Supplementary Materials

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

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Table S1. The concentration of cadmium (Cd) in the blood, liver, and urine of rats receiving the extract from the berries of *Aronia melanocarpa* L. (AE) and/or Cd.[1, 2]

Group	Duration of the Experiment			
	3 Months	10 Months	17 Months	24 Months
	Blood (μg/L)			
Control	0.0691 ± 0.0079	0.0860 ± 0.0091	0.0743 ± 0.0052	0.0834 ± 0.0043
AE	0.0752 ± 0.0046	0.0802 ± 0.0087	0.0718 ± 0.0099	0.0861 ± 0.0038
Cd_1	0.1884 ± 0.0100*	0.1792 ± 0.0198*	0.2425 ± 0.0167***	0.2330 ± 0.0143**
Cd_1 + AE	0.1887 ± 0.0124*	0.1844 ± 0.0137*	0.2375 ± 0.0166***	0.2188 ± 0.0143**
Cd_5	1.0236 ± 0.066***	0.9394 ± 0.0439***	1.0339 ± 0.0266***	1.0467 ± 0.0508***
Cd_5 + AE	0.8298 ± 0.0544*** †††	0.7948 ± 0.0454*** †††	0.9319 ± 0.0413*** †††	0.8503 ± 0.0600*** †††
	Liver (μg/g)			
Control	0.0348 ± 0.0026	0.0231 ± 0.0011	0.0138 ± 0.0016	0.0137 ± 0.0015
AE	0.0312 ± 0.0014	0.0202 ± 0.0014	0.0145 ± 0.0019	0.014 ± 0.0016
Cd_1	0.1447 ± 0.0093***	0.199 ± 0.028***	0.211 ± 0.019***	0.364 ± 0.025***
Cd_1 + AE	0.0968 ± 0.007*** †††	0.179 ± 0.0179***	0.1913 ± 0.0168***	0.2275 ± 0.0335*** †
Cd_5	0.912 ± 0.053***	1.617 ± 0.112***	2.449 ± 0.178***	2.755 ± 0.089***
Cd_5 + AE	0.7427 ± 0.0045*** †††	1.4424 ± 0.0916*** †††	1.8479 ± 0.3144*** ††	2.4911 ± 0.1342*** ††
	Urine (μg/g of creatinine)			
Control	0.1387 ± 0.0103	0.1304 ± 0.0080	0.1491 ± 0.0103	0.1337 ± 0.0154
AE	0.1321 ± 0.0046	0.1364 ± 0.0043	0.1445 ± 0.0116	0.1357 ± 0.0070
Cd_1	0.2184 ± 0.0081**	0.1809 ± 0.0194**	0.2096 ± 0.0215*	0.2053 ± 0.0155*
Cd_1 + AE	0.2193 ± 0.0171**	0.1913 ± 0.0113**	0.2143 ± 0.0268*	0.2084 ± 0.0105*
Cd_5	0.5008 ± 0.0234***	0.4002 ± 0.0480***	0.4147 ± 0.0390***	0.4104 ± 0.0198***
Cd_5 + AE	0.6064 ± 0.0374*** †††	0.4997 ± 0.0400*** †††	0.5773 ± 0.0420*** †††	0.4994 ± 0.0438*** †††

[1] The rats received 0.1% aqueous AE and Cd in diet at the concentration of 1 or 5 mg/kg for 3–24 months.
[2] Data are represented as mean ± SE for 8 rats (except for 7 animals in the AE, Cd_1, and Cd_5 groups after 24 months). Statistically significant differences (ANOVA, Duncan's multiple range test) compared to the control group (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$) and respective group receiving Cd alone († $p < 0.05$, †† $p < 0.01$, ††† $p < 0.001$) are marked.

Table S2. The effect of the extract from the berries of *Aronia melanocarpa* L. (AE) on the concentration of cadmium (Cd) in the blood, liver and urine of rats. [1, 2, 3]

Duration (months)	Exposure to 1 mg Cd/kg diet			Exposure to 5 mg Cd/kg diet		
	Effect of Cd	Effect of Cd + AE	Effect of AE	Effect of Cd	Effect of Cd + AE	Effect of AE
			Blood			
3	↑ 2.7-fold	↑ 2.7-fold	↔	↑ 14.8-fold	↑ 12-fold	↙ 19%
10	↑ 2.1-fold	↑ 2.1-fold	↔	↑ 11-fold	↑ 9.2-fold	↙ 15%
17	↑ 3.3-fold	↑ 3.2-fold	↔	↑ 13.9-fold	↑ 12.5-fold	↙ 9.9%
24	↑ 2.8-fold	↑ 2.6-fold	↔	↑ 12.5-fold	↑ 10.2-fold	↙ 11%
			Liver			
3	↑ 4.2-fold	↑ 2.8-fold	↙ 33%	↑ 26-fold	↑ 21-fold	↙ 18%
10	↑ 8.6-fold	↑ 7.8-fold	↔	↑ 70-fold	↑ 62-fold	↙ 11%
17	↑ 15-fold	↑ 14-fold	↔	↑ 177-fold	↑ 134-fold	↙ 24%
24	↑ 27-fold	↑ 17-fold	↙ 37%	↑ 201-fold	↑ 182-fold	↙ 10%
			Urine			
3	↑ 57%	↑ 58%	↔	↑ 3.6-fold	↑ 4.4-fold	↗ 21.1%
10	↑ 39%	↑ 47%	↔	↑ 3-fold	↑ 3.8-fold	↗ 25%
17	↑ 41%	↑ 44%	↔	↑ 2.8%	↑ 3.9%	↗ 33%
24	↑ 53%	↑ 56%	↔	↑ 3.1-fold	↑ 3.7-fold	↗ 22%

[1] The rats received 0.1% aqueous AE and Cd in diet at the concentration of 1 or 5 mg/kg for 3–24 months.
[2] Table presents changes in Cd concentration ($p < 0.05$) compared to the control group: a percentage or factor of increase (↑), and the respective group receiving Cd alone: a percentage decrease (↙), increase (↗), or lack of statistically significant change (↔) $p > 0.05$; ANOVA, Duncan's multiple range test), are indicated.
[3] Detailed data on the impact of AE on the concentration of Cd in the blood, liver and urine of rats exposed to this heavy metal have already been published [27] and are presented in Table S1.

Table S3. Polyphenolic composition of the extract from the berries of *Aronia melanocarpa* L. (AE). [1, 2]

COMPOUND	Concentration (mg/g)
Total polyphenols	612.40 ± 3.33
Total anthocyanins	202.28 ± 1.28
Total proanthocyanidins	129.87 ± 1.12
Total phenolic acids	110.92 ± 0.89
Total flavonoids	21.94 ± 0.98
Chlorogenic acid	68.32 ± 0.08
Cyanidin 3-O-β-galactoside	80.07 ± 1.05
Cyanidin 3-O-α-arabinoside	33.21 ± 0.01
Cyanidin 3-O-β-glucoside	3.68 ± 0.01

[1] The concentrations of total polyphenols, total phenolic acids, flavonoids, proantocyanidins, and anthocyanins in the aronia extract by Adamed Consumer Healthcare were determined spectrophotometrically [27,28]. Ultra Performance Liquid Chromatography was used to evaluate the polyphenolic profile of the extract and quantificate chlorogenic acid and anthocyanins (cyanidin 3-O-β-galactoside, cyanidin 3-O-α-arabinoside, and cyanidin 3-O-β-glucoside [27].

[2] Data are represented as mean ± SE for 3 independent measurements.

Table S4. The intake of cadmium (Cd) and the extract from the berries of *Aronia melanocarpa* L. (AE) in particular experimental groups. [1, 2, 3, 4]

GROUP	Duration of the Experiment			
	3 Months	**10 Months**	**17 Months**	**24 Months**
	Daily Cd Intake (μg/kg b.w.)			
Control	4.709 ± 0.067	2.716 ± 0.060	2.582 ± 0.047	2.597 ± 0.051
AE	4.615 ± 0.065	2.935 ± 0.063	2.410 ± 0.046	2.764 ± 0.059
Cd_1	77.50 ± 1.264***	43.85 ± 0.60***	43.41 ± 0.64***	43.22 ± 0.85***
Cd_1 + AE	80.93 ± 1.06***	46.90 ± 0.84***	40.15 ± 0.77***	48.12 ± 0.94***
Cd_5	383.65 ± 4.44***	235.78 ± 2.55***	214.54 ± 2.83***	253.75 ± 8.63***
Cd_5 + AE	385.92 ± 4.91***	239.66 ± 1.43***	208.75 ± 3.04***	252.06 ± 9.29***
	Daily AE Intake (mg/kg b.w.)			
AE	91.53 ± 1.12	62.29 ± 1.87	53.22 ± 1.14	47.96 ± 1.56
Cd_1 + AE	97.47 ± 1.45	60.92 ± 1.07	55.00 ± 0.76	49.26 ± 1.57
Cd_5 + AE	92.41 ± 1.30	65.14 ± 1.77	56.02 ± 1.15	48.26 ± 2.07

[1] The rats received 0.1% aqueous AE and Cd in diet at the concentration of 1 or 5 mg/kg for 3–24 months.
[2] Data are represented as mean ± SE for 8 rats (except for 7 animals in the AE, Cd_1, and Cd_5 groups after 24 months).
[3] The intake of Cd in the control group and the group receiving AE alone was calculated based on this metal concentration determined by us in the standard diet (0.0584 mg/kg) [27], whereas this xenobiotic intake in the groups exposed to Cd (1 or 5 mg Cd/kg) was calculated based on its concentration in the feed declared by the manufacturer. Statistically significant differences (ANOVA, Duncan's multiple range test) compared to the control group (*** $p < 0.001$) are marked.
[4] The intake of polyphenols was calculated assuming that the AE contained 65.74% of these compounds (manufacturer's data). The intake of polyphenols in the control group, Cd_1 group, and Cd_5 group was 0. There were no statistically significant differences ($p > 0.05$; ANOVA, Duncan's multiple range test) in the intake of polyphenolic compounds between the AE, Cd_1 + AE, and Cd_5 + AE groups.

Table S5. Main and interactive effects of cadmium (Cd) and the extract from the berries of *Aronia melanocarpa* L. (AE) on the activities of superoxide dismutase (SOD) and catalase (CAT) in the liver of rats. [1, 2]

Duration (months)	Parameter	Exposure to 1 mg Cd/kg diet			Exposure to 5 mg Cd/kg diet		
		Main effect of Cd	Main effect of AE	Main effect of Cd + AE	Main effect of Cd	Main effect of AE	Main effect of Cd + AE
3	**SOD**	23.85***	NS	NS	-	-	-
	CAT	-	-	-	18.95***	NS	NS
10	**SOD**	32.75***	NS	NS	-	-	-
	CAT	NS	6.852*	NS	-	-	-
17	**SOD**	11.21**	NS	NS	NS	4.370*	7.152*
	CAT	5.191*	5.337*	NS	42.79***	NS	5.415*
24	**SOD**	-	-	-	NS	4.650*	8.478**
	CAT	29.48***	NS	7.723*	NS	NS	4.961*

[1] The rats received 0.1% aqueous AE and Cd in diet at the concentration of 1 or 5 mg/kg for 3–24 months.
[2] The results of the ANOVA/MANOVA analysis are presented as F values and the level of statistical significance (p). F values having $p < 0.05$ were considered statistically significant (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$). NS – not statistically significant ($p > 0.05$). In the case when a one way-analysis of variance (ANOVA, Duncan's multiple range test) revealed any influence of the co-administration of Cd and AE on the investigated parameter, a two-way analysis of variance (ANOVA/MANOVA, test F) was conducted in aim to discern possible interactive and independent impact of Cd and AE on this parameter.

Table S6. Main and interactive effects of cadmium (Cd) and the extract from the berries of *Aronia melanocarpa* L. (AE) on the activities of glutathione peroxidase (GPx) and glutathione reductase (GR) and the concentration of glutathione S-transferase (GST) in the liver of rats. [1, 2]

Duration (months)	Parameter	Exposure to 1 mg Cd/kg diet			Exposure to 5 mg Cd/kg diet		
		Main effect of Cd	Main effect of AE	Main effect of Cd + AE	Main effect of Cd	Main effect of AE	Main effect of Cd + AE
3	**GPx**	9.127*	8.329*	NS	61.56***	NS	NS
	GR	NS	21.72***	NS	57.36***	NS	13.24**
	GST	5.970*	NS	NS	16.3***	NS	NS
10	**GPx**	NS	NS	6.242*	9.702**	NS	11.77**
	GR	5.076*	NS	NS	8.671**	NS	NS
	GST	15.17***	9.913**	8.917**	22.58***	NS	NS
17	**GPx**	23.88***	NS	7.996*	12.96**	NS	8.988**
	GR	-	-	-	16.07***	NS	NS
	GST	NS	NS	16.48***	NS	NS	12.11**
24	**GPx**	51.06***	NS	12.78**	18.46***	NS	30.31***
	GR	NS	34.18***	16.08***	5.790*	10.81**	NS
	GST	-	-	-	NS	6.998*	10.43**

[1] The rats received 0.1% aqueous AE and Cd in diet at the concentration of 1 or 5 mg/kg for 3–24 months. [2] The results of the ANOVA/MANOVA analysis are presented as F values and the level of statistical significance (p). F values having $p < 0.05$ were considered statistically significant (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$). NS – not statistically significant ($p > 0.05$). In the case when a one way-analysis of variance (ANOVA, Duncan's multiple range test) revealed any influence of the co-administration of Cd and AE on the investigated parameter, a two-way analysis of variance (ANOVA/MANOVA, test F) was conducted in aim to discern possible interactive and independent impact of Cd and AE on this parameter.

Table S7. Main and interactive effects of cadmium (Cd) and the extract from the berries of *Aronia melanocarpa* L. (AE) on the concentrations of reduced glutathione (GSH), oxidized glutathione (GSSG), and the ratio of GSH/GSSGS, as well as the concentration of total thiol groups (TSH) in the liver of rats. [1, 2]

Duration (months)	Parameter	Exposure to 1 mg Cd/kg diet			Exposure to 5 mg Cd/kg diet		
		Main effect of Cd	Main effect of AE	Main effect of Cd + AE	Main effect of Cd	Main effect of AE	Main effect of Cd + AE
3	**GSH**	-	-	-	-	-	-
	GSSG	-	-	-	NS	NS	15.09^{***}
	GSH/GSSG	-	-	-	NS	25.60^{***}	11.05^{**}
	TSH	NS	6.444^{*}	12.93^{**}	7.320^{*}	NS	47.82^{*}
10	**GSH**	13.83^{***}	NS	NS	NS	NS	7.440^{*}
	GSSG	6.901^{*}	8.813^{*}	4.312^{*}	27.43^{***}	10.04^{**}	NS
	GSH/GSSG	NS	8.450^{*}	9.319^{**}	NS	4.852^{*}	5.543^{*}
	TSH	NS	5.346^{*}	26.05^{*}	NS	NS	19.47^{*}
17	**GSH**	8.085^{*}	NS	NS	10.95^{**}	5.444^{*}	NS
	GSSG	NS	10.98^{**}	NS	NS	33.16^{***}	13.64^{***}
	GSH/GSSG	NS	15.59^{***}	NS	NS	34.03^{***}	15.52^{***}
	TSH	5.267^{*}	NS	14.45^{***}	13.43^{**}	NS	7.760^{**}
24	**GSH**	NS	NS	8.384^{*}	NS	4.752^{*}	10.68^{**}
	GSSG	10.887^{**}	44.01^{***}	NS	11.22^{**}	81.91^{***}	NS
	GSH/GSSG	6.022^{*}	24.41^{***}	NS	NS	41.89^{***}	NS
	TSH	NS	30.21^{***}	NS	NS	35.90^{***}	NS

[1] The rats received 0.1% aqueous AE and Cd in diet at the concentration of 1 or 5 mg/kg for 3–24 months.
[2] The results of the ANOVA/MANOVA analysis are presented as F values and the level of statistical significance (p). F values having $p < 0.05$ were considered statistically significant (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$). NS – not statistically significant ($p > 0.05$). In the case when a one way-analysis of variance (ANOVA, Duncan's multiple range test) revealed any influence of the co-administration of Cd and AE on the investigated parameter, a two-way analysis of variance (ANOVA/MANOVA, test F) was conducted in aim to discern possible interactive and independent impact of Cd and AE on this parameter.

Table S8. Effect of the extract from the berries of *Aronia melanocarpa* L. (AE) on the concentration of thioredoxin (Trx) in the liver of rats exposed to cadmium (Cd). [1, 2]

Group	Duration of the Experiment			
	3 Months	**10 Months**	**17 Months**	**24 Months**
Trx (ng/mg protein)				
Control	0.152 ± 0.015	0.236 ± 0.017	0.152 ± 0.009	0.153 ± 0.016
AE	0.146 ± 0.018	0.239 ± 0.017	0.158 ± 0.015	0.186 ± 0.026
Cd_1	0.163 ± 0.016	0.258 ± 0.007	0.174 ± 0.016	0.173 ± 0.021
Cd_1 + AE	0.138 ± 0.008	0.246 ± 0.019	0.143 ± 0.019	0.199 ± 0.014
Cd_5	0.150 ± 0.017	0.196 ± 0.011	0.168 ± 0.012	0.167 ± 0.007
Cd_5 + AE	0.155 ± 0.017	0.235 ± 0.017	0.162 ± 0.011	$0.243 \pm 0.016^{** \dagger\dagger \ddagger}$

[1] The rats received 0.1% aqueous AE and Cd in diet at the concentration of 1 or 5 mg/kg for 3–24 months. [2] Data are represented as mean ± SE for 8 rats (except for 7 animals in the AE, Cd_1 and Cd_5 groups after 24 months). Statistically significant differences (ANOVA, Duncan's multiple range test) compared to the control group (** $p < 0.01$), group intoxicated with 5 mg Cd/kg diet alone (†† $p < 0.01$), and group receiving AE alone (‡ $p < 0.05$) are marked.

Table S9. Main and interactive effects of cadmium (Cd) and the extract from the berries of *Aronia melanocarpa* L. (AE) on the concentrations of hydrogen peroxide (H_2O_2), myeloperoxidase (MPO), and xanthine oxidase (XOD) in the liver of rats. [1, 2]

Duration (months)	Parameter	Exposure to 1 mg Cd/kg diet			Exposure to 5 mg Cd/kg diet		
		Main effect of Cd	Main effect of AE	Main effect of Cd + AE	Main effect of Cd	Main effect of AE	Main effect of Cd + AE
3	**H_2O_2**	NS	13.93^{***}	NS	NS	9.818^{**}	NS
	MPO	NS	NS	NS	NS	NS	6.790^{*}
	XOD	NS	13.35^{**}	NS	NS	15.45^{***}	NS
10	**H_2O_2**	18.70^{***}	26.93^{***}	21.02^{***}	26.81^{***}	27.75^{***}	21.05^{***}
	MPO	13.01^{**}	7.045^{*}	10.97^{**}	NS	NS	5.710^{*}
	XOD	14.09^{***}	5.003^{*}	11.72^{***}	NS	NS	12.75^{**}
17	**H_2O_2**	29.54^{***}	57.73^{***}	78.96^{***}	61.98^{***}	103.3^{***}	137.6^{***}
	MPO	4.684^{*}	22.84^{***}	8.230^{**}	6.180^{*}	22.83^{***}	8.717^{**}
	XOD	NS	30.02^{***}	11.76^{**}	15.13^{***}	58.18^{***}	36.78^{***}
24	**H_2O_2**	70.28^{***}	71.69^{***}	63.92^{***}	60.62^{***}	67.56^{***}	60.72^{***}
	MPO	NS	7.622^{*}	5.008^{*}	NS	13.00^{**}	7.976^{**}
	XOD	6.310^{*}	13.15^{**}	20.72^{***}	9.778^{**}	23.71^{***}	32.74^{***}

[1] The rats received 0.1% aqueous AE and Cd in diet at the concentration of 1 or 5 mg/kg for 3–24 months.
[2] The results of the ANOVA/MANOVA analysis are presented as F values and the level of statistical significance (p). F values having $p < 0.05$ were considered statistically significant (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$). NS – not statistically significant ($p > 0.05$). In the case when a one way-analysis of variance (ANOVA, Duncan's multiple range test) revealed any influence of the co-administration of Cd and AE on the investigated parameter, a two-way analysis of variance (ANOVA/MANOVA, test F) was conducted in aim to discern possible interactive and independent impact of Cd and AE on this parameter.

Table S10. Main and interactive effects of cadmium (Cd) and the extract from the berries of *Aronia melanocarpa* L. (AE) on the total antioxidative status (TAS), total oxidative status (TOS) and the index of oxidative stress (OSI) in the liver of rats. [1, 2]

Duration (months)	Parameter	Exposure to 1 mg Cd/kg diet			Exposure to 5 mg Cd/kg diet		
		Main effect of Cd	Main effect of AE	Main effect of Cd + AE	Main effect of Cd	Main effect of AE	Main effect of Cd + AE
3	**TOS**	-	-	-	-	-	-
	TAS	-	-	-	-	-	-
	OSI	-	-	-	NS	9.160^{**}	6.344^{*}
10	**TOS**	27.04^{***}	NS	NS	7.025^{*}	NS	NS
	TAS	4.410^{*}	6.670^{*}	6.062^{*}	NS	NS	NS
	OSI	44.87^{***}	101.2^{***}	50.51^{***}	11.05^{**}	17.93^{**}	5.934^{*}
17	**TOS**	26.68^{***}	NS	NS	-	-	-
	TAS	NS	NS	NS	NS	NS	NS
	OSI	22.54^{***}	21.73^{***}	31.99^{***}	13.85^{***}	13.60^{**}	23.47^{***}
24	**TOS**	-	-	-	44.22^{***}	6.251^{*}	NS
	TAS	NS	6.094^{*}	14.79^{***}	13.85^{***}	13.60^{**}	23.47^{***}
	OSI	10.85^{**}	NS	11.87^{**}	21.13^{***}	31.37^{***}	54.51^{***}

[1] The rats received 0.1% aqueous AE and Cd in diet at the concentration of 1 or 5 mg/kg for 3–24 months. [2] The results of the ANOVA/MANOVA analysis are presented as F values and the level of statistical significance (p). F values having $p < 0.05$ were considered statistically significant (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$). NS – not statistically significant ($p > 0.05$). In the case when a one way-analysis of variance (ANOVA, Duncan's multiple range test) revealed any influence of the co-administration of Cd and AE on the investigated parameter, a two-way analysis of variance (ANOVA/MANOVA, test F) was conducted in aim to discern possible interactive and independent impact of Cd and AE on this parameter.

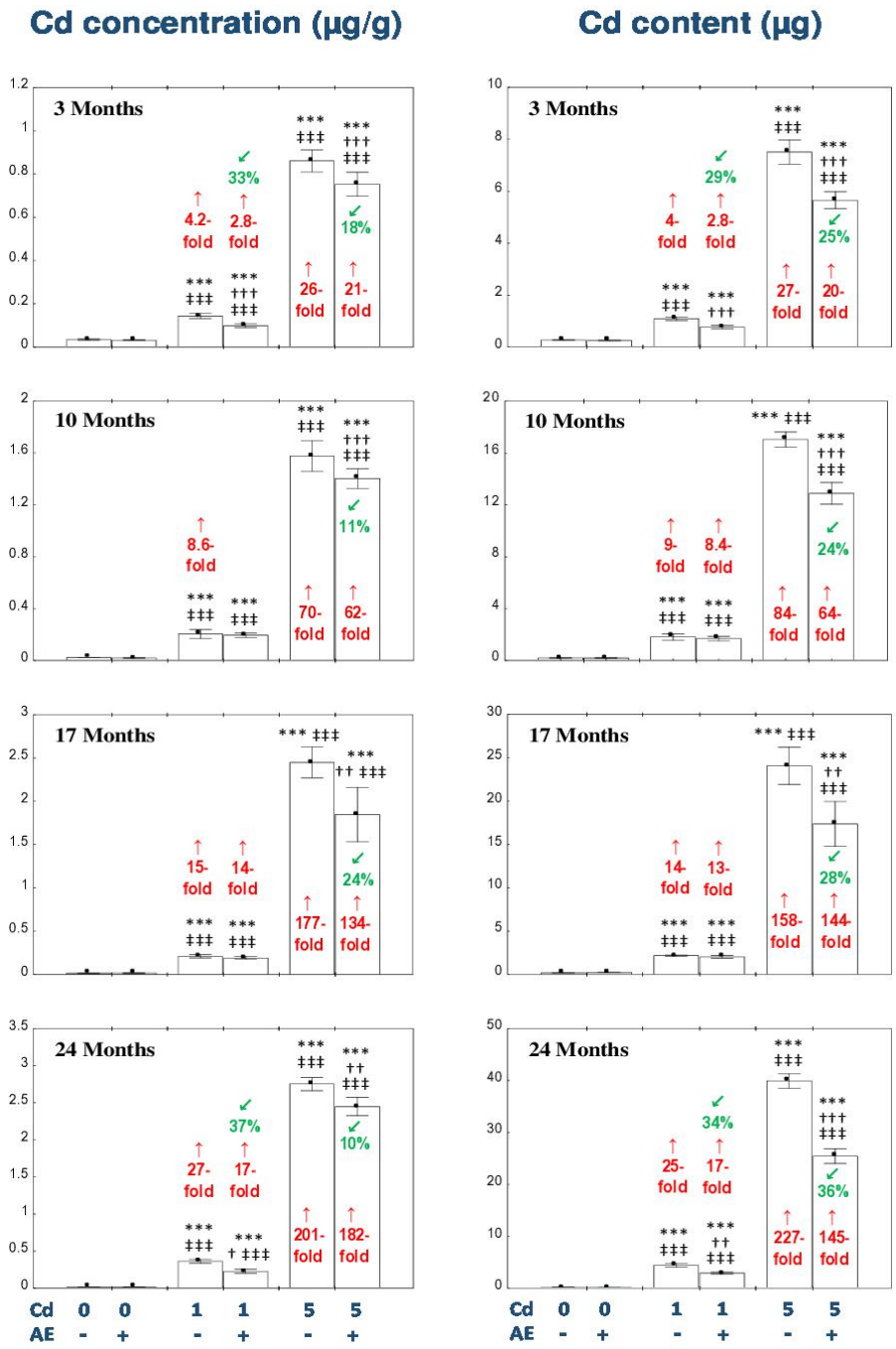

Figure S1. The effect of the extract from the berries of *Aronia melanocarpa* L. (AE) on the concentration and content of cadmium (Cd) in the liver of rats exposed to this metal. The rats received Cd in the diet at the concentration of 0, 1, and 5 mg Cd/kg and/or 0.1% aqueous AE (+) or not (-). Data are presented as mean ± SE for 8 rats, except for 7 animals in the AE, Cd_1, and Cd_5 group after 24 months. Statistically significant differences (ANOVA, Duncan's multiple range test): *** $p < 0.001$ vs. control group; ††$p < 0.01$, †††$p < 0.001$ vs. respective group intoxicated with Cd alone; ‡‡‡$p < 0.001$ vs. group receiving AE alone. Numerical values in bars or above the bars disclose the percentage changes or factors of changes in comparison to the control group (↑, increase) or the respective group receiving Cd alone (↙, decrease). Detailed data on Cd concentration in the liver are presented in Table S1 [27]. Cd content in the liver in the control group reached 0.27510 ± 0.0265 µg, 0.2024 ± 0.0129 µg, 0.1525 ± 0.0216 µg, and 0.1747 ± 0.0231 µg after 3, 10, 17, and 24 months, respectively [27].