test) compared to: a – Control group, b – AM group, c – Cd_1 group, d – Cd_1+AM group, and e – Cd_5 group, where $^*p < 0.05$, and $^\dagger p < 0.01$, are marked. The percentage changes compared to the control group (↓, decrease, ↑, increase) or the adequate group treated with Cd alone (↘, decrease) are indicated by the numerical values below or above the bars.

H_2O_2 concentration

In the animals maintained on the diet containing 1 and 5 mg Cd/kg for 3, 10, and 17 months, the kidney concentration of H_2O_2 was within the range of the control group; however, after 24 months it was increased (4.9- and 4.6-fold, respectively) (Figure 7). Throughout the study, there was no difference in H_2O_2 concentration between the Cd_1 and Cd_5 groups (Figure 7).

In the Cd_1+AM group and the Cd_5+AM group, the concentration of this compound throughout the study was lower (from 31% to 7.2-fold) compared to the respective group treated with Cd alone, and after 3 months it was even lower (by 54% and 63%, respectively) than in the control animals. The 24-month supplementation with the extract at both levels of exposure to Cd prevented this xenobiotic-induced increase in the concentration of H_2O_2 (Figure 7).

H3 XOD and MPO concentrations

The low-level exposure to Cd had no impact on the concentrations of XOD and MPO, except for an increase in MPO concentration after 17 and 24 months (by 81% and 2.4-fold, respectively) (Figure 8). At the moderate exposure to this xenobiotic, both parameters were unaffected after 3 and 10 months but they increased (74% to 4 times) due to the longer (17 and 24 months) treatment (Figure 8). There were no differences in the concentrations of XOD and MPO between the Cd_1 and Cd_5 groups, except for higher (2.1-fold) XOD concentration in the Cd_5 group after 24 months (Figure 8).

The administration of AM prevented the impact of Cd on the concentration of MPO. Moreover, the concentrations of XOD and MPO in the Cd_1+AM group after 10 months and in the Cd_5+AM group after 3 and 10 months were lower (by 40–73%) compared to the respective groups treated with Cd alone

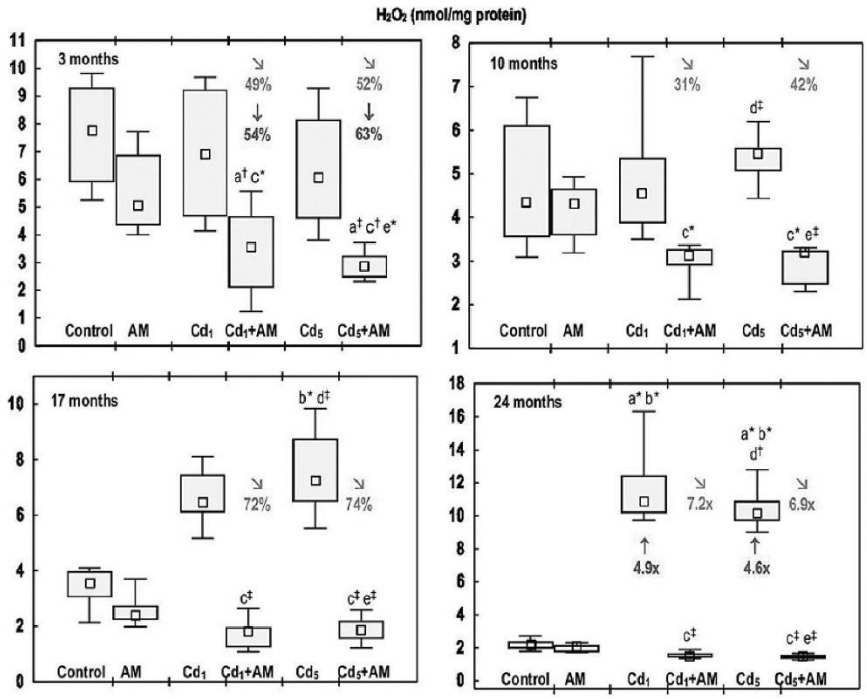

Figure 7. The concentration of hydrogen peroxide (H_2O_2) in the kidney of female rats. The animals were treated with cadmium (Cd) in the diet at the concentration of 0, 1, or 5 mg/kg (Control, Cd_1, and Cd_5 groups) and/or 0.1% extract from the berries of *Aronia melanocarpa* L. (AM, Cd_1+AM, and Cd_5+AM groups) for 3, 10, 17, and 24 months. Data are shown as median, 25–75% confidence interval, and minimum and maximum values for eight animals (except for seven females in the AM, Cd_1, and Cd_5 groups after 24 months). Statistically significant differences (Kruskal-Wallis test) compared to: a – Control group, b – AM group, c – Cd_1 group, d – Cd_1+AM group, and e – Cd_5 group, where $^*p < 0.05$, $^\dagger p < 0.01$, and $^\ddagger p < 0.001$, are marked. The factors of changes or percentage changes compared to the control group (↓, decrease, ↑, increase) or the adequate group treated with Cd alone (↘, decrease) are indicated by the numerical values below or above the bars.

and in the case of the concentration of XOD in the Cd_5+AM group after 10 months also compared to the control group (by 36%) (Figure 8).

The relationships between the indices of the oxidative/reductive status of the kidney and Cd concentration in this organ

In the female rats that were not administered AM (the control group and the Cd_1 and Cd_5 groups), negative dependencies were found between Cd concentration in the kidney and TAS, the activities of SOD, CAT, and GPx, the concentrations of GSH and TRx, and the GSH/GSSG ratio in this organ (Table 1). Moreover, positive correlations were noted between this toxic element concentration and TOS, OSI, GR activity, and the concentrations of GSSG and H_2O_2 in the kidney (Table 1). A lack of dependence between Cd concentration and the concentrations of XOD and MPO was found (Table 1).

In the animals receiving AM alone and during the treatment with Cd (AM, Cd_1+AM, and Cd_5+AM groups), negative dependencies occurred between Cd concentration and TAS, the activities of SOD and GPx, and the concentrations of GSH, GSSG, TRx, XOD, MPO, and H_2O_2 in the kidney (Table 1). TOS and OSI positively correlated with the kidney

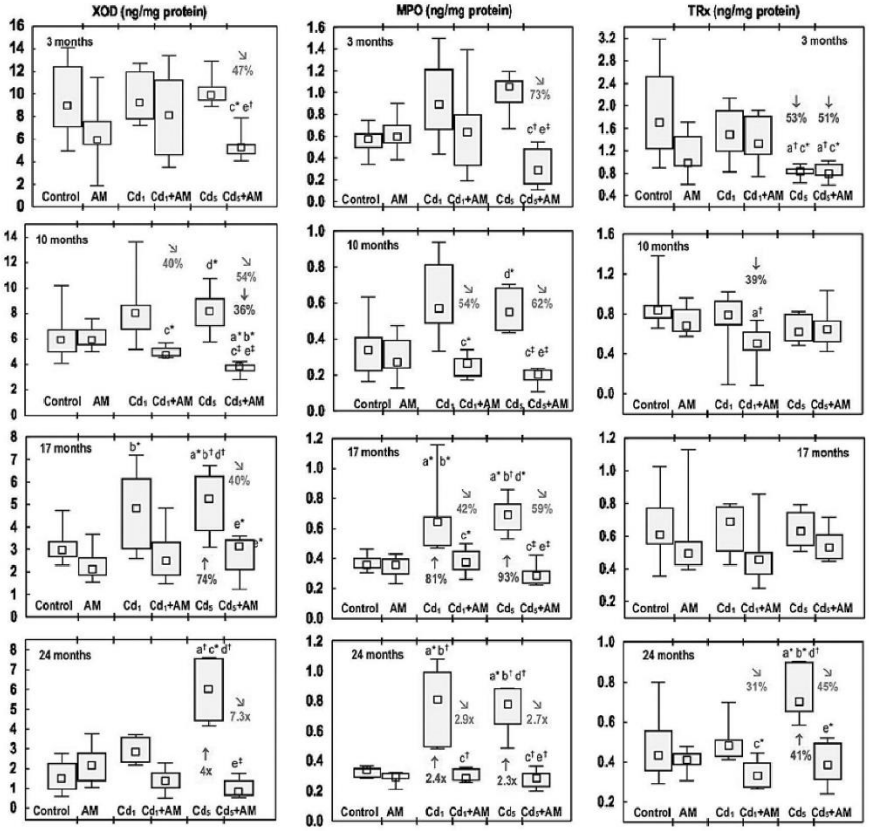

Figure 8. The concentrations of xanthine oxidase (XOD) and myeloperoxidase (MPO) in the kidney of female rats. The animals were treated with cadmium (Cd) in the feed at the concentration of 0, 1, or 5 mg/kg (Control, Cd_1, and Cd_5 groups) and/or 0.1% extract from the berries of *Aronia melanocarpa* L. (AM, Cd_1+AM, and Cd_5+AM groups) for 3, 10, 17, and 24 months. Data are shown as median, 25–75% confidence interval, and minimum and maximum values for eight animals (except for seven females in the AM, Cd_1, and Cd_5 groups after 24 months). Statistically significant differences (Kruskal-Wallis test) compared to: a – Control group, b – AM group, c – Cd_1 group, d – Cd_1+AM group, and e – Cd_5 group, where $^*p < 0.05$, $^\dagger p < 0.01$, and $^\ddagger p < 0.001$, are marked. The factors of changes or percentage changes compared to the control group (↓, decrease, ↑, increase) or the adequate group treated with Cd alone (↘, decrease) are indicated by the numerical values below or above the bars.

Table 1. Relationships between the investigated biomarkers of the oxidative/reductive kidney status and cadmium (Cd) concentration in this organ of female rats administered or not with a 0.1% extract from the berries of *Aronia melanocarpa* L. (AM)

Parameter	Regression analysis	Cd in the kidney of rats	
		Not administered with AM	Administered with AM
TAS	βp R^2	−0.437 ‡ 0.182	−0.450 ‡ 0.194
TOS	βp R^2	0.535 ‡ 0.278	0.443 ‡ 0.188
OSI	βp R^2	0.483 ‡ 0.225	0.502 ‡ 0.244
SOD	βp R^2	−0.446 ‡ 0.190	−0.400 ‡ 0.147
CAT	βp R^2	−0.445 ‡ 0.189	NS
GPx	βp R^2	−0.494 ‡ 0.236	−0.460 ‡ 0.203
GR	β^p R^2	0.242 * 0.048	NS
GSH	βp R^2	−0.516 ‡ 0.258	−0.400 ‡ 0.149
GSSG	β^p R^2	0.286 † 0.072	−0.310 † 0.084
GSH/GSSG	βp R^2	−0.485 ‡ 0.227	NS
TRx	βp R^2	−0.230 * 0.044	−0.290 † 0.071
H_2O_2	βp R^2	0.360 ‡ 0.120	−0.430 ‡ 0.173
XOD	βp R^2	NS	−0.410 ‡ 0.159
MPO	βp R^2	NS	−0.280 † 0.071

Cd concentrations in the kidney of the rats subjected to necropsy after 3, 10, 17, and 24 months have already been published (14). The results of the analysis of regression are presented as the β coefficient, R^2, and the level of statistical significance (p, where $^*p < 0.05$, $^\dagger p < 0.01$, and $^\ddagger p < 0.001$). NS, a lack of relationship ($p > 0.05$). All groups not administered with AM were included in the analysis (the control group that received the fodder containing 0.0584 ± 0.0049 mg Cd/kg and the Cd_1 and Cd_5 groups maintained on the feed containing 1 and 5 mg Cd/kg, respectively). All groups administered with AM were included in the analysis (the AM, Cd_1+AM, and Cd_5+AM groups).

concentration of Cd. Moreover, there were no dependencies between this heavy metal concentration in the kidney and the activities of CAT and GR, and the GSH/GSSG ratio (Table 1).

The relationships between the kidney TAS, TOS, and OSI and markers of this organ damage

Numerous correlations between the main indices of the oxidative/reductive status of the kidney and biomarkers of damage to the tubules and glomeruli, as well as markers of inflammatory processes in this organ were noted in the animals that were not administered with AM (the control group and the Cd_1 and Cd_5 groups) (Table 2). The kidney TAS negatively correlated with the concentrations of KIM-1 and β2-MG, the activities of NAG and ALP, ACR, total protein concentration in the urine (PCR), and urea concentration in the serum, whereas TOS and OSI positively correlated with these markers of kidney injury. A positive dependence occurred between TAS and creatinine clearance and negative relationships were noted between TOS and OSI and this marker of glomerular function. Moreover, positive dependencies were revealed between the kidney TAS and the concentrations of inflammatory markers such as macrophage inflammatory protein 1 alpha (MIP1a) and Bax in this organ, as well

as between TOS and OSI and the concentration of chemerin. In the animals that were supplemented with AM, negative relationships between the kidney TAS and β2-MG concentration in the urine, ALP activity in the urine, ACR, and PCR, and positive dependencies between TAS and urea concentration in the serum, and the kidney concentrations of chemerin, MIP1a, and Bax were noted. Positive correlations occurred between the kidney TOS and OSI and ACR and uric acid concentration in the serum. Moreover, TOS and OSI negatively correlated with the concentrations of MIP1a and Bax in the kidney.

Table 2. Relationships between the kidney total antioxidative status (TAS), total oxidative status (TOS), and oxidative stress index (OSI) and biomarkers of this organ damage in the female rats administered or not with a 0.1% extract from the berries of *Aronia melanocarpa* L. (AM)

Parameter	Regression analysis	Not Administered with AM			Administered with AM		
		TAS	TOS	OSI	TAS	TOS	OSI
Markers of tubular damage							
KIM-1 in the urine	βp R^2	−0.270 † 0.062	0.386 ‡ 0.140	0.333 † 0.101	NS	NS	NS
β2-MG in the urine	βp R^2	−0.540 ‡ 0.279	0.639 ‡ 0.402	0.662 ‡ 0.432	−0.370 ‡ 0.128	NS	NS
NAG in the urine	βp R^2	−0.490 ‡ 0.232	0.677 ‡ 0.452	0.793 ‡ 0.625	NS	NS	NS
ALP in the urine	βp R^2	−0.470 † 0.217	0.469 ‡ 0.212	0.552 ‡ 0.297	−0.250 * 0.053	NS	NS
Markers of tubular damage							
ACR	βp R^2	−0.570 ‡ 0.318	0.764 ‡ 0.580	0.762 ‡ 0.575	−0.330 † 0.118	0.226 * 0.041	0.233 * 0.044
PCR	βp R^2	−0.620 ‡ 0.374	0.646 ‡ 0.411	0.705 ‡ 0.491	−0.360 ‡ 0.118	NS	NS
Creatinine clearance	βp R^2	0.541 † 0.285	−0.330 † 0.096	−0.340 † 0.106	NS	NS	NS
Uric acid in the serum	βp R^2	NS	0.372 ‡ 0.129	0.342 ‡ 0.107	NS	0.336 ‡ 0.103	0.362 ‡ 0.121
Uric acid in the urine	βp R^2	NS	NS	NS	NS	NS	NS
Urea in the serum	βp R^2	−0.250 * 0.050	0.379 ‡ 0.135	0.428 ‡ 0.174	0.220 * 0.318	NS	NS
Urea in the urine	βp R^2	NS	NS	NS	NS	NS	NS
Inflammatory markers							
Chemerin in the kidney	βp R^2	NS	0.259 * 0.057	0.278 † 0.067	0.469 ‡ 0.211	NS	NS
MIP1a in the kidney	βp R^2	0.532 ‡ 0.276	NS	NS	0.866 ‡ 0.747	−0.360 ‡ 0.118	−0.450 ‡ 0.193
Bax in the kidney	βp R^2	0.374 ‡ 0.130	NS	NS	0.784 ‡ 0.610	−0.210 * 0.032	−0.260 † 0.060

The results of the analysis of regression are presented as the β coefficient, R^2, and the level of statistical significance (p, where $^*p < 0.05$, $^\dagger p < 0.01$, and $^\ddagger p < 0.001$). NS, a lack of relationship ($p > 0.05$). All groups not administered with AM were included in the analysis (the control group that received the fodder containing 0.0584 ± 0.0049 mg Cd/kg and the Cd_1 and Cd_5 groups maintained on the feed containing 1 and 5 mg Cd/kg, respectively). All groups administered with AM were included in the analysis (the AM, Cd_1+AM, and Cd_5+AM groups). ACR, albumin concentration in the urine adjusted for creatinine concentration; ALP, alkaline phosphatase; Bax, Bcl2-associated X protein; KIM-1, kidney injury molecule 1; MIP1a, macrophage inflammatory protein 1 alpha; NAG, N-acetyl-β-D-glucosaminidase; PCR, total protein concentration in the urine adjusted for creatinine concentration; β2-MG, beta2-microglobulin

Mutual relationships between the indices of the oxidative/reductive status of the kidney

In both, the rats that were not administered with AM (the control group and the Cd_1 and Cd_5 groups) and those receiving AM alone and during the treatment with Cd (AM, Cd_1+AM, and Cd_5+AM groups), numerous mutual negative relationships were noted between the investigated biomarkers of the antioxidative and oxidative status, including the negative dependencies between TAS of the kidney and TOS and OSI (Table 3).

DISCUSSION

The current study is the first to show that even low-level long-term exposure to Cd leads to disruption of the oxidative/reductive balance and development of oxidative stress in the kidney, whereas consumption of an extract from the berries of *A. melanocarpa* protects against these effects. Since the study was performed in the *in vivo* model well reflecting current exposure of the worldwide general population to Cd, based on the findings it seems possible that environmental exposure to this xenobiotic may disturb the balance between oxidants and antioxidants in the human kidney and lead to oxidative stress in this organ. Furthermore, this article also provides evidence that these outcomes of Cd action may be counteracted by the use of a well-known due to its strong antioxidative properties natural product i.e., extract from *A. melanocarpa* berries.

The results of epidemiological studies (9–13), as well as our recent findings (21) from the research, performed in the experimental model of the current environmental exposure to Cd in industrialized countries used in the present paper, show that the low-level exposure to this toxic element poses a risk of kidney damage. It is known that the mechanism of Cd nephrotoxicity is multidirectional and that oxidative stress is one of the main pathways of the toxic action of this xenobiotic, including damage to the kidney (10, 13, 22). Numerous studies have found, that this heavy metal may induce oxidative stress and oxidative modifications of cellular macromolecules and cellular structures in the kidney, but at higher than in the present study levels of exposure and its higher concentrations in the blood, urine, and/or kidney (26–31). The detailed mechanism of the injurious impact of Cd on this organ at low exposure is still unknown; however, the results of the present study together with numerous dependencies between the main indices of the oxidative/antioxidative status (TAS, TOS, and OSI) and markers of tubular and glomerular damage in the animals exposed to this xenobiotic show that this organ injury may be related to the prooxidative Cd properties and the destruction of the oxidative/reductive balance in the renal tissue.

Under chronic, even low-level, exposure Cd is accumulated in the kidney (10, 14, 32). Initially, it is stored in the epithelial cells of the kidney tubules in the form of unharmful complexes with metallothionein (MT), but these complexes (Cd-MT) are characterized by short half-life and are decomposed with the release of Cd ions (Cd^{2+}) capable of exerting toxic action via various mechanisms, including first of all induction of oxidative stress (10, 33). Although Cd is not a redox-active agent and is unable to generate free radicals (FR) and reactive oxygen species (ROS) directly, this element may destroy the oxidative/reductive balance and lead to the development of oxidative stress indirectly by the pathways such as weakening of the enzymatic and non-enzymatic antioxidative barrier, inducing the activities of oxidases, increasing the concentrations of prooxidants, and causing injury to the mitochondria, which are considered the target cellular organelles for the toxic action of this xenobiotic (10, 13, 17, 22). After penetrating the mitochondrial membranes, Cd^{2+} ions interfere with the electron transport chain, resulting in electron leakage and increased generation of ROS. Moreover, they affect the course of metabolic processes in the mitochondria, such as respiration and the Krebs cycle. As a result, relatively large amounts of H_2O_2 may be released and the oxidative status of cells and tissues can enhance (22). Via the indirect mechanisms, Cd^{2+} ions contribute to the production of nitryl, hydroxyl ($^{\bullet}OH$), and $O_2^{\bullet-}$, which initiate further processes of the generation of FR and ROS like the Fenton reaction (22). The Cd-caused weakening of the enzymatic and non-enzymatic defence mechanisms, including a reduction in the content of GSH, favours the development of oxidative stress (10, 22).

The measurements performed in the present study revealed that the low-level and moderate exposure to Cd weakened both the enzymatic and non-enzymatic antioxidative barriers and enhanced the level of prooxidants in the kidney leading to disruption of the balance between the processes of oxidation and reduction and as a result the development of oxidative stress in this organ. Although there was no difference in the values of particular parameters of the oxidative/antioxidative status of the renal tissue between the Cd_1 and Cd_5 groups, with only a few exceptions, and OSI (the main marker of oxidative stress) at both levels of exposure was increased to almost the same extent compared to the control

Table 3. Mutual relationships between the investigated parameters describing the kidney oxidative/reductive status in the female rats administered (*italic*) or not administered with a 0.1% extract from the berries of *Aronia melanocarpa* L. (AM)

Parameter	Regression analysis	TAS	TOS	OSI	SOD	CAT	GPx	GR	GSH	GSSG	GSH/GSSG	TRx	H_2O_2	XOD	MPO
TAS	βp	———	*−0.250 **	*−0.380* ‡	*0.802* ‡	*NS*	*0.367* ‡	*NS*	*0.519* ‡	*0.412* ‡	*NS*	*0.830* ‡	*0.811* ‡	*0.855* ‡	*0.610* ‡
	R^2		*0.051*	*0.138*	*0.640*		*0.125*		*0.262*	*0.161*		*0.686*	*0.654*	*0.728*	*0.365*
TOS	βp	−0.440 ‡	———	*0.951* ‡	*−0.360* †	*NS*	*−0.270* †	*0.296* †	*NS*	*−0.270* †	*NS*	*−0.210 **	*−0.290* †	*−0.290* †	*NS*
	R^2	0.186		*0.904*	*0.122*		*0.063*	*0.078*		*0.063*		*0.035*	*0.074*	0.076	
OSI	βp	−0.480 ‡	0.971‡	———	*−0.460* †	*NS*	*−0.280* †	*0.352* ‡	*−0.220 **	*−0.340* ‡	*−0.230 **	*−0.310* †	*−0.390* ‡	*−0.400* ‡	*NS*
	R^2	0.227	0.943		*0.200*		*0.069*	*0.114*	*0.040*	*0.107*	*0.041*	*0.085*	*0.140*	*0.153*	
SOD	βp	0.949 ‡	−0.480 ‡	−0.520 ‡	———	*−0.220 **	*0.300* †	*−0.240 **	*0.486* ‡	*0.509* ‡	*NS*	*0.715* ‡	*0.778* ‡	*0.778* ‡	*0.499* ‡
	R^2	0.899	0.219	0.261		*0.038*	*0.080*	*0.046*	*0.228*	*0.251*		*0.506*	*0.601*	*0.601*	*0.241*
CAT	βp	NS	NS	NS	NS	———	*NS*	*NS*	*0.314* †	*NS*	*0.315* †	*NS*	*NS*	*NS*	*NS*
	R^2								*0.088*		*0.089*				
GPx	βp	0.473 ‡	−0.400 ‡	0.360 ‡	0.442 ‡	0.680 ‡	———	*NS*	*0.382* ‡	*NS*	*NS*	*0.262 **	*0.393* ‡	*0.402* ‡	*NS*
	R^2	0.216	0.150	0.123	0.187	0.457			*0.137*			*0.059*	*0.145*	*0.152*	
GR	βp	−0.270 †	0.561 ‡	0.611 ‡	−0.280 †	NS	NS	———	*NS*	*NS*	*NS*	*NS*	*NS*	*NS*	*NS*
	R^2	0.060	0.307	0.367	0.068										
GSH	βp	0.717 ‡	−0.390 ‡	−0.380 ‡	0.640 ‡	0.518 ‡	0.685 ‡	−0.250 *	———	*0.369* ‡	*0.434* ‡	*0.465* ‡	*0.671* ‡	*0.376* ‡	*0.485* ‡
	R^2	0.508	0.140	0.132	0.403	0.260	0.463	0.054		*0.127*	*0.179*	*0.208*	*0.445*	*0.132*	*0.227*
GSSG	βp	−0.330 †	0.316 †	0.409 ‡	−0.310 †	−0.270 †	−0.210 *	0.326 †	−0.290 †	———	*−0.480* ‡	*0.206 **	*0.522* ‡	*0.338* ‡	*NS*
	R^2	0.100	0.090	0.158	0.086	0.064	0.033	0.097	0.077		*0.221*	*0.032*	*0.264*	*0.105*	
GSH/ GSSG	βp	0.641 ‡	−0.380 ‡	−0.390 ‡	0.587 ‡	0.530 ‡	0.615 ‡	−0.290†	0.911 ‡	−0.510 ‡	———	*NS*	*NS*	*NS*	*0.236 **
	R^2	0.405	0.136	0.144	0.337	0.273	0.372	0.072	0.828	0.257					*0.046*
TRx	βp	0.836 ‡	−0.210 *	−0.250 *	0.793 ‡	NS	0.343 ‡	NS	0.667 ‡	NS	0.574 ‡	———	*0.693* ‡	*0.775* ‡	*0.691* ‡
	R^2	0.695	0.033	0.054	0.625		0.108		0.439		0.322		*0.474*	*0.596*	*0.458*
H_2O_2	βp	NS	0.611 ‡	0.636 ‡	NS	−0.280 †	−0.330 †	0.570 ‡	NS	0.382 †	NS	0.244 *	———	*0.703* ‡	*0.477* ‡
	R^2		0.366	0.398		0.070	0.096	0.318		0.137		0.049		*0.488*	*0.219*
XOD	βp	0.617 †	−0.280 †	−0.310 †	0.680 ‡	−0.370 ‡	NS	−0.210 *	0.203 *	NS	NS	0.631 ‡	0.215 *	———	*0.570* †
	R^2	0.374	0.066	0.084	0.456	0.126		0.034	0.031			0.392	0.036		*0.317*
MPO	βp	NS	NS	NS	NS	−0.560 ‡	−0.470 ‡	0.212 *	NS	NS	−0.210 *	0.247 *	0.466 †	0.450 ‡	———
	R^2					0.301	0.211	0.035			0.035	0.051	0.208	0.194	

The groups not administered with AM included in the analysis were the control group that received the fodder containing 0.0584 ± 0.0049 mg Cd/kg and the Cd_1 and Cd_5 groups maintained on the feed containing 1 and 5 mg Cd/kg, respectively. The groups administered with AM included in the analysis were the AM, Cd_1+AM, and Cd_5+AM groups. The results of the analysis of regression are presented as the β coefficient, R^2, and the level of statistical significance (p, where *$p < 0.05$, †$p < 0.01$, and ‡$p < 0.001$). NS, *NS* a lack of relationship ($p > 0.05$)

group, the detailed analysis of the results allowed for the conclusion that the prooxidative impact of Cd was dependent on the level of exposure and its duration and the weakening of the antioxidative defence mechanisms preceded the enhancement in the oxidative status of the renal tissue. Numerous negative dependencies between Cd concentration in the kidney and indices of the antioxidative status (TAS, the activities of SOD, CAT, and GPx, the concentrations of GSH and TRx, and the GSH/GSSG ratio), as well as positive relationships between this xenobiotic concentration and the indices of the oxidative status (TOS, OSI, and the concentrations of GSSG and H_2O_2) confirm the dependence between the level of exposure to this heavy metal and the intensity of oxidative stress in this organ. The fact that in the Cd_5 group, TAS of the renal tissue was decreased after 3 and 10 months, while its TOS and OSI were unaffected shows that Cd may first weaken the antioxidative capacity of the renal tissue. Initially, in spite of decreased TAS, the antioxidative barrier was sufficient to remain the oxidative status at the proper level. However, the continuation of the exposure resulted in further weakening of the enzymatic and non-enzymatic antioxidative barrier and enhanced the presence of FR and ROS in the kidney cells leading to destroying the oxidative/antioxidative balance and development of oxidative stress.

Regardless of the cause, a decrease in the activities of antioxidative enzymes such as SOD, CAT, and GPx results in increased concentrations of FR and ROS in the cells. CAT and GPx are accountable for the detoxification of H_2O_2, whereas SOD catalyses the dismutation of $O_2^{\bullet-}$ (16, 27, 34). In addition, GPx together with GR, are enzymes involved in the metabolism of GSH. The first enzyme promotes the oxidation of GSH to GSSG, while the second is responsible for the reduction of GSSG to GSH (34). Thus, the decrease in the activities of antioxidative enzymes noted in the animals exposed to Cd resulted in an enhancement of the amount of FR and ROS in the renal cells, as was reflected in the increased concentration of H_2O_2 and the value of TOS in the Cd_1 and Cd_5 groups. The Cd-induced decrease in the activities of SOD, CAT, and GPx might result from interactions between this toxic element and bioelements such as zinc (Zn), copper (Cu), and manganese (Mn) present in the active centre of SOD (ZnCu-SOD, Mn-SOD), selenium necessary for GPx activity, and iron inhered in the active centre of CAT (34). Such an explanation seems very probable in the case of SOD activity as previous research by our team has revealed that the maintenance of rats on the diet containing 1 and 5 mg Cd/kg disturbed the kidney homeostasis of Cu, Zn, and Mn (17, 35). In discussing the impact of the low-level and moderate exposure to Cd on the activity of SOD in the kidney it is very important to underline that recently, at both levels of the treatment with Cd we have reported an increase in the activity of Mn-SOD in the mitochondria of the kidney, that might result from both an increased concentration of Mn in these organelles and the stimulation of defense mechanisms and/or adaptive response of these cellular structures against Cd-mediated enhanced generation of ROS (17, 34). However, the measurements performed in the present study have revealed that, in spite of the enhanced activity of the mitochondrial Mn-SOD, the total activity of SOD in the kidney cells was unaffected or decreased due to the exposure to Cd, depending on its intensity and duration. The unchanged or even decreased total SOD activity in the kidney despite the enhanced activity of Mn-SOD seems to indicate that the activity of ZnCu-SOD in the kidney might be importantly decreased.

The results of the present study allow for the conclusion that the involvement of Cd in the development of kidney damage at low-to-moderate exposure, including the induction of oxidative stress, may be related to the decreased concentration of GSH, which is the main non-enzymatic antioxidant playing an important role in the inactivation of organic and inorganic FR, ROS and xenobiotics, including prooxidants such as Cd. Due to the presence of the sulfhydryl group (–SH group) in its structure, GSH is capable of complexing Cd^{2+} ions into inert form preventing in this way their toxic action. It is important to emphasize, that TRx being a protein possessing two –SH groups plays a similar role as GSH (16). The decreased concentration of GSH in the kidney in the Cd_1 and Cd_5 groups might result from the use of this compound in the processes of detoxification of Cd accumulated in the renal tissue and this heavy metal generated FR and ROS. The increased concentration of GSSG and elevated GSH/GSSG ratio confirm enhanced GSH utilization. The increased activity of GR, noted at some time points at both levels of exposure to Cd, might be a compensative mechanism aimed at restoring GSH from its oxidized form.

The increased concentration of H_2O_2 noted in the kidney at both levels of exposure to Cd shows that this compound was not enough sufficiently detoxified due to this heavy metal-induced inhibition of the activities of CAT and GPx. Furthermore, the increase in the concentration of H_2O_2 might result from the increased concentrations of MPO and XOD since reactions catalysed by both enzymes are the

source of H_2O_2 (and other reactive oxidants, including $O_2^{\bullet-}$) (16). Enhanced concentration of H_2O_2 in the kidney cells may have very negative consequences because this compound is the source of the generation of extremely reactive $^{\bullet}OH$ in the presence of ions of transition metals such as ion of Cu(I) i.e. Cu^{+} and Fe^{2+} (the Fenton reaction) (6, 15, 28) and in this way facilitates the development of oxidative stress as was reflected in the enhanced values of TOS and OSI in the renal tissue.

Because the disruption of the oxidative/antioxidative balance in the cells, regardless of the cause, results in oxidative damage to the critical biological macromolecules (lipids, proteins, and nucleic acids) and structures (including cellular organelles and cellular membranes), ultimately leading to the cell death (9, 10, 22), the revealing in the present study that even low-level exposure to Cd induces oxidative stress in the kidney indicates that such exposure can have a damaging impact on the kidney. Thus, it seems possible that the recently reported by us in the rats exposed to the 1 and 5 mg Cd/kg feed (21) damaging impact of Cd on the structure and function of the kidney might result, at least partially, from its prooxidative action and induction of oxidative stress in the renal tissue. The numerous dependencies noted between the main indices of the oxidative/reductive status such as TAS, TOS, and OSI and markers of tubular and glomerular damage in the animals that did not receive AM under trace-to-moderate-level exposure to Cd (control group, Cd_1 group, and Cd_5 group) indicate the involvement of oxidative stress in the development of this heavy metal-induced kidney damage and this issue will be the subject of our further paper. Moreover, it is worth emphasizing that oxidative stress might also contribute to the development of inflammatory processes noted in the kidney of these animals (21). It is known that there exists a dependence between the oxidative/antioxidative balance and inflammatory processes in the cells (36, 37). Moreover, the coexistence of oxidative stress and inflammation in the kidney leads to stimulating the damage to this organ as a result of synergic deleterious processes (37). The oxidative stress can induce inflammation through the activation of multiple pathways, including up-regulation of the production of proinflammatory cytokines like chemerin and acute phase proteins, whereas the state of inflammation can trigger oxidative stress (36, 37). Thus, the positive correlations between the kidney TOS and OSI and the concentration of chemerin in this organ together with the finding that at both levels of exposure to Cd, the destroying the oxidative/antioxidative balance preceded the increase in chemerin concentration (as well as in Bax concentration) (21) suggest that the prooxidative action of Cd might result in the development of inflammation in the kidney. On the other hand, the positive correlation between the kidney TAS and inflammatory markers such as MIPa or Bax may indicate that Cd-induced mild inflammation might trigger defence mechanisms in this organ, resulting in an increase in the antioxidative capacity.

The current study provided new data on the impact of low-to-moderate exposure to Cd on the oxidative/antioxidative balance in the kidney. The revealing that even low exposure to this xenobiotic, corresponding to the current general population exposure, can induce oxidative stress in the kidney and that under such exposure the antioxidative barrier may be the first target of its toxicity is an important finding. However, our primary interest and thus the most important finding consist in the protective effect of the administration of AM on the oxidative/antioxidative status of this organ during chronic low-level and moderate treatment with Cd.

The fact that in the animals that received AM and were maintained on the fed without Cd addition, the oxidative/antioxidative balance of the kidney was unaffected indicates that the enhanced intake of aronia products in the case of a lack of exposure to an agent characterized by prooxidative capacity did not influence the oxidative/reductive balance in the renal tissue. This confirms the safety of the prolonged supplementation with the AM shown in our previous studies (14–21). The 3-fold higher, compared to the control group, GSH/GSSG ratio in the AM group after 24 months may imply more effective antioxidative protection of GSH in this organ as a result of the extract intake.

It is very important to emphasize, that the intake of AM in the amount that did not influence the oxidative/antioxidative balance in the absence of exposure to a prooxidative agent such as Cd was effective in the complete counteraction of this toxic element-induced destroying the oxidative/antioxidative balance. The administration of AM during the low-level and moderate repeated exposure to Cd improved both the enzymatic and nonenzymatic (prevented disruption in glutathione homeostasis) antioxidative barrier. It is also interesting to note that the administration of AM under exposure to Cd, which resulted in the increase in TOS and the development of oxidative stress completely prevented these effects. Although not always all parameters of the oxidative/antioxidative status were within the range of proper values in the animals co-administered with Cd and AM, the finding that

long-term (17- and 24-month) administration of AM prevented the Cd-induced changes in the values of TAS, TOS, and OSI, which are the best indicators of the oxidative/antioxidative status, allows for the conclusion that an enhancement of daily consumption of aronia polyphenols by several times compared to their average intake among the worldwide general population, may be effective in counteracting oxidative stress in the kidney under low-level and moderate exposure. The extract administration under the exposure to Cd not only improved the antioxidative potential of the kidney but also decreased the concentration of a reactive oxygen compound such as H_2O_2 and the concentrations of oxidases (MPO and XOD) involved in its generation in this organ. It is worth mentioning that the beneficial impact of the co-administration of AM under the treatment with Cd on the oxidative/antioxidative balance in the kidney might be related to the lower concentration of H_2O_2 compared to the animals treated with this toxic element alone. Even if the exposure to Cd alone had no impact on the concentration of H_2O_2, the extract administration resulted in its decrease.

Taking into account the results of the present study and the findings of our previous investigations conducted in these animals, the beneficial influence of AM on the oxidative/reductive status of the kidney under the treatment with Cd may be explained by a direct effect of the extract resulting from its high antioxidative potential (2, 3, 7, 15, 16, 19) and an indirect action related to interactions between the extract ingredients and this toxic element (2, 3, 6, 14). The first effect can be caused, first of all, by a variety of polyphenolic compounds present in the extract, which can act as hydrogen donors capable of inactivating oxidative species and playing a major role in the restoration of nonenzymatic antioxidants. Most common polyphenolic compounds found in AM belong to the group of anthocyanins, proanthocyanidins, phenolic acids, and flavonoids. These compounds are characterized by high antioxidative capacity due to the direct neutralization of FR and ROS by donating an electron or hydrogen atom and acting as radical scavengers of the lipid peroxidation chain reactions by donating an electron to the FR, making radicals less reactive, resulting in the suppression of the chain reactions (1–4, 18). It is important to note that the beneficial effect of AM may also be due to the presence within the extract of components other than polyphenols that have been shown to combat the toxic action of Cd, such as β-carotene, essential microelements, fiber, pectin, triterpenes, and vitamins C and E (1–4, 18).

The protective impact of the administration of AM under the exposure to Cd may also be, at least partially, explained by the indirect action resulting in lower accumulation of this xenobiotic in the kidney (14). It has been noted, that Cd concentration in this organ in the animals supplemented with AM in the case of feeding with the diet containing 1 mg Cd/kg for 3 and 10 months was lower by 29% and 9.5% compared to the animals that did not receive the extract, whereas in the case of the 3–24-month treatment with the 5 mg Cd/kg feed, Cd concentration was lower by 5.6–14% (14). Due to the presence of hydroxyl (–OH) groups, these compounds are capable of chelating ions of divalent metals, including Cd^{2+} ions, and thus may decrease gastrointestinal absorption of this xenobiotic. Moreover, the increased urinary excretion of Cd, previously noted in the animals exposed to 5 mg Cd/kg feed due to the administration of AM (14, 21), shows that polyphenols may also form complexes with Cd^{2+} ions absorbed from the gastrointestinal tract and in this way accelerate their elimination from the body. Due to the lower body burden of Cd, the concentration of this xenobiotic in the kidney was lower, and as a result, the effects of its toxic action, including destroying the oxidative/antioxidative balance, were also less advanced. Because the destructive impact of Cd on the oxidative/antioxidative status is involved in the mechanisms of kidney damage, revealing in the present study that the co-administration of AM prevented oxidative stress in this organ allows for the conclusion that it will also counteract, at least partially, its damage by Cd. The negative relationships between the kidney TAS and some of the markers of tubular (β2-MG concentration and ALP activity in the urine) and glomerular (ACR and PCR) damage noted in the rats supplemented with AM confirm that the beneficial impact of the extract on the kidneys recently reported by us in these animals (21) might be related to its antioxidative properties.

Although in the available literature, there are no other studies investigating the protective action of natural-based agents against the damaging impact of Cd on the kidney in the animal model well reflecting current exposure of the general population to this heavy metal, there are numerous reports showing protective action of isolated polyphenols (ferulic acid, quercetin) or products rich in these compounds (extract from *Fragaria ananassa* – strawberry, fruit extract from *Cleistocalyx nervosum var. paniala* – Ma Kiang, stem bark extract from *Irvingia gabonensis* – Bush mango, leaf extract from *Coriandrum sativum* – coriander, aqueous

extract from the bulb of *Allium cepa* – onion and leaf infusions from *Camelia sinensis* – tea) against Cd-induced disruption of the oxidative/antioxidative barrier and oxidative stress development in this organ (26–31, 38–40).

We are aware of both the scientific value and practical implications of our research, as well as of its limitations. Because females are more susceptible to the toxic action of Cd than males, the experimental model used in our research used female rats, hence the findings apply to the female kidney. Thus, for a more complete investigation of the unfavourable impact of the low-to-moderate exposure to Cd on the kidney and the beneficial effect of AM, it seems necessary to perform a similar study on male rats. Furthermore, it is necessary to investigate the involvement of the ability of AM to counteract Cd-induced oxidative stress in the kidney in the protection from this organ damage. A study of this kind has been carried out by us and the findings will be published shortly.

CONCLUSIONS

The results of the present study provide the first evidence, that moderate and even low-level prolonged exposure to Cd can disrupt the oxidative/reductive balance in the kidney and lead to the development of oxidative stress in the organ. The mechanism of the harmful action of this xenobiotic involves a decrease in the activity of antioxidative enzymes, disruption of glutathione homeostasis, and enhancement of the level of oxidants. Moreover, it has been revealed, for the first time, that products from the berries of *A. melanocarpa*, due to the abundance of compounds possessing antioxidative capacity, can protect the kidney from the development of oxidative stress caused by exposure to Cd. Based on the findings it seems possible that the recently noted by us in the experimental model protective effect of the administration of AM against the damaging impact of Cd on the structure and function of the kidney might result, at least partially, from its antioxidative potential and prevention of the development of oxidative stress in the renal tissue.

Acknowledgments

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11. Zgoda Lokalnej Komisji Etycznej do Spraw Doświadczeń na Zwierzętach

UCHWAŁA NR 60 /2009 w sprawie wniosku nr 2009/57
z dnia 21.12.2009 r.
Lokalnej Komisji Etycznej do Spraw Doświadczeń na Zwierzętach w Białymstoku

§ 1

Na podstawie art. 30 ust. 1 pkt 1 ustawy z dnia 21 stycznia 2005r. o doświadczeniach na zwierzętach (Dz. U. Nr 33, poz. 289) i § 14 ust. 3 rozporządzenia Ministra Nauki i Informatyzacji z dnia 29 lipca 2005r. w sprawie Krajowej Komisji Etycznej do Spraw Doświadczeń na Zwierzętach oraz lokalnych komisji etycznych do spraw doświadczeń na zwierzętach (Dz. U. Nr 153, poz. 1275), po rozpatrzeniu wniosku, pt.:

Ocena możliwości zastosowania związków polifenolowych z *Aronia melanocarpa* w zapobieganiu uszkodzeniom układu kostnego w warunkach chronicznej ekspozycji na kadm – badania w modelu doświadczalnym *in vivo*.

z dnia 14.12.2009 r. złożonego przez **dr hab. n. med. Małgorzatę Michalinę Brzóska**, z Zakładu Toksykologii Uniwersytetu Medycznego w Białymstoku lokalna komisja etyczna,

WYRAŻA ZGODĘ / ~~NIE WYRAŻA ZGODY~~

na przeprowadzenie doświadczeń na zwierzętach w zakresie wniosku.

§ 2

W wyniku rozpatrzenia wniosku, o którym mowa w § 1, lokalna komisja etyczna ustaliła, że:
1. Wniosek należy zaliczyć do kategorii:
Badania naukowe na zwierzętach
2. Najwyższy stopień inwazyjności proponowanych procedur nie przekracza wartości 2
3. Doświadczenia będą przeprowadzone na zwierzętach: szczury *Wistar-Crl:WI(Han)*, samice, 240 szt.
4. Doświadczenia będą przeprowadzone przez: dr hab. n. med. Małgorzatę Michalinę Brzóska, dr hab. n. med. Marię Jurczuk, dr n. biol. Małgorzatę Gałażyn – Sidorczuk, dr n. med. Joannę Rogalską, dr n. med. Alicję Roszczenko z Zakładu Toksykologii UMwB.

§ 3

Integralną część niniejszej uchwały stanowi uzasadnienie i kopia wniosku, o którym mowa w § 1.

Podpisy członków lokalnej komisji etycznej biorących udział w głosowaniu:

1. ..... Przewodnicząca LKE w Białymstoku
2. .....
3. .....
4. .....
5. .....
6. .....
7. .....
8. .....
9. .....

[Pieczęć lokalnej komisji etycznej]
UNIWERSYTET MEDYCZNY W BIAŁYMSTOKU
LOKALNA KOMISJA ETYCZNA
do Spraw Doświadczeń na Zwierzętach w Białymstoku
ZAKŁAD FIZJOLOGII DOŚWIADCZALNEJ
15-222 Białystok, ul. A. Mickiewicza 2A
tel./fax 0-85 748 56 99

Otrzymują:
1. Wnioskodawca,
2. Prorektor ds. Nauki UMwB
3. a/a

Pouczenie
Strona niezadowolona z niniejszej uchwały może wnieść odwołanie do Krajowej Komisji Etycznej do Spraw Doświadczeń na Zwierzętach w terminie 14 dni od dnia otrzymania uchwały.
Odwołanie składa się za pośrednictwem lokalnej komisji etycznej, która wydała uchwałę zgodnie z § 20 rozporządzenia Ministra Nauki i Informatyzacji z dnia 29 lipca 2005r. w sprawie Krajowej Komisji Etycznej do Spraw Doświadczeń na Zwierzętach oraz lokalnych komisji etycznych do spraw doświadczeń na zwierzętach (Dz. U. Nr 153 poz. 1275).

12. Oświadczenie autora rozprawy doktorskiej (*)

(*) – oryginał oświadczenia autora publikacji wchodzących w skład rozprawy doktorskiej znajduje się w przedmiotowej dokumentacji w Dziekanacie Wydziału Farmaceutycznego z Oddziałem Medycyny Laboratoryjnej Uniwersytetu Medycznego w Białymstoku

Mgr Nazar M. Smereczański
Zakład Toksykologii UMB

Białystok, 20.07.2023 r.

Oświadczenie autora

Oświadczam, iż mój udział w przygotowaniu publikacji:

1. **Smereczański N.M.**, Brzóska M.M.: Current levels of environmental exposure to cadmium in industrialized countries as a risk factor for kidney damage in the general population: A comprehensive review of available data. International Journal of Molecular Sciences, 2023, 24, 8413; doi.: 10.3390/IJMS24098413

wchodzącej w skład mojej rozprawy doktorskiej polegał na tworzeniu koncepcji pracy, dokonaniu przeglądu elektronicznych baz publikacji naukowych i zgromadzeniu piśmiennictwa w zakresie tematyki pracy, krytycznym przeglądzie zgromadzonego piśmiennictwa, tworzeniu manuskryptu oraz opracowywaniu tabel i schematów zamieszczonych w pracy i suplemencie do pracy, co określam jako 75% udziału w przygotowaniu wyżej wymienionej publikacji.

2. **Smereczański N.M.**, Brzóska M.M., Rogalska J., Hutsch T.: The protective potential of *Aronia melanocarpa* L. berry extract against cadmium-induced kidney damage: A study in an animal model of human environmental exposure to this toxic element. International Journal of Molecular Sciences, 2023, 24, 11647; doi.: 10.3390/ijms241411647

wchodzącej w skład mojej rozprawy doktorskiej polegał na tworzeniu koncepcji pracy, dokonaniu przeglądu piśmiennictwa w zakresie tematyki pracy, przygotowywaniu materiału do badań, przeprowadzaniu oznaczeń badanych parametrów w moczu, surowicy i homogenatach nerki, opracowywaniu wyników badań łącznie z ich analizą statystyczną, interpretacji wyników badań i ich przedstawianiu w pracy i suplemencie do pracy w formie rycin i tabel oraz pisaniu manuskryptu, co określam jako 60% udziału w przygotowaniu wyżej wymienionej publikacji.

3. **Smereczański N.M.**, Brzóska M.M., Rogalska J.: Protective effect of the extract from *Aronia melanocarpa* L. berries against cadmium-induced oxidative stress in the kidney: A study in an *in vivo* experimental model. Acta Poloniae Pharmaceutica – Drug Research, 2023, 80; doi.: 10.32383/appdr/169782

wchodzącej w skład mojej rozprawy doktorskiej polegał na tworzeniu koncepcji pracy, dokonaniu przeglądu piśmiennictwa w zakresie tematyki pracy, przeprowadzaniu oznaczeń badanych parametrów, opracowywaniu wyników badań łącznie z ich analizą statystyczną, interpretacji wyników badań i ich przedstawianiu w pracy w formie rycin i tabel oraz pisaniu manuskryptu, co określam jako 65% udziału w przygotowaniu wyżej wymienionej publikacji.

Nazar Smereczański

Podpis autora rozprawy doktorskiej (czytelny)

Małgorzata M. Brzóska

Podpis promotora (czytelny)

13. Oświadczenia współautorów rozprawy doktorskiej (*)

(*) – oryginały oświadczeń współautorów publikacji wchodzących w skład rozprawy doktorskiej znajdują się w przedmiotowej dokumentacji w Dziekanacie Wydziału Farmaceutycznego z Oddziałem Medycyny Laboratoryjnej Uniwersytetu Medycznego w Białymstoku

Prof. dr hab. Małgorzata M. Brzóska
Zakład Toksykologii
Uniwersytet Medyczny w Białymstoku

Białystok, 20.07.2023 r.

Oświadczenie współautora

Oświadczam, iż mój udział w przygotowaniu publikacji:

1. Smereczański N.M., **Brzóska M.M.**: Current levels of environmental exposure to cadmium in industrialized countries as a risk factor for kidney damage in the general population: A comprehensive review of available data. International Journal of Molecular Sciences, 2023, 24, 8413. doi.: 10.3390/IJMS24098413

wchodzącej w skład rozprawy doktorskiej Pana mgr Nazara M. Smereczańskiego polegał na współtworzeniu koncepcji pracy, nadzorze merytorycznym podczas przygotowywania pracy (pisania pracy oraz opracowywania rycin i tabel) oraz edycji finalnej wersji manuskryptu

2. Smereczański N.M., **Brzóska M.M.**, Rogalska J., Hutsch T.: The protective potential of *Aronia melanocarpa* L. berry extract against cadmium-induced kidney damage: A study in an animal model of human environmental exposure to this toxic element. International Journal of Molecular Sciences, 2023, 24, 11647. doi.: 10.3390/ijms241411647

wchodzącej w skład rozprawy doktorskiej Pana mgr Nazara M. Smereczańskiego polegał na współtworzeniu koncepcji pracy, pomocy i nadzorze merytorycznym podczas wykonywania badań, opracowywania i interpretacji wyników, przygotowywania manuskryptu (pisania pracy oraz opracowywania rycin i tabel) oraz edycji finalnej wersji pracy

3. Smereczański N.M., **Brzóska M.M.**, Rogalska J.: Protective effect of the extract from *Aronia melanocarpa* L. berries against cadmium-induced oxidative stress in the kidney: A study in an *in vivo* experimental model. Acta Poloniae Pharmaceutica – Drug Research, 2023, 80 (4). doi.: 10.32383/appdr/169782

wchodzącej w skład rozprawy doktorskiej Pana mgr Nazara M. Smereczańskiego polegał na współtworzeniu koncepcji pracy, pomocy i nadzorze merytorycznym podczas wykonywania badań, opracowywania i interpretacji wyników, przygotowywania manuskryptu (pisania pracy oraz opracowywania rycin i tabel) oraz edycji finalnej wersji pracy

Jednocześnie wyrażam zgodę na przedłożenie wyżej wymienionych prac przez Pana mgr Nazara M. Smereczańskiego jako część rozprawy doktorskiej w formie spójnego tematycznie cyklu prac opublikowanych w czasopismach naukowych.

Małgorzata Brzóska
Podpis (czytelny)

Dr n. med. Joanna Rogalska
Zakład Toksykologii
Uniwersytet Medyczny w Białymstoku

Białystok, 24.07.2023 r.

Oświadczenie współautora

Oświadczam, iż mój udział w przygotowaniu publikacji:

1. Smereczański N.M., Brzóska M.M., **Rogalska J.**, Hutsch T.: The protective potential of *Aronia melanocarpa* L. berry extract against cadmium-induced kidney damage: A study in an animal model of human environmental exposure to this toxic element. International Journal of Molecular Sciences, 2023, 24, 11647. doi: 10.3390/ijms241411647

wchodzącej w skład rozprawy doktorskiej Pana mgr Nazara M. Smereczańskiego polegał na pomocy podczas wykonywania badań oraz analizy statystycznej i graficznego opracowywania wyników

2. Smereczański N.M., Brzóska M.M., **Rogalska J.**: Protective effect of the extract from *Aronia melanocarpa* L. berries against cadmium-induced oxidative stress in the kidney: A study in an *in vivo* experimental model. Acta Poloniae Pharmaceutica – Drug Research, 2023, 80 (4). doi: 10.32383/appdr/169782

wchodzącej w skład rozprawy doktorskiej Pana mgr Nazara M. Smereczańskiego polegał na pomocy podczas wykonywania badań oraz analizy statystycznej i graficznego opracowywania wyników

Jednocześnie wyrażam zgodę na przedłożenie wyżej wymienionej pracy/prac przez Pana mgr Nazara M. Smereczańskiego jako część rozprawy doktorskiej w formie spójnego tematycznie cyklu prac opublikowanych w czasopismach naukowych.

Rogalska
.....
Podpis (czytelny)

dr n. med. Tomasz Hutsch
Katedra Patologii i Diagnostyki Weterynaryjnej,
Szkoła Główna Gospodarstwa Wiejskiego
w Warszawie
ALAB Bioscience

Białystok, 20.07.2023 r.

Oświadczenie współautora

Oświadczam, iż mój udział w przygotowaniu publikacji:

Smereczański N.M., Brzóska M.M., Rogalska J., Hutsch T. The protective potential of Aronia melanocarpa L. Berry Extract against Cadmium-induced Kidney Damage: A Study in an Animal Model of Human Environmental Exposure to this Toxic Element, International Journal of Molecular Sciences (MDPI), 2023, 24, 11647.

wchodzącej w skład rozprawy doktorskiej Pani/Pana mgr Nazara M. Smereczańskiego polegał na wykonaniu badań histopatologicznych nerek (przygotowanie szkiełek histopatologicznych, ocena mikroskopowa przygotowanych preparatów wraz z dokumentacją zdjęciową)

Jednocześnie wyrażam zgodę na przedłożenie wyżej wymienionej pracy/prac przez Pana mgr Nazara M. Smereczańskiego jako część rozprawy doktorskiej w formie spójnego tematycznie cyklu prac opublikowanych w czasopismach naukowych.

Tomasz Hutsch
.....
Podpis (czytelny)

14. Dorobek naukowy, dydaktyczny i organizacyjny

Wykształcenie

2018 – Uniwersytet Medyczny w Białymstoku, Wydział Farmaceutyczny z Oddziałem Medycyny Laboratoryjnej, Studia doktoranckie w dziedzinie nauk medycznych i nauk farmaceutycznych

2012 – 2018 – Uniwersytet Medyczny w Białymstoku, Wydział Farmaceutyczny z Oddziałem Medycyny Laboratoryjnej, Studia magisterskie, kierunek: Farmacja

Szkolenia i staże zawodowe

01.06.2017 r. – 30.09.2017 r. – staż w aptece Day Lewis (Liverpool, Wielka Brytania)

01.10.2017 r. – 30.03.2018 r. – staż w Aptece pod Lwem (Białystok)

10.11.2020 r. – szkolenie dla osób odpowiedzialnych za planowanie procedur i doświadczeń oraz ich przeprowadzanie, wykonujących procedury i dla osób uśmiercających zwierzęta wykorzystywane w procedurach (Białystok)

15.09.2021 r. – Warsztaty pt. „Model Zebrafish – przyszłość i wyzwanie. Warsztaty na temat metod utrzymywania i pracy z modelem Zebrafish" (Olsztyn)

Doświadczenie zawodowe

01.04.2018 r. – 30.06.2018 r. – Apteka Sieciowa, Góra Kalwaria – stanowisko: magister farmacji

01.07.2018 r. – 30.01.2020 r. – Apteka pod Lwem, Białystok – stanowisko: magister farmacji

01.10.2021 r. – obecnie – Zakład Toksykologii, Uniwersytet Medyczny w Białymstoku – stanowisko: asystent dydaktyczny

Działalność dydaktyczna

- ✓ Ćwiczenia laboratoryjne z toksykologii dla studentów Wydziału Farmaceutycznego z Oddziałem Medycyny Laboratoryjnej (kierunek: Farmacja, Analityka Medyczna, Kosmetologia I i II stopnia), Wydziału Nauk o Zdrowiu (kierunek: Ratownictwo Medyczne, Dietetyka, Zdrowie Publiczne, Zdrowie Publiczne i Epidemiologia) i Wydziału Lekarskiego (kierunek: lekarski)
- ✓ Ćwiczenia z toksykologii w języku angielskim dla studentów Medical Faculty na Wydziale Lekarskim z Oddziałem Stomatologii i Oddziałem Nauczania w Języku Angielskim
- ✓ Ćwiczenia z toksykologii w języku polskim i angielskim z wykorzystaniem platform nauczania zdalnego (Blackboard i MS Teams)

Działalność społeczna na rzecz promocji Uniwersytetu Medycznego w Białymstoku

2018 – 2023 – koordynator i aktywny uczestnik wszystkich akcji „Skonsultuj z Farmaceutą!" Polskiego Towarzystwa Studentów Farmacji (oddział w Białymstoku), mających na celu popularyzację postaw prozdrowotnych i udzielania informacji na temat zagrożeń dla zdrowia, jak astma, cukrzyca, nadciśnienie (lokalizacja akcji: Atrium Biała, Galeria „Alfa", Galeria „Jurowiecka")

20.05.2020 r. – Prowadzenie webinaru pt. „Farmaceuta za granicą: Wielka Brytania" we współpracy z Polskim Towarzystwem Studentów Farmacji

28.10.2020 r. – Prowadzenie webinaru pt. „Uzależnienia od leków OTC"

Wykaz publikacji stanowiących rozprawę doktorską

1) **Smereczański N.M.**, Brzóska M.M.: Current levels of environmental exposure to cadmium in industrialized countries as a risk factor for kidney damage in the general population: A comprehensive review of available data. International Journal of Molecular Sciences, 2023, 24 (9), 8413. doi.: 10.3390/IJMS24098413
IF = 5,6; MEiN = 140

2) **Smereczański N.M.**, Brzóska M.M., Rogalska J., Hutsch T.: The protective potential of *Aronia melanocarpa* L. berry extract against cadmium-induced kidney damage: A study in an animal model of human environmental exposure to this toxic element. International Journal of Molecular Sciences, 2023, 24 (14), 11647. doi.: 10.3390/ijms241411647
IF = 5,6; MEiN = 140

3) **Smereczański N.M.**, Brzóska M.M., Rogalska J.: Protective effect of the extract from *Aronia melanocarpa* L. berries against cadmium-induced oxidative stress in the kidney: A study in an *in vivo* experimental model. Acta Poloniae Pharmaceutica – Drug Research, 2023, 80 (4), praca w druku. doi.: 10.32383/appdr/16982
IF = 0,4; MEiN = 140

Wykaz innych publikacji naukowych

1) Brzóska M.M., Kozłowska M., Rogalska J., Gałażyn-Sidorczuk M., Roszczenko A., **Smereczański N.M.**: Enhanced zinc intake protects against oxidative stress and its consequences in the brain: a study in an *in vivo* rat model of cadmium exposure. Nutrients, 2021, 13 (2), 478. doi.: 10.3390/nu13020478
IF = 5,9; MEiN = 140

2) Brzóska M.M., Gałażyn-Sidorczuk M., Kozłowska M., **Smereczański N.M.**: The body status of manganese and activity of this element-dependent mitochondrial superoxide

dismutase in a rat model of human exposure to cadmium and co-administration of *Aronia melanocarpa* L. extract. Nutrients, 2022, 14 (22), 4773. doi.: 10.3390/nu14224773
IF = 5,9; MEiN = 140

3) Grudzińska M., Jakubowska K., Koda M., Kańczuga-Koda L., Kisielewski W., **Smereczański N.M.**, Rogoz-Jezierska N., Famulski W.: Systemic inflammation markers in blood samples of colorectal cancer patients. Progress in Health Sciences, 2020, 10 (1), 92-101. doi.: 10.5604/01.3001.0014.1917
MEiN = 20

4) Grudzińska M., Jakubowska K., Kańczuga-Koda L., Kisielewski W., Famulski W., **Smereczański N.M.**, Lomperta K., Płoński M.A., Rogoz-Jezierska N., Koda M.: Correlation of clinico-pathologic data with inflammatory cells infiltration in colorectal cancer. Progress in Health Sciences, 2020, 10 (1), 69-76. doi.: 10.5604/01.3001.0014.1902
MEiN = 20

Łączny dorobek naukowy
Sumaryczny wskaźnik oddziaływania Impact Factor (IF) = **24,206**
Sumaryczna liczba punktów MEiN = **740**

Wykaz doniesień zjazdowych

1) **Smereczański N.M.**, Brzóska M.M.: Wpływ ekstraktu z owoców *Aronia melanocarpa* L. na całkowity potencjał oksydacyjny i antyoksydacyjny nerek szczurów poddanych niskiej ekspozycji przewlekłej na kadm. VI Ogólnopolska Konferencja Studentów Medycyny Laboratoryjnej i Młodych Diagnostów „Wschodząca Diagnostyka", Białystok, 13.04.2019 r.
Prezentacja ustna w sesji doktoranckiej. Praca zajęła **2 miejsce** w sesji doktoranckiej.

2) **Smereczański N.M.**, Brzóska M.M.: The influence of the extract from *Aronia melanocarpa* L. berries on the total antioxidative and oxidative status of the rats' kidneys moderately exposed to cadmium. 14th Białystok International Medical Congress for Young Scientists, Białystok, 17.05.2019 r.
Prezentacja ustna podczas PhD Session. Praca zajęła **1 miejsce** w PhD Session.

3) **Smereczański N.M.**, Brzóska M.M.: Wpływ ekstraktu z owoców *Aronia melanocarpa* L. na stężenie glutationu zredukowanego i utlenionego w nerkach szczurów poddanych niskiej długotrwałej ekspozycji na kadm. VII Ogólnopolska Konferencja Studentów Medycyny Laboratoryjnej i Młodych Diagnostów „Wschodząca Diagnostyka", Białystok, 05.06.2021 r.
Prezentacja ustna w sesji doktoranckiej. Praca **wyróżniona**.

4) **Smereczański N.M.**, Brzóska M.M., Rogalska J.: Ekstrakt z owoców *Aronia melanocarpa* L. chroni przed uszkodzeniami oksydacyjnymi kwasów nukleinowych w nerkach

szczurów długotrwale eksponowanych na kadm. XIII Konferencja Naukowo - Szkoleniowa Polskiego Towarzystwa Toksykologicznego, Gdańsk, 16–17.09.2021 r. ePoster

5) **Smereczański N.M.**, Brzóska M.M.: Ocena wpływu ekstraktu z owoców aronii czarnoowocowej na stężenie wybranych metaloproteinaz i ich inhibitorów tkankowych w nerce w warunkach ekspozycji na kadm. XIV Naukowy Zjazd Polskiego Towarzystwa Farmaceutycznego, Lublin, 22–24.09.2021 r.
Prezentacja plakatowa

6) **Smereczański N.M.**, Brzóska M.M.: Wpływ ekstraktu z owoców *Aronia melanocarpa* L. na stopień nasilania stresu oksydacyjnego i uszkodzeń oksydacyjnych w nerce w warunkach ekspozycji przewlekłej na kadm – badania w modelu doświadczalnym *in vivo* narażenia środowiskowego człowieka na ten ksenobiotyk. Seminarium „Pierwiastki fizjologicznie istotne i toksyczne", Gugny, 13.05.2022 r.
Prezentacja ustna

Wykaz innych aktywności naukowych

- ✓ Kierownik czterech projektów realizowanych ze środków Uniwersytetu Medycznego w Białymstoku przeznaczonych na utrzymanie potencjału badawczego oraz na badania naukowe lub prace rozwojowe oraz zadania z nimi związane, służące rozwojowi młodych naukowców oraz uczestników studiów doktoranckich:

` ,,Ocena wpływu wodnego ekstraktu z owoców *Aronia melanocarpa* L. na stężenie 8-hydroksy-2'-deoksyguanozyny w nerkach szczurów w warunkach niskiej i umiarkowanej ekspozycji na kadm'' (nr projektu SUB/2/DN/19/001/2221)

,,Wpływ ekstraktu z owoców *Aronia melanocarpa* L. na stan oksydacyjno – redukcyjny nerki – badania w modelu doświadczalnym *in vivo* narażenia na kadm'' (nr projektu SUB/2/DN/20/002/2221)

,,Wpływ ekstraktu z owoców (*Aronia melanocarpa* L. na stężenie białka KIM-1w nerce i moczu – badania w modelu doświadczalnym narażenia populacji generalnej na kadm" (nr projektu SUB/2/DN/21/002/2221)

„Ocena wpływu ekstraktu z owoców *Aronia melanocarpa* L. na stężenie endotelialnej syntazy tlenku azotu – badania w modelu doświadczalnym *in vivo* narażenia populacji generalnej na kadm" (nr projektu SUB/2/DN/22/002/2221)

- ✓ Członek zespołu badawczego czterech projektów realizowanych ze środków Uniwersytetu Medycznego w Białymstoku przeznaczonych na utrzymanie potencjału badawczego oraz na badania naukowe lub prace rozwojowe oraz zadania z nimi

związane, służące rozwojowi młodych naukowców oraz uczestników studiów doktoranckich w roku 2019, 2020, 2022, 2023:

„Ocena wpływu ekstraktu z owoców *Aronia melanocarpa* na uszkodzenia oksydacyjne białek, lipidów i DNA w mózgu oraz strukturę i wybrane biomarkery funkcji nerek w warunkach ekspozycji na kadmu – badania w modelu doświadczalnym *in vivo* narażenia populacji generalnej na ten ksenobiotyk." Kierownik: Prof. dr hab. Małgorzata M. Brzóska (nr projektu: SUB/2/DN/19/006/2221)

„Ocena wpływu ekstraktu z owoców aronii czarnoowocowej (*Aronia melanocarpa* L.) na stężenie wybranych metaloproteinaz i ich inhibitorów tkankowych w nerce w warunkach ekspozycji na kadm – badania w modelu doświadczalnym *in vivo* narażenia populacji generalnej na ten ksenobiotyk." Kierownik: Prof. dr hab. Małgorzata M. Brzóska (nr projektu: SUB/2/DN/20/001/2221)

„Ocena wpływu ekstraktu z owoców aronii czarnoowocowej (*Aronia melanocarpa* L.) na stężenie wybranych cytokin antyresorpcyjnych i białek niekoloagenowych w tkance kostnej oraz markerów osteoliozy w surowicy i moczu w warunkach ekspozycji na kadm – badania w modelu doświadczalnym *in vivo* narażenia populacji generalnej na ten ksenobiotyk." Kierownik: Prof. dr hab. Małgorzata M. Brzóska (nr projektu: SUB/2/DN/22/001/2221)

„Ocena wybranych mechanizmów ochronnych wpływu ekstraktu z owoców aronii czarnoowocowej (*Aronia melanocarpa* L.) na tkankę kostną podczas narażenia na kadm – badania w modelu doświadczalnym *in vivo* niskiej i umiarkowanej ekspozycji środowiskowej człowieka na ten ksenobiotyk". Kierownik: Prof. dr hab. Małgorzata M. Brzóska (nr projektu: B.SUB.23.313)

- ✓ Nagroda naukowa III stopnia Rektora Uniwersytetu Medycznego w Białymstoku za osiągnięcia naukowe w 2021 r.