European Journal of Chemistry 4 (1) (2013) 74-84

European Journal of Chemistry

Journal homepage: www.eurjchem.com

On the effect of chelating agents and antioxidants on cadmium-induced organ toxicity. An overview

Juliana Ivanova [a,*], Yordanka Gluhcheva [b], Denitsa Tsanova [a], Angelina Piskova [a], Radostina Djaleva [a], Steliana Mokresheva [a], Dimitrina Kamenova [a], and Mariana Mitewa [c]

[a] *Faculty of Medicine, Sofia University "St. Kl. Ohridski", 1407 Sofia, Bulgaria*
[b] *Institute of Experimental Morphology, Pathology and Anthropology with Museum, BAS, 1113, Sofia, Bulgaria*
[c] *Faculty of Chemistry and Pharmacy, Sofia University, "St. Kl. Ohridski", 1164 Sofia, Bulgaria*

**Corresponding author at: Faculty of Medicine, Sofia University "St. Kl. Ohridski", 1407 Sofia, Bulgaria. Tel.: +359.2.8161247; fax: +359.2.9625438. E-mail address: dkji@chem.uni-sofia.bg (J. Ivanova).*

REVIEW INFORMATION

Received: 31 January 2013
Accepted: 16 February 2013
Online: 31 March 2013

KEYWORDS

Vitamins
Cadmium
Toxicology
Ionophores
Antioxidants
Medicinal chemistry

ABSTRACT

Cadmium (Cd) has been classified as a human carcinogen. The World Health Organization (WHO) reported that the concentration of Cd in the environment has rapidly increased in the last few years. In many epidemiological studies, the correlation between environmental exposure of humans to Cd and diseases such as stroke, ischemia, renal and hepatic dysfunction, anemia, osteoporosis and diabetes has been discussed. For the treatment of heavy metal intoxications a therapy with chelating agents has been applied. A chelating agent is a compound that binds the toxic metal ion thus promoting its excretion by the living organisms. Recently, it has been found that Cd-induced toxicity is a result of formation of reactive oxygen species (ROS). These results increased the interest towards the antioxidants as possible agents for the treatment of Cd-induced organ toxicity. Herein, we present summary and discussion of the literature data for the influence of chelating agents and antioxidants on Cd-induced pathological conditions in Cd-intoxicated animals.

1. Introduction

Cadmium (Cd) is a toxicant, classified as a human carcinogen by the International Agency for Research on Cancer [1]. According to the data provided by the WHO, the daily Cd intake varies in the interval from 40 μg (for people living in unpolluted regions) to 200 μg (for people from polluted areas) [2,3]. The level at which chronic exposure to Cd is not likely to cause cancer or adverse health effects is 14 μg per day [2,3]. Cd is a very dangerous environmental pollutant due to its ability to cause severe organ toxicity. When accumulated in the body it induces hepatotoxicity [4], renal dysfunction [5,6], distortion of the reproductive function [7,8] and cardiovascular injury [9-14].

Compounds that possess low toxicity, good absorbability by the gastrointestinal tract and bind toxic metal ions have been used in the chelation therapy for the treatment of toxic metal intoxication [15-17]. Many chelating agents have been tested to mobilize Cd but no effective chelation therapy is available so far for humans, exposed to Cd intoxication [18].

It has been discussed that the oxidative stress is one of the mechanisms for the Cd-induced toxicity [19-21]. Albeit Cd does not generate free radicals by itself it replaces the iron (Fe) and copper (Cu) from cytoplasmic proteins and metalloenzymes [19]. Free iron and copper ions participate in Fenton type reactions leading to the generation of ROS [19-21]. Other possible mechanism for Cd-induced oxidative stress includes direct interaction of Cd with SH groups of the thiols [20,21]. In the last years enormous data regarding the application of the antioxidants for the treatment of Cd-induced toxicity have been published [3].

Herein we present a summary of the antioxidants and chelating agents screened on animal models to decrease Cd concentrations in the body and to prevent Cd-induced oxidative stress. The data presented in this review comprise the period 1992-2012 years. Discussion of the antioxidants and chelating agent tested to inhibit Cd-induced hepatotoxicity, renal dysfunction, testicular toxicity and cardiac impairment is provided.

2. Effect of antioxidants and chelating agents on Cd-induced hepatotoxicity

The liver and kidneys are critical organs in Cd-induced intoxications. When accumulated in the liver Cd induces inflammatory cell infiltrations, dilation of sinosoids and disorganization of the normal radiating pattern of the hepatocytes around the central vein [22-24]. At acute and long-lasting chronic Cd-intoxications necrosis of the central vein of the liver has been observed [22].

2.1. Antioxidants, tested on animal models for the treatment of Cd-induced hepatotoxicity

The antioxidants tested on animal models to prevent Cd-induced hepatotoxicity are presented in Tables 1 and 2. As could be seen from the data, presented in both tables, there is an enormous diversity of experimental models, published by authors of studies dealing with the effect of the antioxidants on Cd-induced hepatotoxicity.

ISSN 2153-2249 (Print) / ISSN 2153-2257 (Online)
DOI:10.5155/eurjchem.4.1.74-84.739

Table 1. Antioxidants, tested on animal models for the treatment of Cd-induced hepatotoxicity, caused by acute and subacute Cd-exposure.

Antioxidant	Route of administration	Dose and duration of administration	Animal model	Effect	Ref.
Alpha lipoic acid (α-LA)	i.p.	LA:Cd = 5:1. The antioxidant is co-administrated with Cd(II) salt	Male mice, acute intoxicated with 40 μmol/kg Cd(II) salt (s.c.)	Cd accumulation is not affected by LA. The antioxidant does not recover the activity of CAT and GSH level, but the mortality associated with Cd-intoxication is decreased by LA.	[25]
Curcumin	p.o.	50 mg/kg b.w., applied before Cd-intoxication for 3 days	Male mice and male rats, acute intoxicated with 0,025 and 0,03 mmol/kg b.w. Cd(II) chloride (s.c.)	LPO is abolished by the antioxidant, but Cd-induced GSH depletion in the mice liver is not significantly affected. Cd concentration and trace elements homeostasis are not changed by the antioxidant.	[26]
Curcumin and Mn-curcumin complex	p.o.	0,14 mmol/kg b.w, applied for 3 days before intoxication	Male mice, intoxicated s.c. with 33 μmol/kg Cd(II) chloride	Both compounds prevent Cd-induced decrease of GSH but do not affect Cd accumulation. The treatment with Mn complexes causes an increase of Fe and Mn in the kidneys in the control and Cd-treated mice and Fe and Cu in the brain of the control mice.	[27]
Curcumin, resveratol, melatonin	p.o.	50 mg/kg b.w. curcumin; 20 mg/kg b.w - resveratrol; melatonin - 12 mg/kg, dispersed in 0.5% methylcellulose. Duration - 3 days	Male mice, intoxicated with Cd(II) chloride (7 mg/kg b.w., s.c.)	The Cd-induced decrease of GSH level in the liver is not prevented by the antioxidants. Resveratol recovers CAT activity.The MDA and GPx level is returned to normal in the pretreated animals. The antioxidants do not affect Cd accumulation.	[28]
Vitamin E	Information not found	100 mg/kg, applied before intoxication	Male rats, intoxicated i.v. with 2 mg/kg Cd (II)	Vit. E improves SOD and CAT activity in the liver as well as GSH level. The antioxidant has been shown to recover Hb and HCT in the blood.	[32]
Diallyl tetrasulfide (DTS)	p.o.	40 mg/kg (optimum dose) for 3 weeks, applied together with Cd(II)	Male rats, intoxicated with 3 mg/kg Cd(II) s.c. for 3 weeks	DTS significantly reduces the accumulation of Cd and restores the levels of the antioxidant defense in the liver. DTS improves the hepatocytes morphology.	[29, 30]
Endomorophin 1 (EM1)	i.p.	50 μM /kg per day for 6 days - optimum dose. The antioxidant has been coadministrated with Cd (II)	Male mice, injected i.p. with Cd(II) chloride (1 mg/kg per day) for 6 days	EM1 reduces the Cd accumulation up to 28 % compared to the toxic control. EM1 attenuates Cd-induced alterations in MDA levels, GSH, SOD, and CAT but MDA values remain higher than the controls. On the morphological level, the degenerative changes are reduced by EM1.	[31]
Taurine	p.o.	150 mg/kg body weight, once daily for 5 days prior Cd-intoxication	Mice received Cd (II) chloride orally through drinking water at a dose of 4 mg/kg b.w. for 6 days, once daily	Taurine significantly increases SOD and CAT activity but at optimum dose the values remains 15% and 20% lower than the controls. Taurine prevents Cd accumulation by 50% and preserves the homeostasis of the trace elements. Considerable improvement of the liver morphology by the antioxidant is observed.	[22]
Panax ginseng (Pg)	p.o.	10 mg/kg b. w. for 10 days prior to Cd-intoxication and continued up to 30 days after Cd(II) chloride administration	Male mice, acute intoxicated with Cd(II) chloride (1 mg/kg b. w., i.p. once)	The antioxidant recovers GSH and ALP. TBARS is partly affected by Pg. TBARS levels remain higher than the control (about 20 %).	[34]
Carnosine	i.p.	10 mg/kg/day is applied for three consecutive days, starting one day before Cd-administration	Male mice, acute intoxicated with 6.5 mg/kg Cd(II) chloride i.p.	The antioxidant partly reduces MDA, and increases GSH, CAT and SOD. CAT, SOD and GSH remain lower with 15 %, 20 %, and 10 % respectively than the controls. Carnosine decreased by 87% Cd concentration and recovered Zn homeostasis. On the morphological level a significant improvement of the radiating hepatocytes structure is observed in carnosine treated mice.	[23]
Solublequercetin-50-sulfonic acid sodiumsalt (NaQSA); andmorin-50-sulfonic acid sodium salt (NaMSA)	i.p.	20 mg/kg b.w. after Cd-intoxication 10 mg/kg in combination of both flavanoids	Male mice, intoxicated with 0.64 mg/kg b.w Cd(II) chloride s.c.	Both antioxidants increased the level of SOD and GSH, but the values remained lower than the controls (30 % and 20 % respectively).	[35]
Garlic and Ascorbic acid	p.o.	100 mg/kg, pretreated for 4 weeks	Male rats - acute intoxicated with Cd (II) chloride 4 mg/kg b.w. (i.p.).	The antioxidants recover SOD and CAT activity and return to normal the LPO level.	[33]

In most papers, however, the effect of the antioxidants on Cd-induced oxidative stress in the liver is monitored by the analysis of superoxide dismutase activity (SOD), catalase activity (CAT), gluthathione level (GSH) and level of lipid peroxidation (LPO), expressed as thiobarbituric acid reactive species (TBRAS) or malondyaldehyde (MDA). Cd decreases the level of GSH, and the activity of SOD and CAT in the liver of Cd-intoxicated animals and induces a significant increase of LPO. All antioxidants studied have been demonstrated to prevent in some extent Cd-induced oxidative stress in the liver of Cd-treated animals.

2.1.1. Effect of antioxidants on Cd-induced hepatoxicity in animals, subjected to acute and subacute Cd-intoxications

The antioxidants, screened on animal models for the treatment of Cd-induced hepatotoxicity, caused by acute and subacute Cd-exposure, are presented on Table 1.

Table 2. Antioxidants, tested on animal models for the treatment of Cd-induced hepatotoxicity in subchronic and chronic Cd-intoxications.

Antioxidant	Route of administration	Dose	Animal model	Effect	Ref.
Hydroxytyrosol, (DPE)	Information not found	9 mg/kg, applied after Cd-intoxication	Male rats	DPE inhibits Cd-induced toxicity in the liver	[42]
Diphenyl diselenide ($(PhSe)_2$)	s.c.	5 μmol/kg	Male mice, subchronically intoxicated with 10 μmol Cd(II) salt five times per week for four weeks	The antioxidant restores the activity of AST and TBARS to normal values. Diphenyl diselenide decreases ALT activity in the plasma of Cd-treated mice but the value remains higher than the controls (70 %). The effect of the antioxidant on the Cd and trace elements level is not studied.	[36]
Melatonin	p.o	4 μg/mL for 8 weeks applied together with Cd(II)	Female and male mice, subchronically intoxicated with 50 μg Cd/mL as Cd(II) chloride for 8 weeks	Melatonin decreases Cd level by 24 % compared to the toxic control; the antioxidant however decreases hepatic iron to 14% compared to the toxic control. Cd diminishes Fe level to 20 % compared to the control; TBRAS levels are recovered by the antioxidant and are even lower than the normal control.	[38]
Hibiscus sabdariffa L petal	Information not found	Applied before intoxication	Male rats, chronically intoxicated with Cd(II)	The antioxidant decreases the levels of AST and ALT in the blood plasma.	[43]
Diphenyl diselenide, $(PhSe)_2$	p.o.	5 μmol/kg, applied together with Cd(II) administration for 30 days	Male rats, orally intoxicated with 10 μmol/kg Cd(II) chloride for 30 days	The antioxidant reduces the levels of the hepatic enzymes; MDA, urea and bilirubin.	[36]
Hesperetin (HTN)	p.o. in 0.1% carboxymethyl cellulose	40 mg/kg/day for 21 days	Male rats, intoxicated s.c. for 21 days with 3 mg/kg b.w. Cd (II) chloride	Hesperetin increases SOD and CAT levels but the values remain lower than the controls - 30 and 15% respectively. The hepatic enzymes were partially recovered. TBARS are affected by HTN but the values remain higher than the control (30 %). Normal hepatic architectural pattern with mild dilation of sinusoids in Cd+HTN treated animals was observed.	[24]
Organo-selenocyanates	p.o.	3 mg/kg for 20 days applied concomitant or prior to Cd-intoxication.	Female mice, intoxicated i.p. with 2 mg/kg Cd(II) chloride for 20 days	The tested compounds do not correct SOD level. GSH is completely recovered by the antioxidants, CAT - partially (values remain 10 % lower than the controls). TBARS level is 20 % higher compared to the controls in the Cd+antioxidant treated mice. The antioxidants recover partially the hepatic enzymes. Normal central vein and hepatocytes structure in antioxidant-treated animals is observed.	[39]
Naringenin (NGN)	p.o.	50 mg/kg for 4 weeks, administrated together with Cd(II)	Male rats, orally intoxicated with Cd(II) (5mg/kg) for 4 weeks	NGN recovers the levels of the hepatic and antioxidant enzymes near to their normal values and preserves the normal histological architecture.	[40]
Naringenin+Vitamin C+VitaminE	p.o.	50 mg/kg for 28 days of each compound, administrated together with Cd(II)	Male rats, intoxicated orally with Cd(II) chloride 5 mg/kg b.w. for 28 days	The combination of three antioxidant results in complete recovery of SOD, CAT, GSH, TBARS and the hepatic enzymes. Normal hepatic architecture is observed in the animals treated with the three antioxidants.	[41]

Among the antioxidants, presented in the Table 1, alpha lipoic acid (α-LA), curcumin, Mn-curcumin complex, resveratol and melatonin have been shown not to prevent Cd-accumulation in the liver of Cd-treated animals [25-28].

Alpha lipoic acid (α-LA), resveratol and melatonin did not affect Cd-induced decrease of GSH level in the liver, suggesting that these antioxidants might not be the best choice for the treatment of Cd-induced oxidative stress in the liver of animals, subjected to subacute and acute Cd-intoxications [25,28]. Mn-curcumin complex recovers GSH level in the liver but the effect of this antioxidant on the trace element homeostasis especially Fe and Cu should be taken into account when Mn-curcumin complex is applied for the therapy of Cd-induced hepatotoxicity [27].

Diallyl tetrasulfide [29,30], endomorophin 1 [31], taurine [22] and carnosine [23] have been demonstrated to prevent Cd accumulation in the liver of animals, subjected to acute and subacute Cd-intoxications, as in the case of carnosine administration the effect reaches 87% compared to the normal control. Considerable improvement of the liver morphology by these antioxidants has been observed.

Vitamin E (applied before Cd-intoxication) has been shown to improve the level of GSH in male rats, subjected to acute intoxication with Cd(II) salt [32]. Panax ginseng, ascorbic acid and garlic also improve the total antioxidant capacity of the liver in Cd-treated animals [33,34]. Detail histological studies about the effect of these antioxidants on the liver morphology of Cd-intoxicated animals are needed to make a conclusion regarding the possible application of these compounds for the treatment of Cd-induced hepatotoxicity.

2.1.2. Effect of antioxidants on Cd-induced hepatotoxicity in subchronically or chronically Cd-intoxicated animals

Diphenyl diselenide [36,37], melatonin [38] and hesperetin [24] have been proven to inhibit the level of LPO in the liver of Cd-treated mice. The effect of melatonin [38] however on iron homeostasis should be considered when this antioxidant is utilized for the treatment of Cd-induced hepatotoxicity. Organoselenocyanates [39] have been also effective in restoring the activity of CAT in the liver of Cd-intoxicated animals but the compounds do not recover SOD values. Among the antioxidants screened on animals subchronically or chronically exposed to Cd, naringenin and the combination of naringenin (NGN), vitamin C (Vit C) and vitamin E (Vit E) seem to be the most effective in restoring CAT and SOD activity of the liver (Table 2) [40,41]. Histopathological analysis of the liver of Cd-intoxicated animals treated with the combination of NGN, Vit C and Vit E has revealed that the antioxidants preserve the normal hepatic architecture.

2.2. Effect of chelating agents on Cd-induced hepatotoxicity in mice, subjected to acute and chronic Cd-intoxication

A comparative study on the effect of the chelating agents *N*-(4-methylbenzyl)-4-*o*-(beta-*D*-galactopyranosyl)-*D*-glucamine-

Table 3. Chelating agents, tested on animal models for the treatment of Cd-induced hepatotoxicity.

Chelating agent	Route of administration	Dose	Animal model	Effect	Ref.
Mi-ADMS MeBLDTC	Information not found	Every 48 h for 12 days	Male mice, injected i.v. with Cd(II) chloride	MeBLTDC is more effective on equimolar basis; Mi-ADMS however could be applied orally. Both compounds improve trace elements homeostasis in Cd-treated mice.	[44]
DDC, DMDC, CYCLAM, TACPD, DMSA, DMPS	Information not found	Information not found	Mice	DMDS, CYCLAM, TACPD reduce Cd in the liver, but are not as effective as DMSA and DMPS. The effect on trace elements is not studied.	[45]
Mi-PDMA; Mi-BDMA Mi-ADMA	i.p.	4x1.5 mmol/kg	Female rats, exposed to chronic Cd-intoxication	The effectiveness of the chelating agents increases in the order: M*i*-ADMA < M*i*-BDMA < M*i*-PDMA for the hepatic Cd. The most effective agent was the most toxic as well.	[46]
Carbodithioate analogue BLDTC and CaDTPA	i.p.	Injected every 48 h for 16 days	Male mice intoxicated with 20 doses of Cd (II) chloride (single dose of 3 mg kg^{-1} i.p.)	The antidotes restore partially the levels of Cu and Zn. BLDTC is more effective than CaDTPA.	[49]
Monensin	p.o.	14 mg/kg, applied after Cd-intoxication	Male mice, subacute intoxicated with 20 mg/kg Cd (II) acetate for 14 days	Monensin recovers AST, ALT and ALP values, Cu homeostasis. Zn homeostasis is partially recovered by the chelating agent. Monensin reduces Cd concentration in liver by 50 % compared to the toxic control and recovers the hepatic architecture.	[47,48]

Abbreviations: Mi-ADMS: monoisoamyl meso-2,3-dimercaptosuccinate; MeBLDTC: N-(4-methylbenzyl)-4-O-(beta-D-galactopyranosyl)-D-glucamine-N-carbodithioate; DDC: diethyl dithiocarbamate; DMDC: dimethyl dithiocarbamate; CYCLAM: 1,4,8,11-tetraazacyclotetradecane; TACPD: 1,4,8,12-tetraazacyclopentadecane; DMSA: 2,3 dimercaptosuccinic acid; DMPS: 2,3-dimercapto-1-propane sulfonate; M*i*-PDMA: *meso*-2,3-dimercaptosuccinic acid monoisopropylamide; M*i*-BDMA: *meso*-2,3-dimercaptosuccinic acid monoisobutylamide; M*i*-ADMA: *meso*-2,3-dimercaptosuccinic acid monoisoamylamide; BLDTC: N-benzyl-4-O-(beta-D-galactopyranosyl)-D-glucamine-N-carbodithioate CaDTPA: calcium trisodium pentetate.

N-carbodithioate (MeBTDTC) and monoisoamyl meso-2,3-dimercaptosuccinate (Mi-ADMS) on Cd concentration in Cd-treated animals has demonstrated that the chelating agent MeBTDTC reduces more effectively the concentration of the toxic metal in the liver of animals, subjected to acute Cd-intoxication but could not be applied orally in contrast to Mi-ADMS (Table 3) [44].The effect of the chelating agents diethyl dithiocarbamate (DDC), dimethyl dithiocarbamate (DMDC), 1,4,8,11-tetraazacyclotetradecane (CYCLAM), 1,4,8,12-tetraaza cyclopentadecane (TACPD), 2,3-dimercaptosuccinic acid (DMSA), and 2,3-dimercapto-1-propane sulfonate (DMPS) on Cd concentration in the liver, kidney and brain of mice, exposed to Cd-intoxication has been compared. The results by Srivastava et al. demonstrate that DMSA and DMPS are most effective in reducing Cd [45], compared to DDC, DMDC, CYCLAM, TACPD. DMSA and DMPS however are hydrophilic compounds and their ability to bind the toxic metal ion, accumulated in the intracellular space is limited.

The meso-2,3-dimercaptosuccinic acid mono-*N*-alkylamides: M*i*-PDMA (*meso*-2,3-dimercaptosuccinic acid monoisopropylamide); M*i*-BDMA (*meso*-2,3-dimercapto-succinic acid monoisobutylamide) and M*i*-ADMA (*meso*-2,3-dimercaptosuccinic acid monoisoamylamide) are effective in mobilizing Cd from the liver but are not that much effective in reducing the Cd concentration in the kidneys. At optimum dose M*i*-BDMA reduces renal Cd concentration to 40% compared to the toxic control [46].

Our studies have demonstrated that the polyether ionophorous antibiotic monensin (applied p.o. as tetraethyl ammonium salt) to mice, subjected to subacute Cd-intoxication reduces the concentration of the toxic metal ion in the liver up to 50 %. Furthermore this chelating agent abolished Cd-induced alterations in iron homeostasis and recovered Cu and Zn levels. The data from the histopathological studies showed that monensin attenuated Cd-induced inflammation in the liver confirming the positive effect of the antibiotic on the liver of mice, exposed to subacute Cd intoxication [47,48].

3. Antioxidants and chelating agents, screened on animal models for the treatment of Cd-induced renal dysfunction

3.1. Antioxidants

3.1.1. Antioxidants, tested on animal models for the treatment of renal dysfunction, induced by subacute and acute Cd-intoxication

The kidneys are major organs that accumulate metal ions in cases of metal intoxications. Cd has been shown to induce alterations in creatinine (CR) and urea in the urine and serum of Cd-intoxicated animals [50]. A decrease of the level of the antioxidant enzymes and GSH accompanied with an increase of the LPO in the kidneys of Cd-intoxicated animals has been reported [51,52]. On the morphological level Cd induces swelling of the epithelial cells of the renal tubules; degeneration and thickening of the basement membrane. Necrosis of the proximal renal tubules has been reported in animals, subjected to acute Cd-intoxication [52]. The antioxidants tested in the period 1992-2012 years for the treatment of Cd-induced renal dysfunction in animals, subjected to acute Cd-intoxication, are presented in Table 4. Diallyl tetrasulfide recovers the level of CR and urea in the serum and urine of Cd-intoxicated rats. The antioxidant has been demonstrated to act as a chelating agent, reducing the concentration of the toxic metal ion by 70 % compared to the normal control [30].

Garlic and onion extracts also effectively restore the total antioxidant capacity of the kidneys of Cd-intoxicated animals [50].

The honey bee, taurine, vitamin C and isoquercetin have been shown to improve the antioxidant defense in the kidneys of animals, exposed to acute Cd-intoxication [51-53]. Taurine and vitamin C have been demonstrated to recover CR concentration in the serum of mice, subjected to acute Cd-intoxication. Both antioxidants decrease MDA level, increase GSH and inhibit the accumulation of Cd in the kidneys. The histopathalogical analysis of kidneys of mice treated with taurine and Vitamin C prior to Cd-intoxication demonstrates that the antioxidants preserve the normal renal architecture. Considering that Vitamin C is an oral drug it might be the best choice for the treatment of acute Cd-intoxication [52]. Caffeic acid also reduces Cd in the kidneys of animals, exposed to subacute Cd-intoxication [54].

Analysis of the effect of caffeic acid on Cd-induced elevation of Zn concentration in kidneys of Cd-treated animals demonstrates that the antioxidant increases Zn concentration but the values remain lower than the normal controls.

Table 4. Antioxidants, tested on animal models for the treatment of renal dysfunction induced by acute and subacute Cd-intoxications.

Antioxidant	Route of administration	Dose	Animal model	Effect	Ref.
Diallyl tetrasulfide (DTS)	p.o.	40 mg/kg b.w./day for 3 weeks, co-administrated with Cd(II)	Rats, s.c. intoxicated with Cd(II) chloride (3 mg/kg/d for 3 weeks)	DTS recovers CR and urea in the serum and in urine. DTS decreases Cd by 70 %. DTS also inhibits Cd-induced LPO and increases GSH and antioxidant enzymes.	[30]
Garlic and onion extract	p.o.	0, 5 mL/100 g b.w. and 1 mL/100 g b.w. extract prior to Cd- intoxication. The treatment continued with Cd(II) administration.	Rats - intoxicated p.o. with 1,5 mg Cd/100 g b.w. for 3 weeks	Both extracts restore the antioxidant defense in the kidneys but onion is more effective.	[50]
Honey bee (HB)	p.o.	100; 250 mg/kg b.w., co-administrated with Cd(II)	Mice, acutely intoxicated with Cd(II) chloride - 2 mg/kg b.w.	Recovery of the GSH level has been observed in the animals treated with HB accompanied with decrease of the LPO.	[51]
Taurine, Vitamin C	Taurine -i.p. Vitamin C - orally	100 mg/kg b.w. once daily for 5 days, applied prior to treatment with Cd(II)	Mice, acutely intoxicated with 4 mg/kg Cd(II) chloride (i.p. for 3 days)	Both antioxidants cause 50% reduction of urea in the serum and recover CR in the Cd-treated animals. The antioxidants decrease MDA, but the values remain 10% higher than the control. A 70% decrease of Cd in the kidneys has been observed as a result of the administration of the antioxidants. The histological analysis has been demonstrated normal appearance of the glomeruli and tubules in the kidneys of Cd-treated animals, receiving antioxidants.	[52]
Isoquercetin	Information not found	Information not found	Mice, acutely intoxicated i.p with Cd(II) chloride - 2 mg/kg b.w.	The antioxidant has been shown to attenuate Cd-induced alterations in kidneys and liver.	[53]
Caffeic acid	i.p.,	10 μmol/kg per day for 7 days coadministrated with Cd(II)	Mice, exposed i.p. to Cd(II) chloride (1 mg/kg/day for 7 days)	Caffeic acid completely recovers antioxidant defense system in the kidneys; reduces Cd level (22 %); recovers partially Zn concentration. Zn concentration remains 20 % lower than the control.	[54]

Table 5. Antioxidants, applied for the treatment of Cd-induced oxidative stress in the kidneys of animals, subjected to subchronic and chronic Cd-intoxications.

Antioxidant	Route of administration	Dose	Animal model	Effect	Ref.
Glycyrrhizin, cysteine, glycine	Information not found	Information not found	Rats, subjected to chronic intoxication with 5 μM Cd(II) chloride/kg b.w. /day 5 times a week for 22 weeks	The antioxidants reduce chronic Cd-toxicity.	[62]
Melatonin	p.o	4 μg/mL (optimum dose) for 8 weeks applied together with Cd(II)	Female and male, subchronically intoxicated with 50 μg Cd/mL as Cd(II) chloride for 8 weeks	Melatonin decreases Cd level by 23 % compared to toxic control; the antioxidant however decreases hepatic iron to 14 % compared to the toxic control. The Cd-induced alteration in Fe homeostasis was not recovered by the antioxidant.	[38]
Quercetin (QE)	i.p.	50 mg/kg/day for 9 weeks along with Cd(II)	Rats, intoxicated s.c. 1.2 mg/kg/day Cd(II) for 9 weeks	QE prevents Cd-induced overexpression of iNOS and COX-2.	[58]
N-acetylcysteine (NAC)	p.o.	0,5 % when applied together with Cd; 2 % when used after intoxication. The antioxidant has been applied in co-administration with cadmium and after the intoxication	Rats, intoxicated with $CdCl_2$ for 3 months	NAC recovers SOD and CAT. GSH is no affected by the antioxidant. In both cases NAC preserves the normal appearance of the renal proximal tubules	[55]
NAC	p.o.	120 mg/kg/day	Female rats, chronically exposed to 20 mg/L Cd(II)	NAC decreases LPO, but the concentration of Cd in the kidney is not affected. GSH, SOD and CAT have not been recovered by the antioxidant.	[56]
Naringenin (NGN)	p.o.	50 mg/kg/day for 4 weeks, together with Cd(II) administration	Rats, exposed to subchronic intoxication with Cd(II) chloride 5 mg/kg for 4 weeks	NGN preserves Cd-induced alterations in the antioxidant enzymes; GSH and LPO. NGN ameliorates Cd-induced alterations in the renal architecture.	[57]
QE	p.o.	50 mg/kg/ day for 4 weeks, co-administrated with Cd(II)	Rats, exposed to subchronic intoxication with Cd(II) chloride 5 mg/kg for 4 weeks, co-administrated with Cd(II)	QE recovers CR and urea and attenuates Cd-induced alterations in the antioxidant status in the kidneys; QE ameliorates Cd-induced pathology of the kidneys.	[59]
QE; Vitamin C and Vitamin E	p.o.	Information not found	Rats	The combination of QE plus vitamin C and vitamin E improves the renal morphology and attenuates Cd-induced alterations in antioxidant defence system.	[60]
Telmisartan	p.o.	1 mg/kg/daily started one week before Cd administration and continued for 10 weeks	Mice, treated with 1.2 mg Cd/kg day for 9 weeks	The antioxidant improves Zn homeostasis, decreases the concentration of Cd and inhibits the LPO. Telmisaran also decreases TNF-a and NO elevated by Cd.	[63]
Herbal adaptogens	p.o. after Cd-intoxication	0, 1 % powder in the feed, applied for 2 weeks	Chicks, exposed to chronic cadmium intoxication 100 ppm/ day for 28 days	Asperagus recemosus, Angrographis paniculata are most effective among eight adaptogens tested. These adaptogens recover GSH; reduce LPO to normal values and decrease Cd in the kidneys by 50 %. The serum CR however remains higher than the normal control.	[61]

Table 6. Chelating agents, screened on animal models for mobilization of Cd in kidneys.

Chelating agent	Administration route	Dose	Animal model	Effect	Ref.
MPhEDMS MPhPDMS MPhOEDMS	i.p.	0,5 mmol/kg/day for 4 days	Rats	The chelating agents significantly deplete hepatic and renal Cd compared with the toxic control	[64]
MBzDMS	i.p.; i.v.; p.o.		Rats/mice	MBzDMS was found to be less effective than Mi-ADMS in mobilizing Cd.	[68]
TRIEN, TREN, TETREN, PENTEN			Rats, intoxicated with Cd 4 days before chelation	The chelating agents cause significant renal damage.	[65]
Mi-PDMA; Mi-BDMA Mi-ADMA	i.p.	4x1.5 mmol/kg	Famale rats, exposed to chronic Cd-intoxication	The chelating agents do not possess optimized structure between LD50 and effectiveness.	[46]
DDC, DMDC, CYCLAM, TACPD, DMSA, DMPS	Information not found	Information not found	Mice	DMDS, CYCLAM, TACPD reduce Cd in the kidneys. The therapeutic index of the chelating agents decreases in the order: DMSA>DMPS>DMDC	[45]
MFA; MSFA; MAFA	Information not found	Information not found	Rats	The chelating agents affect the trace elements homeostasis.	[66]
Diethylcarbamate	p.o.	100 mg/kg b.w.	Rats, subjected to acute intoxication with Cd for 60 days (40 mg/kg/day)	The chelating agent reduces Cd in kidney by 60 %.	[69]
DFO	p.o.	Information not found	Rats, subjected to acute intoxication with Cd for 60 days (20 and 40 mg/kg/day)	DFO improves Fe homeostasis and reduces Cd in the body.	[67]
Monensin	p.o.	14 mg/kg	Mice, subjected to subactute Cd intoxication for 2 weeks	Monensin improves iron homeostasis and reduces Cd from 50 % (liver) to 90 % (heart).	[47]

Abbreviations: Monoaralkyl esters of meso-2,3-dimercaptusuccinic acid: HOOCCH(SH)CH(SH)COOR, where R = phenylethyl $(CH_2)_2C_6H_5$, MPhEDMS; R = 3-phenylpropyl $((CH_2)_3C_6H_5)$, MPhPDMS; and R = 2-phenoxyethyl $(CH_2)_2OC_6H_5$, MPhOEDMS; Triethylenetetramine dihydrochloride (TRIEN), *Tris*(2-aminoethyl)amine trihydrochloride (TREN), Tetraethylenepentamine pentahydrochloride (TETRAEN), Pentaethylenehexamine hexahydrochloride (PENTAEN); Alpha-mercapto-beta-(2-furyl) acrylic acid (MFA), alpha-mercapto-beta-(5-sodiumsulfonate, 2-furyl) acrylic acid (MSFA) and alpha-mercapto-beta-(5-acetoxymethyl, 2-furyl) acrylic acid (MAFA); Desferrioxamine (DFO).

It should be pointed out that in contrast to the antioxidants discussed above the caffeic acid has been applied i.p., therefore it might not be the best choice for the treatment of Cd-intoxication [54].

3.1.2. Antioxidants, tested on animal models for the treatment of renal dysfunction, induced by subchronic and chronic Cd-intoxications

The antioxidants screened on animals, exposed to subchronic and chronic Cd-intoxications, are summarized in Table 5.

N-acetylcysteine (NAC) salt is effective in improving renal function of male and female rats, exposed to chronic Cd-intoxication. In both cases however NAC does not recover GSH [55,56].

Naringenin (NGN), Quercetin (QE), QE+Vitamin C+Vitamin E have been demonstrated to recover CR and urea levels in serum and urine in animals, subjected to subchronic Cd-intoxication [57-60]. Furthermore, these antioxidants decrease LPO in the kidneys and improve GSH and antioxidant enzymes levels. The histopatological analysis of the renal tissue of Cd-treated animals, receiving NGN or QE reveals that these antioxidants preserve the normal renal architecture [57-60].

The herbal adaptogens: Withania somnifera, Ocimum sanctum, Asperagus recemosus, Andrographis paniculata, Asphaltum panjabinum (Shilajith), Gymnema sylvestre, Spirulina platensis, and Panex ginseng have been tested to prevent Cd bioaccumulation in chicks, exposed to chronic Cd-intoxication [61]. The herbal adaptogens Asperagus recemosus and Andrographis paniculata, applied p.o., reduce Cd concentration in kidney by 50% compared to the control; inhibit LPO, and recover GSH level. CR values in the serum of Cd-treated animals, receiving antioxidants, however remain higher than the normal control (25%) [61].

3.2. Chelating agents

The monoaralkyl esters (HOOCCH(SH)CH(SH)COOR, where R = phenylethyl $((CH_2)_2C_6H_5)$, MPhEDMS; R = 3-phenylpropyl $((CH_2)_3C_6H_5)$, MPhPDMS; and R = 2-phenoxyethyl $((CH_2)_2OC_6H_5)$. MPhOEDMS) of meso-2,3-dimercaptusuccinic acid have been effective in reducing the hepatic and renal Cd concentration in Cd-intoxicated rats, but no information regarding the effect of the chelating agents on the trace elements homeostasis has been presented. Furthermore, the esters of DMSA have been administrated i.p., which could be a disadvantage of these agents over others chelators for the treatment of Cd-intoxication (Table 6) [64].

Among the chelating agents, the polyamines: triethylenetetraamine dihydrochloride (TRIEN), *tris*(2-amino ethyl)amine trihydrochloride (TREN), tetraethylene pentamine pentahydrochloride (TETRAEN), and pentaethylene hexamine hexahydrochloride (PENTAEN) increase Cd in the urine of Cd-exposed rats (Table 5) [65]. Oliguria and anuria induced as a result of the application of these chelating agents have been observed in Cd-treated animals, suggesting a significant renal damage [65].

DMSA and DMPS reduce Cd in the kidneys of mice, exposed to Cd intoxication, but DMSA is not effective in eliminating Cd from intracellular deposits. Therefore this agent is not very suitable for the treatment of chronic Cd-intoxication [46].

The chelating agents alpha-mercapto-beta-(2-furyl) acrylic acid (MFA), alpha-mercapto-beta-(5-sodiumsulfonate, 2-furyl) acrylic acid (MSFA) and alpha-mercapto-beta-(5-acetoxy methyl, 2-furyl) acrylic acid (MAFA) have been found to be effective in mobilizing Cd in Cd-treated animals, but their affect on trace element homeostasis, especially Cu and Zn, should be considered in chelation therapy with these agents [66].

Table 7. Antioxidants, tested on animal models for the treatment of Cd-induced toxicity in the testes (acute Cd-intoxication).

Antioxidant	Route of administration	Dose	Animal model	Effect	Ref.
Ascorbic acid	p.o.	200 mg/100 g b.w.	Rats, exposed to acute intoxication with 0.2 and 0.3 mg/100 g b.w. Cd(II) (s.c.)	The antioxidant prevents germ cell apoptosis; restores testosterone; 3-beta and 17-beta HSD.	[71,72]
Vitamin C Vitamin E	i.p.	Vitamin C 10 mg/kg b.w.; Vitamin E - 100 mg/kg b.w., concurrent with Cd(II)	Mice, intoxicated with Cd(II) chloride 1 mg/kg b.w. i.p.	Both vitamins improve sperm count, decrease sperm abnormality percentage and partially improve total antioxidant capacity in the testes.	[73]
Hemin	p.o.	40 μmol/kg b.w., applied one day before Cd-intoxication and continued for 2 days after Cd-administration	Rats, exposed to acute intoxication with 2 mg/kg Cd(II) chloride, i.p.	Hemin restores the active spermatogenesis but the values of testosterone remain lower than the normal control. The antioxidant preserves the normal histoarchitectute of the testes of Cd-treated rats.	[74]
$(PhSe)_2$	p.o.	400 μmol/kg b.w., applied 30 min after Cd-administration	Mice, exposed to Cd(II) chloride (5 mg/kg b.w., i.p.)	The antioxidant partially decreases Cd-induced LPO and increases ascorbic acid in the testes. Moderate edema is observed in the testicular tissues of Cd-treated animals, receiving $(PhSe)_2$.	[75]
Vitamin E and Q10	Information not found	Information not found	Rats, exposed to 0,4 mg/kg	Both antioxidants recover GSH, SOD and GR and GSH-Px to normal values.	[76]
Melatonin	i.p.	5 mg/kg b.w. every 8th hour, beginning 8 hours before Cd-administration	Mice, exposed to Cd(II) chloride (2 mg/kg), i.p.	The antioxidant inhibits Cd-induced testicular germ cell apoptosis.	[77]

DFO and monensin are most effective in the elimination of Cd in animals, exposed to acute and subactute Cd-intoxication. Both agents have been demonstrated to improve iron homeostasis and could be applied orally [46,67].

4. Antioxidant and chelating agents, tested on animal models for the treatment of Cd-induced testicular dysfunction

Cd induces reduction of sperm motility, testosterone, 3-β-hydroxysteroiddehydrogenase (3-β-HSD), 17-β-hydroxysteroid dehydrogenase (17-β-HSD) and an increase of the percentage of abnormal sperm in animals, subjected to Cd intoxication. Histopathological analysis of testicular tissue of animals, administrated Cd, reveals that the toxic metal causes severe lesions in the form of diffuse necrosis affecting the germinal layer in the seminiferous tubules and interstitial tissue [70]. Higher levels of lipid peroxidation in testes compared to the normal control have been reported in animals, receiving Cd. Cd decreases the activity of antioxidant enzymes: SOD, CAT and GSH level in the testes [70].

4.1. Antioxidants

4.1.1. Antioxidants, screened on animal models for the treatment of oxidative stress in testes of animals, exposed to acute Cd-intoxication

The antioxidants, screened on animal models for the treatment of oxidative stress in testes of animals, exposed to acute Cd-intoxication, are summarized in Table 7. Vitamins C and E alone or in combination, applied concurrent with Cd-intoxication have been shown to restore testosterone and testicular key androgenic enzymes 3-β-hydroxysteroid dehydrogenase and 17-β-hydroxysteroid dehydrogenase [71,72]. Depending on the dose of Cd, Vitamins C and E have been demonstrated to recover partially or completely the total antioxidant capacity in the testes. Both vitamins decrease sperm abnormality percentage and improve sperm count [71-73,76].

4.1.2. Antioxidants, screened on animals for the treatment of oxidative stress in testes of animals, exposed to subchronic and chronic Cd-intoxication

The antioxidants tested on Cd-induced oxidative stress in animals, exposed to subchronic and chronic Cd intoxication, are presented in Table 8.

Vitamin E and β-carotene improve some hematological paramets (Hb, TEC, TLC) in rats, subjected to subchronic Cd-intoxication and significantly recover sperm quality [80,81]. α-tocopherol reduces the degree of necrosis of the seminiferous tubules induced by 2 mg/kg b.w. Cd(II) chroride, injected i.p. in rats for 5 weeks [78,79]. The antioxidant however has been demonstrated not to have protective effect in higher doses of Cd(II) chloride [80] and applied before Cd-intoxication.

α-Lipoic acid, applied along with Cd, also recovers testicular key androgenic enzymes and preserves the normal testicular architecture [81].

Among the antioxidants, screened on animal models for the treatment of Cd-induced toxicity in the testes of animals exposed to subchronic or chronic Cd-intoxication, hesperetin (HP), diallyl tetrasulfide (DTS) and α-tocopherol possess chelating effect, reducing the concentration of toxic metal ion [70,82-84]. The protection by these antioxidants is further substantiated by reduction of Cd-induced pathological changes in the testicular tissue.

4.2. Chelating agents

The chelating agents screened on animal models for the treatment of Cd-induced toxicity in testes are summarized in Table 9. Diethyldithiocarbamate (DED) alone decreases Cd concentration in testes, but redistribution of the toxic metal ion to the kidneys and brain has been observed [91,92]. The combinations of DED and *N*-benzyl-*D*-glucamine dithio carbamate (BGD) or DED and *N*-*p*-isopropylbenzyl-*D*-glucaminedithiocarbamate (PBGD) diminish Cd level in testes without causing its redistribution in other tissues [91,92]. However, these combinations of the chelating agents increase Cd concentration in the blood and Cd distribution in the red blood cells [91,92]. Disulfiram (DSF) has been demonstrated to be more effective compated to DED in reducing the testicular damage, caused by Cd [93].

Meso-2,3-dimercaptosuccinic acid (DSMA) applied alone or in combination with $(PhSe)_2$ inhibits the toxicity caused by 2.5 mg/kg b.w. Cd(II) chloride in mice. Both compounds however are ineffective in restoring the SOD activity and the level of ascorbic acid, induced by 5 mg/kg Cd(II) chloride [94]. Diphenyl diselenide applied s.c. 30 min after Cd administration in mice elevates Cd-induced injury in the testes, but when administrated at higher dose (100 μmol/kg), applied alone or together with 2,3-dimercapto-1-propanesulfonic acid (DMPS) decreases Cd-induced toxicity and improves the level of δ-aminolevulinic acid dehydratase (δ-ALA-D) [95,96].

Table 8. Antioxidants, screened on animal models for the treatment of Cd-induced oxidative stress in testes of animals, exposed to subchonic and chronic Cd-intoxication.

Antioxidant	Route of administration	Dose	Animal model	Effect	Ref.
β-carotene	i.g.	250 IU/kg, concurrent with Cd(II)	Rats, intoxicated with Cd(II) chloride (2 mg/kg b.w. - intragastrically for 3 and 6 weeks)	The antioxidant restores the activity of SOD and GSH in the testes and brain.	[78]
Vitamin E-, β-carotene	p.o. by gavage	100 mg/kg Vitamin E; 10 mg/kg β-carotene for 15 days, concurrent with Cd(II)	Rats, exposed to Cd(II) chloride (5mg/kg b.w. for 15 days	Both vitamins alone or in combination improve sperm concentration, motility (%) and recover the weight of the testes and epididymis, and decrease the abnormal sperm.	[79]
α-tocopherol	i.p.	0,1 mL applied five days before Cd-intoxication	Rats, exposed to Cd i.p. Doses of Cd(II) chloride - 1, 2 , 4, 9 mg/kg b.w. for 5 weeks	The antioxidant does not show protective effect on higher doses of Cd(II) chloride - 4 and 8 mg/kg.	[80]
Organoselenium compound (Ebselen)	Information not found	Information not found	Mice, administrated i.p. Cd(II) chloride	Ebselen recovers the levels of ascorbic acid; the activity of delta-ALA-D and inhibits LPO in the testes.	[85]
Diallyl sulphide (DAS)	Information not found	Information not found	Rats, exposed to 2, 5 mg/kg Cd(II) chloride five times a week for 4 weeks	DAS restores total antioxidant capacity in the testes and restores testosterone levels; 3-beta-HSD and 17-beta-HSD.	[86]
Onion and garlic	p.o.	0, 5 mL/100 g bw. 1 mL/100 g b.w.; applied 1 week before intoxication and continued with Cd-administration	Rats, intoxicated with 1,5 mg Cd/100 g b.w. daily p.o. for 3 weeks	The onion and combination of onion and garlic are more effective than garlic alone. The antioxidants improve sperm motility, decrease abnormal sperm rate. They partially recovered GSH, SOD and reduce LPO.	[87]
α-lipoic acid	p.o.	35 mg/kg, concurrent with Cd(II)	Rats, exposed to 2 mg/kg Cd(II) chloride, i.p. for 28 days	The antioxidant ameliorates testicular key androgenic enzymes and preserves the normal histoarchitecture of the testes.	[81]
Guarana (Paullinia cupana)	p.o. or i.p.	Pretreatment for 56 days with 2 mg/kg b.w., 1.15 mg/kg i.p. single dose	Rats	P. Cupana reduces inflammatory cell response and improves the morphology of Leidyg cells.	[88]
Tribulus terrestris Linn (TT); α-tocopherol	p.o.	TT - 5 mg/kg b.w. daily; -tocopherol - 75 mg/kg b.w. daily for 6 weeks	Rats, exposed to Cd(II) chloride treatment (3 mg/kg b.w.) once a week for 6 weeks	TT reduces Cd by 50 %, α-tocopherol - 80 %. Both antioxidants improve total antioxidant capacity of the testes and preserve the normal histoarchitecture of the testes. The α-tocopherol is more effective than TT at the doses applied.	[82,83]
Quercetin (QE)	p.o.	75 mg/kg daily for 2 weeks, concurrent with Cd(II)	Mice, treated with Cd(II) chloride (4 mg/kg b.w.) p.o. for 2 weeks	The antioxidant inhibits germ cell apoptosis and LPO induced by Cd. QE recovers SOD and improves GSH level.	[89]
Ginger	p.o.	Information not found	Rats	The antioxidant reverses the Cd-induced toxicity in the testes.	[90]
Hesperetin (HP)	p.o.	40 mg/kg b.w. along with Cd(II)	Rats, intoxicated with 3 mg/kg b.w. Cd (II) chloride s.c. for 21 days	HP reduces Cd concentration in the testes by 50 %; abolishes Cd-induced pathology in testicular tissue and improves the antioxidant total capacity.	[70]
Diallyl tetrasulfide (DTS)	p.o.	40 mg/kg b.w. concurrent with Cd(II)	Male rats, intoxicated with 3 mg/kg Cd s.c. for 3 weeks	DTS reduces Cd accumulation in the testes, improves the activity of antioxidant enzymes and GSH level in the testes, and preserves the normal histoarchitecture of the testes.	[84]

Table 9. Chelating agents, applied for treatment of Cd concentration in testes of animals, subjected to Cd-intoxication.

Chelating agent	Route of administration	Dose	Animal model	Effect	Ref.
DED; BGD; PBGD	i.p.	0.4, 1 or 3 mmol/kg b.w., after Cd-treatment	Rats, intoxicated with 3 mg/kg Cd(II) s.c.	Combinations of DED and BGD or DED and PBGD prevented sterility caused by Cd and decrease Cd in the testes.	[91,92]
Mi-ADMS	i.p.	0,5 mmol/kg b.w., after Cd-intoxication	Rats, intoxicated with 0,03 mmol/kg Cd(II) chloride, i.p.	The chelating agent, applied 1 h after Cd-administration reduces Cd-induced apoptosis and DNA fragmentation.	[98,99]
DSF; DED	i.p.	DED - 0, 1; 1 mmol/kg b.w.; DSF - 0,05; 5 mmol/kg b.w. applied after Cd-intoxication	Rats, injected (s.c.) with 3 mg/kg Cd	DSF inhibits Cd-induced LPO; reduces testicular damage caused by Cd and restores Fe homeostasis.	[93]
$(PhSe)_2$; DMSA	Information not found	Information not found	Mice, exposed to 2.5 mg/kg b.w. Cd(II) chloride or 5 mg/kg b.w. Cd(II) chloride (i.p)	Both compounds in combination or alone do not improve the ascorbic acid and SOD activity in mice, exposed to 5 mg/kg Cd(II) chloride.	[94]
$(PhSe)_2$; DMPS	Information not found	Information not found	Mice, exposed to 2.5 mg/kg b.w. Cd(II) chloride or 5 mg/kg b.w. Cd(II) chloride (i.p)	The chelating agents partially inhibit Cd-induced toxicity caused by 2.5 mg/kg Cd(II) chloride. Combined therapy decreases Cd concentration in testes and recovers plasma AST, elevated by 5 mg/kg Cd(II) chloride.	[95]
$(PhSe)_2$	s.c.	5 micromol/kg b.w., in 30 min after Cd five weekly, 4 weeks	Mice, exposed to subchronic intoxication with Cd(II) chloride (s.c. 2.5 mg/kg, five times weekly, 4 weeks)	The antioxidants, enhanced Cd-induced testicular injury.	[96]
Monensin	p.o.	14 mg/kg, applied after Cd-intoxication	Mice, exposed to subacute intoxication with Cd(II) acetate for 2 weeks (10 mg/kg b.w.)	Monensin reduces Cd concentration in the testes by 50 % compared to the normal control and recovers the histology of the testes to a great extent.	[97]

Abbreviations: Diethyldithiocarbamate (DED), *N*-benzyl-D-glucamine dithiocarbamate (BGD), *N*-*p*-isopropylbenzyl-D-glucamine dithiocarbamate (PBGD); Disulfiram (DSF); Diphenyl diselenide $(PhSe)_2$.

Table 10. Antioxidants and chelating agents, tested on animal model to reduce Cd-induced cardiac impairment.

Compound	Route of administration	Dose	Animal model	Effect	Ref.
DMPS	i.v.	n.a.	Rabbits, intoxicated with Cd(II) chloride 50 mg/kg b.w./week for 10 weeks	The chelating agent decreases Ca and Mg concentration in the cardiac muscle.	[105]
Caffeic acid phenyl ester	i.p.	10 μmol/kg b.w., for 15 days along with Cd	Rats, intoxicated with Cd(II) chloride 1 mg/kg/day for 15 days (i.p.)	The antioxidant decreases MDA level in the cardiac muscle in the Cd-treated rats; increases NO in the serum and inhibits Cd-induced myocardial hypertrophy.	[100]
Vitamin E and coenzyme Q (10)	i.m.	Vitamin E - 20 IU/kg/b.w. Q10 - 20 mg/kg b.w. concurrent with Cd	Rats, exposed to 0.4 mg/kg Cd (i.p.)	The combination of both oxidants diminishes the toxic effects induced by Cd.	[101]
Taurine Vitamin C	p.o.	100 mg/kg b.w. for 5 days, prior to Cd-intoxication	Mice, received Cd(II) chloride for 6 days (4 mg/kg b.w.)	Both antioxidants decrease Cd concentration in the cardiac muscle, improve antioxidant defence system and preserve the normal histoarchitecture of the heart.	[103]
Diethyl carbamate	Data not available	Data not available	Rats, exposed to 20 mg/kg b.w. Cd or 40 mg/kg b.w. Cd/ daily for 60 days	The chelating agent reduces Cd concentration by 36% compared to the control and recovers Fe homeostasis.	[69]
DMSA	Intragastrically	25 mg/kg 50 mg/kg for 5 days after Cd-intoxication	Mice, treated with 100 mg/L for 8 weeks	DMSA reduces the concentration of Cd in blood, heart, liver and kidneys, improves blood pressure and antioxidant defence system in the heart.	[104]
α-Lipoic acid (ALA) melathonin	Information not found	ALA - 25 mg/kg b.w. /day; Melathonin - 10 mg/kg b.w./day, applied together with Cd(II)	Rats, subjected to subchronic Cd intoxication 5 mg/kg b.w. /day for 15 days	The combination of both agents inhibits Cd-induced cardiac impairment.	[102]
Monensin	p.o.	14 mg/kg after Cd intoxication for 2 weeks	Mice, exposed to 10 mg/kg daily Cd(II) acetate for 2 weeks	Monensin reduces Cd concentration in the heart by 90% compared to the control and recovers the morphology of the heart.	[47]

Our results have demonstrated that monensin (applied as tetraethylammonium salt) reduces Cd concentration in the testes of Cd-treated mice by 50 % compared to the control. The administration of the chelating agent to the Cd-intoxicated mice restored the histology of the testes to normal to a great exent [97].

5. Antioxidants and chelating agents, tested to inhibit Cd-induced cardiac impairment

Cd is a vascular toxicant that induces hypertension and myocardial hypertrophy. The generation of ROS either by affecting Fe homeostasis or NO level in the endothelial cells has been considered as a primary mechanism for Cd-induced cardiac toxicity.

Cd induces expression of stress genes which in turn alters the activity of the antioxidant enzymes [100]. The antioxidants and chelating agents, screened on animal models to prevent Cd-induced cardiac toxicity, are summarized in Table 10.

The combination of coenzyme Q(10) and Vitamin E has been demonstrated to reverse Cd-induced alterations in the antioxidant defense system of heart of rats, exposed to acute Cd-intoxication [101].

Caffeic acid phenyl ester (CAPE) administrated concurrent with Cd prevents the morphological alterations, induced by Cd in the heart of Cd-treated rats by decreasing lipid peroxidation and improving NO homeostasis [100].

The combination of α-lipoic acid and melatonin applied along with the Cd treatment also inhibits Cd-induced cardiac impairment in rats, subjected to subchronic Cd-intoxication [102].

Vitamin C and taurine, applied before Cd-intoxication, decrease Cd accumulation in the heart of mice, exposed to subacute Cd-intoxication. Vitamin C is more effective than taurine in preventing the activity of the antioxidant enzymes and the level of GSH in the cardiac muscle. The cardioprotective effect of both antioxidants is substantiated by the reduction of Cd-induced pathological changes in the cardiac tissue [103].

Among the chelating agents, DMSA and monensin seem to be the most effective in decreasing Cd concentration in the heart of animals, intoxicated with Cd [47,104].

2,3-Dimercapto-1-propanesulphonate (DMPS) also decreases Cd concentration in the cardiac muscle of Cd-treated animals. However, precautions should be taken in its application in patients with cardiotoxicity because of its affect on the Ca and Mg concentration in the heart [105].

6. Conclusion

There are many published reviews on chelating agents and antioxidants tested for the treatment of intoxications with toxic metal ions. To the best of our knowledge however this is the first review that summarizes the literature information published in the last 20 years for the antioxidants and chelating agents, tested on animal models for the treatment of Cd-induced organ toxicity. Detailed information regarding the Cd-induced pathological conditions (hepatotoxicity, renal dysfunction, reproductive disorder and cardiac impairment) is also presented. The data discussed in the review demonstrate the necessity of development of effective scheme for therapy of humans, exposed to Cd-intoxication. Based on the data, presented in this paper, it could be concluded that the simultaneous application of antioxidants and chelating agents could be a good approach for the treatment of Cd-induced organ toxicity. In order to identify the best antioxidant and chelating agent for the treatment of Cd-induced pathological conditions, detailed studies for their effects on different organs in animals, exposed to Cd-intoxications are needed.

References

[1]. IARC, International Agency for Research on Cancer, Beryllium, cadmium, mercury, and exposures in the glass manufacturing industry. In International Agency for Research on Cancer Monographs on the Evaluation of Carcinogenic Risk to Humans; IARC Scientific publications; Lyon, France, 1993; Volume 58, 119-237.
[2]. WHO, Cadmium: Air Quality Guidelines, 2nd ed. World Health Organization, Regional office for Europe, Copenhagen, Denmark, Chapter 6.3., 2000.
[3]. Patrick, L. *Altern. Med. Rev.* **2003**, *8(2)*, 106-128.
[4]. Djukovic-Cosic, D. C.; Jovanovic, M.; Bulat, Z. P.; Ninkovic, M.; Malicevic, Z.; Matovic, V. *J. Trace Elem. Med. Biol.* **2008**, *22*, 67-72.
[5]. Thijssen, S.; Lambrichts, I.; Maringwa, J.; Kerkhove, E. V. *Toxicol.* **2007**, *238*, 200-210.

[6]. Thijssen, S.; Maringwa, J.; Faes, Ch.; Lambrichts, I.; Kerkhove, E. V. *Toxicol.* **2007**, *229*, 145-156.
[7]. Oliviera, H.; Spano, M.; Santos, C.; Pereira, M. *Reproductive Toxicol.* **2009**, *28*, 550-555.
[8]. Monsefi, M.; Alaee, S.; Moradshahi, A.; Rohani, L. *Environ. Toxicol.* **2010**, *25*, 94-102.
[9]. Prozialeck, W. C.; Edwards, J. R.; Woods, J. M. *Life Sci.* **2006**, *79*, 1493-1506.
[10]. Prozialeck, W. C.; Edwards, J. R.; Nebert, D. W.; Woods, J. M.; Barchowsky, A.; Atchison. W. D. *Toxicol. Sci.* **2008**, *102*, 207-218.
[11]. Satarug, S.; Nishijo, M.; Ujjin, P.; Vanavanitkun, Y.; Moore, M. R. *Toxicol. Lett.* **2005**, *157*, 57-68.
[12]. Smetana, R.; Glogar, D.; Weidinger, F.; Meisinger, V. *Wien. Med. Wochensch.* **1987**, *137*, 553-557.
[13]. Teller-Plaza, M.; Navas-Acien, A.; Crainiceanu, C. M.; Sharrett, A. R.; Guallar , E. *Am. J. Epidemiol.* **2010**, *172*, 671-681.
[14]. Peters, J. L.; Perlstein, T. S.; Perry, M. L.; McNeely, E.; Weuve, J. *Environ. Res.* **2010**, *110*, 199-206.
[15]. Flora, S. J.; Mittal, M.; Mehta, M. A. *Indian J. Med. Res.* **2008**, *128*, 501-523.
[16]. Blanusa, M.; Varnai, V. M.; Piasek, M.; Kostial, K. *Current Med. Chem.* **2005**, *12*, 2771-2794.
[17]. Sinicropi, M. S.; Amantea, D.; Caruso, A.; Sturnino, C. *Arch. Toxicol*. **2010**, *84*, 501-520.
[18]. Andjuar, P.; Bensefa-Colas, L.; Descatha, A. *Rev. Med. Interne.* **2010**, *31*, 107-115.
[19]. Jurczuk, M.; Galazyn-Sidorczuk, M.; Brzorska, M. M.; Moniuszko-Jakoniuk, J. *Polish J. Environ. Studies* **1997**, *6*, 74-76.
[20]. Snaikh, Z. Z.; Vu, T. T.; Zaman, K. *Toxicol. Appl. Pharmacol*. **1999**, *154*, 256-263.
[21]. Ercal, N.; Guerer-Orhan, H.; Aykin-Burns, N. *Current Topics in Med. Chem.* **2001**, *1*, 529-539.
[22]. Sinha, M.; Manna, P.; Sil, P. C. *J. Trace Elem. Med. Biol.* **2009**, *23*, 300-313.
[23]. Fouad, A. A.; Qureshi, H. A.; Yacoubi, M. T.; Al-Melhim, W. N. *Food Chem. Toxicol.* **2009**, *47*, 863-870.
[24]. Pari, L.; Shagirtha, K. *Exp. Toxicol. Pathol.* **2012**, *64*, 513-520.
[25]. Bludovska, M.; Kotyzova, D.; Koutensky, J.; Eybl, V. *Gen Physiol Biophys*. **1999**, *18*, 28-32.
[26]. Eybl, V.; Kotyzova, D.; Bludovska, M. *Toxicol Lett.* **2004**, *151*, 79-85.
[27]. Eybl, V.; Kotyzova. D.; Leseticky, L.; Bludovska, M.; Koutensky, J. *J Appl. Toxicol.* **2006**, *26*, 207-212.
[28]. Eybl, V.; Kotyzova, D.; Koutensky, J. *Toxicol.* **2006**, *225(2-3)*, 150-156.
[29]. Murugavel, P.; Pari, L. *Hum. Exp. Toxicol.* **2007**, *26*, 527-534.
[30]. Pari, L.; Murugavel, P.; Sitasawad, S. L.; Kumar, K. S. *Life Sci.* **2007**, *80*, 650-658.
[31]. Gong, P.; Chen, F. X.; Ma, G. F.; Feng, Y.; Zhao, Q.; Wang, R. *Toxicol.* **2008**, *251*, 35-44.
[32]. Nemmiche, S.; Chabane-Sari, D.; Guiraud, P. C*hem. Biol. Interact*. **2007**, *170*, 221-230.
[33]. Olalekan, L. A.; Lawal, A. F.; Ologundudu, A.; Adeniran, O. Y.; Omonkhua, A.; Obi, F. *Toxicol. Sci.* **2011**, *36*, 549-557.
[34]. Shukla, R.; Kumar, M. *Food Chem. Toxicol.* **2009**, *47*, 769-773.
[35]. Chlebda, E.; Magdalan, J.; Merwid-Lad, A.; Trocha, M.; Kopacz. M.; Kuźniar, A.; Nowak, D.; Szelag, A. E*xp. Toxicol. Pathol.* **2010**, *62*, 105-158.
[36]. Santos, F. W.; Zeni, G. , Rocha, J. B.; Weis, S. N. , Fachinetto, J. M.; Favero, A. M.; Nogueira, C. W. *Chem. Biol. Interact.* **2005**, *151*, 159-65.
[37]. Borges, L. P.; Brandao, R.; Godoi, B.; Nogueira, C. W.; Zeni, G. *Chem. Biol. Interact.* **2008**, *171*, 15-25.
[38]. Chwelatiuk, E.; Wlostowski, T.; Krasowska, A.; Bonda, E. *J. Trace Elem. Med. Biol.* **2006**, *19*, 259-265.
[39]. Sk, U. H.; Sharma, A. K.; Ghosh, S.; Bhattacharya, S. *Eur. J. Med. Chem.* **2010**, *45*, 3265-3273.
[40]. Renugadevi, J.; Prabu, S. M. *Exp Toxicol Pathol.* **2010**, *62*, 171-181.
[41]. Prabu, S. M.; Shagirtha, K.; Renugadevi, J. J. *Nutr. Sci. Vitaminol (Tokyo)* **2011**, *57*, 177-185.
[42]. Casalino, E.; Calzaretti, G.; Sblano, C.; Landriscina, V.; Felice, Tecce M.; Landriscina, C. *Comp. Biochem. Physiol. C. Toxicol. Pharmacol.* **2002**, *133*, 625-632.
[43]. Asagba, S. O.; Adaikpoh, M. A.; Kadiri, H.; Obi, F. O. *Biol. Trace Elem. Res.* **2007**, *115*, 47-57.
[44]. Eybl, V.; Kotyzova, D.; Koutensky, J.; Jones, M. M.; Singh, P. K. *Analyst* **1995**, *120*, 855-857.
[45]. Srivastava, R. C.; Gupta, S.; Ahmad, N.; Hasan, S. K.; Farookh, A.; Husain, M. M. *J. Toxicol. Environ. Health* **1996**, *47*, 173-182.
[46]. Singh, P. K.; Jones, M. M.; Rostial, K.; Blanusa, M.; Piasek, M.; Restek-Samarzija, N. *Chem. Res. Toxicol.* **1996**, *9*, 965-969.
[47]. Ivanova, J.; Gluhcheva, Y.; Kamenova, K.; Arpadjan, S.; Mitewa, M. *J. Trace Elem. Med. Biol.* **2012**, *26*, 279-284.
[48]. Ivanova, J.; Gluhcheva, Y.; Kamenova, K.; Arpadjan, S.; Mitewa, M. *Biotechnol. Biotechnol. Eq.* **2013**, in press.
[49]. Eybl, V.; Kotyzova, D.; Koutensky, J.; Míckova, V.; Jones, M. M.; Singh, P. K. *Analyst* **1998**, *123*, 25-26.
[50]. Suru, S. M. *Biometals.* **2008**, *21*, 623-633.
[51]. Cavusoglu, K.; Yapar, K.; Yalcin, E. *J. Med. Food.* **2009**, *12*, 1286-1292.
[52]. Manna, P.; Sinha, M.; Sil, P. C. *Amino Acids* **2009**, *36*, 417-428.
[53]. Li, R.; Yuan, C.; Dong, C.; Shuang, S.; Choi, M. M. *Naunyn Schmiedebergs Arch. Pharmacol.* **2011**, *383*, 437-445.
[54]. Gong, P.; Chen, F.; Liu, X.; Gong, X.; Wang. J.; Ma, Y. *J. Toxicol. Sci.* **2012**, *37*, 415-425.
[55]. Kaplan, M.; Atakan, I. H.; Aydogdu, N.; Aktoz, T.; Ozpuyan, F.; Seren, G.; Tokuç, B.; Inci, O. *Pediatr. Nephrol.* **2008**, *23*, 233-241.
[56]. Wang, L.; Chen, D.; Cao, J.; Liu, Z. *Hum. Exp. Toxicol.* **2009**, *28*, 221-229.
[57]. Renugadevi, J.; Prabu, S. M. *Toxicol*. **2009**, *256*, 128-134.
[58]. Morales, A. I.; Vicente-Sanchez, C.; Jerkic, M.; Santiago. J. M.; Sanchez-Gonzalez, P. D.; Perez-Barriocanal, F.; López-Novoa, J. M. *Toxicol. Appl. Pharmacol.* **2006**, *210*, 128-135.
[59]. Renugadevi, J.; Prabu, S. M. E*xp. Toxicol. Pathol.* **2010**, *62*, 471-481.
[60]. Prabu, S. M.; Shagirtha, K.; Renugadevi, J. *Rev. Med. Pharmacol. Sci.* **2010**, *14*, 903-914.
[61]. Bharavi, K.; Reddy, A. G.; Rao, G. S.; Kumar, P. R.; Kumar, D. S.; Prasadini, P. P. *Indian J. Pharmacol.* **2011**, *43*, 45-49.
[62]. Shaikh, Z. A.; Vu, T. T.; Zaman, K. *Toxicol. Appl. Pharmacol.* **1999**, *154*, 256-263.
[63]. Fouad, A. A.; Jresat, I. *Life Sci.* **2011**, *89*, 29-35.
[64]. Jones, M. M.; Singh, P. K.; Basinger, M. A.; Gale, G. R.; Smith, A. B. *Pharmacol. Toxicol.* **1994**, *74*, 76-83.
[65]. Jones, M. M.; Xu, C.; Singh, P. K.; Walker, E. M. Jr. *J. Toxicol. Environ. Health.* **1996**, *48*, 71-80.
[66]. Tandon, S. K.; Prasad, S.; Singh, S. *Biomed. Environ. Sci.* **2000**, *13*, 205-212.
[67]. Fatemi, S. J.; Saljooghi, A. S.; Balooch, F. D.; Iranmanesh, M.; Golbafan, M. R. *Toxicol. Ind. Health.* **2012**, *28*, 35-41.
[68]. Jones, M. M.; Singh, P. K.; Basinger, M. A.; Gale, G. R.; Smith, A. B.; Harris, W. R. *Chem. Res. Toxicol.* **1994**, *7*, 367-373.
[69]. Fatemi, S. J.; Tubafard, S.; Nadi, B. *Med. Chem Res.* **2009**, *18*, 179-186.
[70]. Shagirtha, K.; Pari, L. *Ecotoxicol. Environ. Saf.* **2011**, *74*, 2105-2111.
[71]. Sen Gupta, R.; Kim, J.; Gomes, C.; Oh, S.; Park, J.; Im, W. B.; Seong, J. Y.; Ahn, R. S.; Kwon, H. B.; Soh, J. *Mol Cell Endocrinol.* **2004**, *221*, 57-66.
[72]. Sen Gupta, R.; Sen Gupta, E.; Dhakal, B. K.; Thakur, A. R.; Ahnn, J. *Mol. Cells* **2004**, *17*, 132-139.
[73]. Acharya, U. R.; Mishra, M.; Patro, J.; Panda, M. K. *Reprod. Toxicol.* **2008**, *25*, 84-88.
[74]. Fouad, A. A.; Qureshi, H. A.; Al-Sultan, A. I.; Yacoubi, M. T.; Ali, A. A. *Toxicol.* **2009**, *257*, 153-160.
[75]. Brandao, R.; Santos, F. W.; Oliveira, R.; Roman, S. S.; Nogueira, C. W. *J. Trace Elem. Med. Biol.* **2009**, *23*, 324-333.
[76]. Ognjanovic, B. I.; Markovic, S. D.; Ethordevic, N. Z.; Trbojevic, I. S.; Stajn, A. S.; Saicic, Z. S. *Reprod. Toxicol.* **2010**, *29*, 191-197.
[77]. Ji, Y. L.; Wang, H.; Meng, C.; Zhao, X. F.; Zhang, C.; Zhang, Y.; Zhao, M.; Chen, Y. H.; Meng, X. H.; Xu, D. X. *J. Pineal Res.* **2012**, *52*, 71-79.
[78]. El-Missiry, M. A.; Shalaby, F. *J. Biochem. Mol. Toxicol.* **2000**, *14*, 238-243.
[79]. El-Demerdash, F. M.; Yousef, M. I.; Kedwany, F. S.; Baghdadi, H. H. *Food Chem. Toxicol.* **2004**, *42*, 1563-1571.
[80]. Yang, H. S.; Han, D. K.; Kim, J. R.; Sim, J. C. *J. Korean Med. Sci.* **2006**, *21(3)*, 445-451.
[81]. El-Maraghy, S. A.; Nassar, N. N. *J. Biochem. Mol. Toxicol.* **2011**, *25*, 15-25.
[82]. Rajendar, B.; Bharavi, K.; Rao, G. S.; Kishore, P. V.; Kumar, P. R.; Kumar, C. S.; Kumar, D. S. *Indian J. Physiol. Pharmacol.* **2011**, *55*, 213-220.
[83]. Rajendar, B.; Bharavi, K.; Rao, G. S.; Kishore, P. V.; Kumar, P. R.; Kumar, C. S.; Patel T. P. *Indian J. Pharmacol.* **2011**, *43*, 568-573.
[84]. Ponnusamy, M.; Pari, L. *Toxicol. Ind. Health* **2011**, *27*, 407-416.
[85]. Ardais, A. P.; Santos, F. W.; Nogueira, C. W. *J. Appl. Toxicol.* **2008**, *28*, 322-328.
[86]. Sadik, N. A. *J. Biochem. Mol. Toxicol*. **2008**, *22*, 345-353.
[87]. Ola-Mudathir, K. F.; Suru, S. M.; Fafunso, M. A.; Obioha, U. E.; Faremi, T. Y. *Food Chem. Toxicol.* **2008**, *46*, 3604-3611.
[88]. Leite, R. P.; Wada, R. S.; Monteiro, J. C.; Predes, F. S.; Dolder, H. *Biol. Trace Elem. Res.* **2011**, *141*, 262-274.
[89]. Bu, T.; Mi, Y.; Zeng, W.; Zhang, C. *Anat. Rec. (Hoboken).* **2011**, *294*, 520-526.
[90]. Onwuka, F. C.; Erhabor, O.; Eteng, M. U.; Umoh, I. B. *J. Med. Food* **2011**, *14*, 817-821.
[91]. Kojima, S.; Sugimura, Y.; Hirukawa, H.; Kiyozumi, M.; Shimada, H.; Funakoshi, T. *Toxicol. Appl. Pharmacol.* **1992**, *116*, 24-29.
[92]. Kojima, S.; Sugimura, Y.; Ono, H.; Shimada, H.; Funakoshi, T. *Biol Pharm Bull.* **1993**, *16*, 244-247.
[93]. Ono, H.; Funakoshi, T.; Shimada, H.; Kojima, S. *J. Toxicol. Environ. Health* **1997**, **50**, 389-399.
[94]. Santos, F. W.; Oro, T.; Zeni, G.; Rocha, J. B.; do Nascimento, P. C.; Nogueira, C. W. *Toxicol. Lett.* **2004**, *152*, 255-263.
[95]. Santos, F. W.; Graça, D. L.; Zeni, G.; Rocha, J. B.; Weis, S. N.; Favero, A. M.; Nogueira, C. W. *Reprod Toxicol.* **2006**, *22*, 546-550.
[96]. Santos, F. W.; Zeni, G.; Rocha, J. B. , do Nascimento, P. C.; Marques, M. S.; Nogueira, C. W. *Food Chem. Toxicol*. **2005**, *43*, 1723-1730.
[97]. Pavlova, E.; Ivanova J.; Dimova, D.; Gluhcheva, Y.; Atanasova, N. *Eur. Chem. Bull.* **2012**, *1*, 463-465.
[98]. Xu, C.; Holscher, M. A.; Jones, M. M.; Singh, P. K. J. *Toxicol. Environ. Health.* **1995**, *45*, 261-277.

[99]. Xu, C.; Johnson, J. E.; Singh, P. K.; Jones, M. M.; Yan, H.; , Carter, C. E. *Toxicol*. **1996**, *107*, 1-8.
[100]. Mollaoglu, H.; Gokcimen, A.; Ozguner, F.; Oktem, F.; Koyu, A.; Kocak, A.; Demirin, H.; Gokalp, O.; Cicek, E. *Toxicol*. **2006**, *227*, 15-20.
[101]. Ognjanovic, B. I.; Markovic, S. D.; Pavlovic, S. Z.; Zikic, R. V.; Stajn, A. S.; Saicic, Z. S. *Environ. Toxicol. Pharmacol.* **2006**, *22*, 219-224.
[102]. Mukherjee**,** R.; Banerjee, S.; Joshi, N.; Singh, P. K.; Baxi, D, Ramachandran, A. V. *Cardiovasc. Toxicol.* **2011**, *11*, 78-88.
[103]. Manna, P.; Sinha, M.; Sil, P. C. *Chem Biol Interact.* **2008**, *174*, 88-97.
[104]. Sompamit, K.; Kukongviriyapan, U.; Donpunha, W.; Nakmareong, S.; Kukongviriyapan, V. *Toxicol. Lett.* **2010**, *198*, 77-82.
[105]. Hrdina, R.; Gersl, V.; Vavrova, J.; Holeckova, M.; Palicka, V.; Voglova, J.; Mazurova, Y.; Bajgar, J. *Hum. Exp. Toxicol.* **1998**, *17*, 221-224.