



Different Routes of Administration Lead to Different Oxidative Damage and Tissue Disorganization Levels on the Subacute Cadmium Toxicity in the Liver

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Received: 8 October 2020 / Accepted: 28 December 2020

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Abstract

The toxic effects of cadmium (Cd) on hepatic parameters are widely described in the literature. Experimental models often make use of the intraperitoneal route (i.p.) because it is easier to apply, while in the oral route, Cd poisoning in humans is best represented by allowing the metal to pass through the digestive system and be absorbed into the bloodstream. Thus, this study investigated the Cd exposure impact on the liver, by comparing both i.p. and oral routes, both in single dose, in addition to the oral route in fractional doses. Swiss adult male mice received CdCl₂ 1.5 mg/kg i.p., 30 mg/kg oral single dose, and 4.28 mg/kg oral route in fractional doses for 7 consecutive days. Cd bioaccumulation was observed in all animals exposed to Cd. Hepatic concentrations of Ca and Fe increased only in the fractionated oral route. Liver activities of SOD and CAT increased only by oral single dose. GST decreased in all forms of oral administration, while MDA decreased only in i.p. route. Liver weight and HSI increased in the i.p. route, while organ volume increased in all forms of oral administration, and liver density increased in all animals exposed to Cd. In hepatic histomorphometry, the changes were more evident in oral administration, mainly in exposure to metal in a single dose. Thus, the subacute administration of Cd in different routes of administration leads to different changes in liver poisoning.

Keywords Histomorphometry · Heavy metal · Toxicology

Introduction

Cadmium (Cd) is a toxic metal, with long biological life (20 years) [1]. Its presence in the environment occurs by natural sources (e.g., volcanic activity, weathering of rocks, and forest fires), human activities (e.g., derived from batteries, pigments, plastic stabilizers, pesticides, and fertilizers) [2], or environmental disasters [1, 3–5]. Occupational exposure (e.g., manufacture of paints, metal alloys) and non-occupational exposure (e.g., inhalation, cigarettes, and

ingestion of contaminated water and food) are the main causes of high levels of Cd in the human body [1, 6, 7].

In mammals, Cd affects the kidneys, testes, stomach, intestines, and especially the liver. In the long run, it favors the appearance of cancer in the liver, kidneys, and hematopoietic and reproductive systems [1]. Oral poisoning (i.e., via water or food) is expected to grow in regions bathed by the Doce and Paraopeba rivers, in Minas Gerais, Brazil, after the rupture of two mining tailings dams in recent years. Studies in these regions have shown an increased concentration of heavy metals, including Cd, in the water of these rivers [4], plants of agro-economic interest [8], and fish [9]. After consuming contaminated water and food, Cd is absorbed in the gastrointestinal tract, enters the bloodstream, and is later distributed. The liver is one of the first organs to be exposed to the metal [10]. In this organ, Cd is associated with metallothionein and forms a complex that is secreted into the bloodstream (CdMT) and can be transported to other organs [1, 11].

Due to its chemical characteristics, Cd can compete with essential elements, such as calcium (Ca), zinc (Zn), and iron (Fe), in binding sites of transport and enzymatic proteins [12],

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[13]. Such competition causes functional losses due to the reduced availability of essential elements for the organism (i.e., in the absorption process), or the functional impairment of Cd-linked enzymes [6, 13, 14]. In addition, in the liver, this metal leads to metabolic impairment, oxidative damage, vacuolization, tissue disorganization, inflammation, and fibrosis [15–17], which disrupt its normal function.

Experimentally, in the study on heavy metal toxicology, Cd is generally administered by two routes, oral and intraperitoneal (i.p.) [6, 15, 18]. The choice for the intraperitoneal route has been preferred because it is easier to apply, less stressing to the laboratory animal, and quick to manage, besides guaranteeing the delivery of the complete studied dose [19]. On the other hand, the oral route (e.g., gavage) is also used in research [20, 21]. Although greater care is needed when administering the dose orally, Cd poisoning in humans is best represented by this route, since it allows the metal to pass through the digestive system and be absorbed into the bloodstream [19].

In our previous study, we demonstrate that the intensity of the damage caused by Cd in the testis depends on the dose [13], the route, and duration of exposure [21, 22]. However, Cd processing by the liver is necessary for it to reach other organs through the blood. It is also known that different routes of administration and time of exposure lead to different toxic effects in the liver [18, 23, 24]. Yet, some questions still lack evidence to support the selection of an administration route that leads to results that can be safely extrapolated to human intoxication with Cd and make it possible to directly compare the results found in toxicological research. Therefore, we aim to study the effects of subacute exposure to Cd by i.p. and oral routes (single dose and fractional dose) in the liver of Swiss mice.

Materials and Methods

Animal Model and Ethics Statement

Twenty adult male Swiss mice, aged 56 days, were obtained from the Central Bioterium of the Biological and Health Sciences Center at the Federal University of Viçosa, and kept under controlled conditions of temperature ($22 \pm 2^\circ\text{C}$) and light-dark cycles (12/12 h). The animals received water and standard diet for rodents (Presence Alimentos, Paulínea, SP, Brazil) ad libitum. The research protocol was approved by the Ethics Committee on Animal Use of the Federal University of Viçosa (CEUA/UFV—protocol 058/ 2016) and was carried out in compliance with the guidelines issued by the National Council for the Control of Animal Experimentation (CONCEA).

Experimental Design and Tissue Collection

After 30 days of bioterium acclimation, the animals were randomly distributed into four experimental groups ($n = 5$ animals/group), a control group, and three groups exposed to Cd. The control group received distilled water via gavage and the other groups were treated with cadmium chloride (CdCl_2 , Sigma, St Louis, MO, USA) at the following concentrations: 1.5 mg/kg of bodyweight (0.92 mg Cd/kg) intraperitoneally (i.p.), single dose (i.p. SD); 30.00 mg/kg (18.33 mg Cd/kg) via gavage, single dose (oral SD); and 4.28 mg/kg/day (2.62 mg Cd/kg) via gavage for 7 consecutive days (fractionated dose, oral FD).

The administration of the CD was performed at 06:00 am in both experimental groups. The dosage of 1.5 mg/kg was determined based on previous studies [21]. Since only 5% of ingested Cd is absorbed [6, 25, 26], the dosage of 30.00 mg/kg via gavage corresponds to the 1.5 mg/kg i.p. dose. In addition, this amount is considered a safe standard rate of absorption after Cd exposure [27]. The dose of 4.28 mg/kg is equivalent, at the end of the 7 days of treatment. Thus, after 1 week, both groups exposed to Cd orally (gavage) received a total of 30.00 mg/kg of CdCl_2 .

The 7-day period was defined so as to observe probable changes caused by a subacute effect, before a possible recovery process could alter the degree of damage caused by the Cd [28]. On the eighth day of the experiment, the animals were euthanized. The animals were anesthetized with sodium thiopental (30 mg/kg i.p.), and euthanized by deepening anesthesia (sodium thiopental, 150 mg/kg i.p.), followed by cardiac puncture and exsanguination [21]. The liver of each animal was removed, cleaned, weighed, and dissected. A fragment was immersed in Karnovsky fixative solution [29] for histomorphological analysis, a second fragment was frozen in liquid nitrogen and stored in ultra-freezer (-80°C) for oxidative and nitrosative stress analysis, and a third fragment was reserved to determine the dosage of Cd and microminerals.

Biometric Analysis

To calculate the variation in body weight (WV), the animals were weighed at the beginning and at the end of the experiment, so that the weight variation was obtained by subtracting the final body weight from the initial one. The hepatosomatic index (HSI) was obtained by the relation between the liver weight (LW) and the final body weight (BWf), with $\text{HSI} = \text{LW} / \text{BWf} \times 100$. To determine the volume of the liver, a hepatic lobe was removed from the material already fixed, weighed on an analytical balance, subsequently placed in a millimeter beaker containing 1 mL of water, and then the displacement of water inside the beaker was observed. This displacement was considered as the volume occupied by the

lobe (LobV). The value obtained was used to calculate the total organ volume (LV), thus $(LV) = \text{LobV} \times \text{LW} / \text{LobW}$, where LW = liver weight and LobW = lobe weight. The liver density (LD) was obtained using the formula $LD = LW/LV$ [30].

Cadmium Bioaccumulation and Micromineral Dosage

The determination of cadmium (Cd), zinc (Zn), calcium (Ca), magnesium (Mg), copper (Cu), and iron (Fe) concentrations were determined in an atomic absorption spectrophotometer (SpectrAA 220FS Varian), as previously described [21].

Oxidative and Nitrosative Stress Marker Assays

The frozen livers were homogenized in potassium phosphate-buffered saline solution (pH 7.4, 0.1 M) containing 1 M EDTA and centrifuged at 12000 rpm, for 10 min. The supernatant was used for the analyses, which were performed in duplicate. The activity of antioxidant enzymes catalase (CAT), superoxide dismutase (SOD) and glutathione-S-transferase (GST), and oxidative stress markers, such as malondialdehyde (MDA) and nitric oxide (NO), was evaluated, as previously described [21].

Serological Biochemical Analysis

Blood samples collected during the euthanasia were centrifuged at 4600 rpm, for 15 min, at 4 °C, and the serum was separated for biochemical quantifications. We performed the analysis for the quantification of total cholesterol, HDL cholesterol, LDL cholesterol, triglycerides, total protein, and albumin in serum using biochemical kits (Bioclin Laboratories, Belo Horizonte, MG, Brazil) at the BS-200 equipment (Bioclin Laboratories, Belo Horizonte, MG, Brazil), following the manufacturer's instructions.

Histological Processing and Evaluation

The liver was immersed in Karnovsky solution [31] for 24 h, and then transferred to 70% ethanol. Subsequently, liver fragments were dehydrated in a growing solution of ethanol and included in methacrylate (Historesin, Leica Microsystems, Nussloch, Germany). Semi-serial sections of 3 µm were obtained in a rotating microtome (RM 2255, Leica Biosystems, Nussloch, Germany), respecting the interval of at least 40 µm between the cuts, and the histological preparations were stained with toluidine blue + sodium borate (1%). Images captured in a photomicroscope (Olympus BX-53, Tokyo, Japan) equipped with a digital camera (Olympus DP73, Tokyo, Japan) were used for morphometric analysis.

Liver Histomorphometry

The proportion of liver components was determined by using a grid with 266 intersections (points) in 10 digital photos per animal, at a magnification of 200×, totaling 2660 points per animal. The points incident on blood vessels, nucleus of mononucleated hepatocytes, nucleus of binucleated hepatocytes, hepatocyte cytoplasm, sinusoid capillaries, and other cell types were counted. In addition, 30 hepatocyte nuclei were randomly measured, per animal, to determine the diameter in the hepatocyte nucleus. The morphometric volume (mL) was determined by the ratio between the percentage of the component in the organ (PCO)%, multiplied by the total organ volume (TOV), using the following formula; $(PCO/100) \times TOV$. All analyses were performed using the Image J program [15].

Statistical Analysis

The statistical analysis was performed using the GraphPad Prism 7.0 software system. The data distribution was tested by the Shapiro-Wilk method. The percentage values were transformed by angular transformation before analysis, given the nature of the variables. The data obtained from the quantitative analysis were evaluated by one-way analysis of variance (ANOVA), followed by the Student-Newman-Keuls post hoc method (SNK). Differences were considered significant when $P \leq 0.05$. The results were expressed as mean ± standard deviation.

Results

Biometric Analysis

Liver weight and hepatosomatic index (HSI) increased in animals that received Cd via i.p., in comparison to the other experimental groups. The liver volume decreased in all animals exposed to Cd by gavage, compared to the control and i.p. route. Liver density increased in the three groups exposed to Cd, when compared to the control group (Table 1). There were no differences in body weight and its variation between the groups.

Cadmium Bioaccumulation and Micromineral Dosage

The concentration of hepatic Cd was higher in all animals exposed to the metal when compared to the control group. Ca was higher in fractionated oral administration than in the control group. The Mg concentration decreased in the animals that received the Cd orally, both in a single and fractional dose, compared to the i.p. route. The concentration of Fe was found in greater amounts in the group that received

Table 1 Body and liver biometric parameters of mice exposed to cadmium (CdCl_2) i.p. and oral routes

	Control	i.p. SD	Oral SD	Oral FD
BWi (g)	36.70 ± 1.54 ^a	38.75 ± 4.14 ^a	35.75 ± 3.28 ^a	34.90 ± 3.64 ^a
BWf (g)	38.33 ± 0.70 ^a	39.60 ± 4.0 ^a	35.27 ± 5.55 ^a	36.57 ± 4.20 ^a
WV (g)	1.63 ± 1.86 ^a	0.85 ± 1.54 ^a	-0.48 ± 4.27 ^a	1.67 ± 1.64 ^a
LW(g)	1.85 ± 0.06 ^a	2.32 ± 0.37 ^b	1.69 ± 0.32 ^a	1.76 ± 0.36 ^a
HSI (%)	4.83 ± 0.16 ^a	5.83 ± 0.39 ^b	4.77 ± 0.26 ^a	4.81 ± 0.98 ^a
LV (mL)	2.81 ± 0.39 ^a	2.56 ± 0.99 ^a	1.55 ± 0.30 ^b	1.42 ± 0.25 ^b
LD (g/mL)	0.67 ± 0.08 ^a	1.00 ± 0.33 ^b	1.10 ± 0.16 ^b	1.24 ± 0.16 ^b

Mean ± SD; control = distilled water; i.p. SD = CdCl_2 intraperitoneal single dose; oral SD = CdCl_2 oral single dose; oral FD = CdCl_2 oral fractional dose; BWi, initial body weight; BWf, final body weight; WV, weight variation; LW, liver weight; HSI, hepatosomatic index; LV, liver volume; LD, liver density. ^{a,b}Different letters on the same line differ by the test of Student-Newman Keuls ($P \leq 0.05$)

fractional oral Cd in relation to the other experimental groups, while zinc increased only in animals that received a single dose of oral Cd, when compared to those that received Cd i.p.. The levels of Cu and Mn did not differ between the experimental groups (Fig. 1).

Oxidative and Nitrosative Stress Marker Assays

SOD activity increased in the groups that received oral single dose of Cd when compared to all other experimental groups, while the CAT activity was higher in relation to the control group. GST activity was reduced in both groups exposed to Cd orally, either receiving single dose or fractioned dose, compared to the control and i.p. Cd-exposed group. MDA was reduced in the i.p. Cd-exposed group, compared to the control and fractional oral Cd (Fig. 2).

Serological Biochemical Analysis

All serological parameters evaluated did not differ between the exposed groups and the control. The data referring to this analysis are shown in Fig. 3.

Histological Evaluation

The liver of the control group presented normal tissue architecture, with strands of hepatocytes without degeneration (Fig. 4), while the animals that received the Cd through both i.p. and oral administration presented degenerations in the tissue, disorganization in the hepatocyte cords, and vacuolization. The animals that received fractional oral Cd showed greater tissue disorganization, compared to the other experimental groups (Fig. 4).

Liver Histomorphometry

The percentage of mononucleated hepatocytes decreased in animals exposed to Cd i.p., when compared to the other

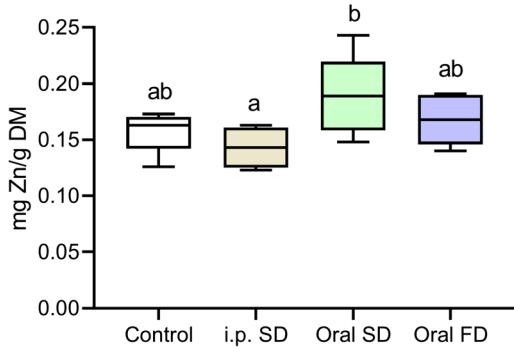
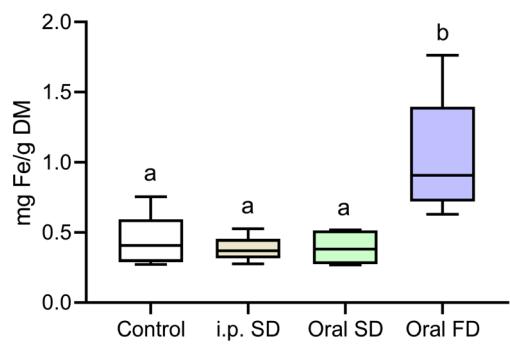
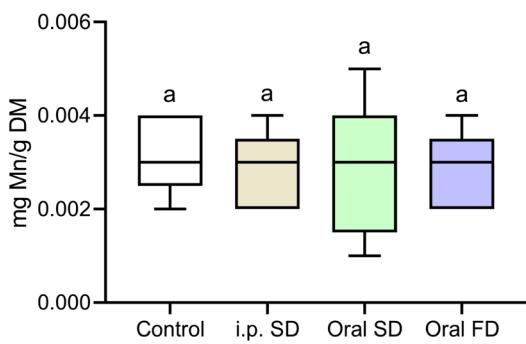
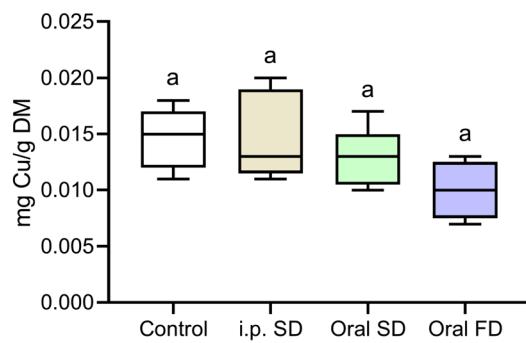
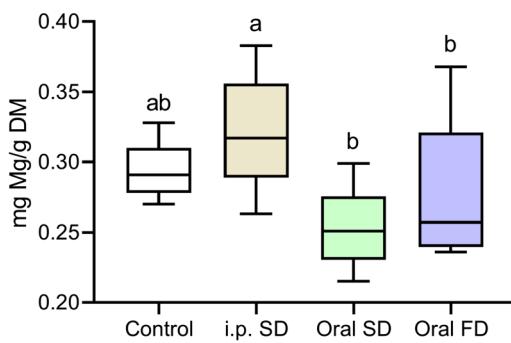
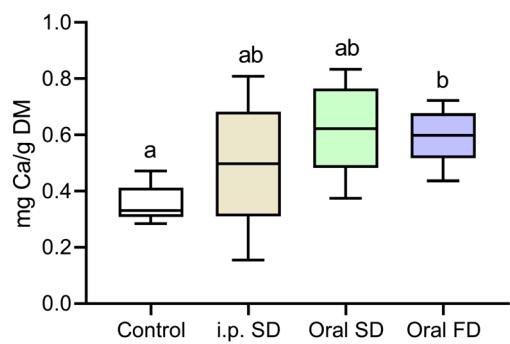
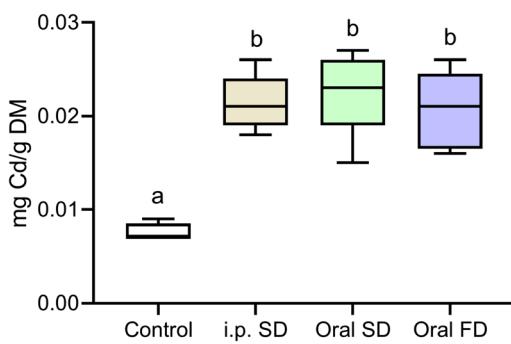
experimental groups, while those of binucleated hepatocytes decreased in animals that received the metal by oral route, for both single and fractioned doses, in relation to the control and Cd i.p.. The percentage of cytoplasm decreased in oral administration with a single dose, while blood vessels increased in the same group, when compared to the other experimental groups. There were no differences in the density of other cells between the groups (Table 2).

The volumes of mononucleated, binucleated hepatocytes, and other cells decreased in all animals exposed to Cd in relation to the control group (Table 2). Regarding the volume of binucleated hepatocytes and other cells, oral administrations of Cd also presented reduced volume, compared to the i.p.. The volume of the cytoplasm decreased in both oral administrations, compared to the control and administration of Cd i.p.. The blood vessel volume decreased in the animals that received fractioned oral Cd, compared to the other experimental groups. No significant differences were found in the nuclear diameters of hepatocytes (Table 2). Figure 5 summarizes all the relevant results when compared to the control found in this study.

Discussion

The effects of liver exposure to Cd and its accumulation in this organ are known and has been discussed in the scientific literature for a long time [25, 32, 33]. It is also known that different forms of administration in experimental research can lead to different pathophysiological outcomes [24]. In

Fig. 1 Levels of cadmium and hepatic essential minerals (mg/g DM) of mice exposed to cadmium chloride (CdCl_2). Control = distilled water; i.p. SD = CdCl_2 intraperitoneal single dose; oral SD = CdCl_2 oral single dose; oral FD = CdCl_2 oral fractional dose; DM = dry mass; Ca = calcium; Mg = magnesium; Cu = copper; Mn = manganese; Fe = iron; Zn = zinc; Cd = cadmium. ^{a,b}Different letters indicate significant differences by Student test Newman Keuls ($P \leq 0.05$)



order to clarify the extent of the changes promoted by Cd in three possible experimental protocols for the toxicological research of this metal, we evaluated liver mineral content, oxidative stress markers, serum lipid profile, and tissue architecture modifications. Our findings corroborate that route and frequency of exposure can promote different results in toxicology research. While all tested forms led to structural disorganization of the tissue, the oral single dose route led to a more aggravated oxidative response than in other forms (Fig. 5).

In our study, exposure to Cd did not change the final body weight of the animals. However, it caused wide individual variations that could not be observed in group effect. In all

experimental groups, we observed positive values for weight variation, except in animals that received single oral Cd dose, which obtained negative average variation. In two studies by Abarikwu et al. [34, 35], no changes in body weight were observed in rats which received 50 mg/kg of CdCl_2 by oral administration route, 3 times a week, for 15 or 30 days. These authors suggest that the absence of alterations in biometric parameters indicate the non-occurrence of general toxicity of Cd, which may indicate that toxicity is higher in the i.p. and that this may present greater toxicity when administered by i.p. route. In contrast, the literature reports the absence of changes in body weight, liver, and HSI of rats exposed to

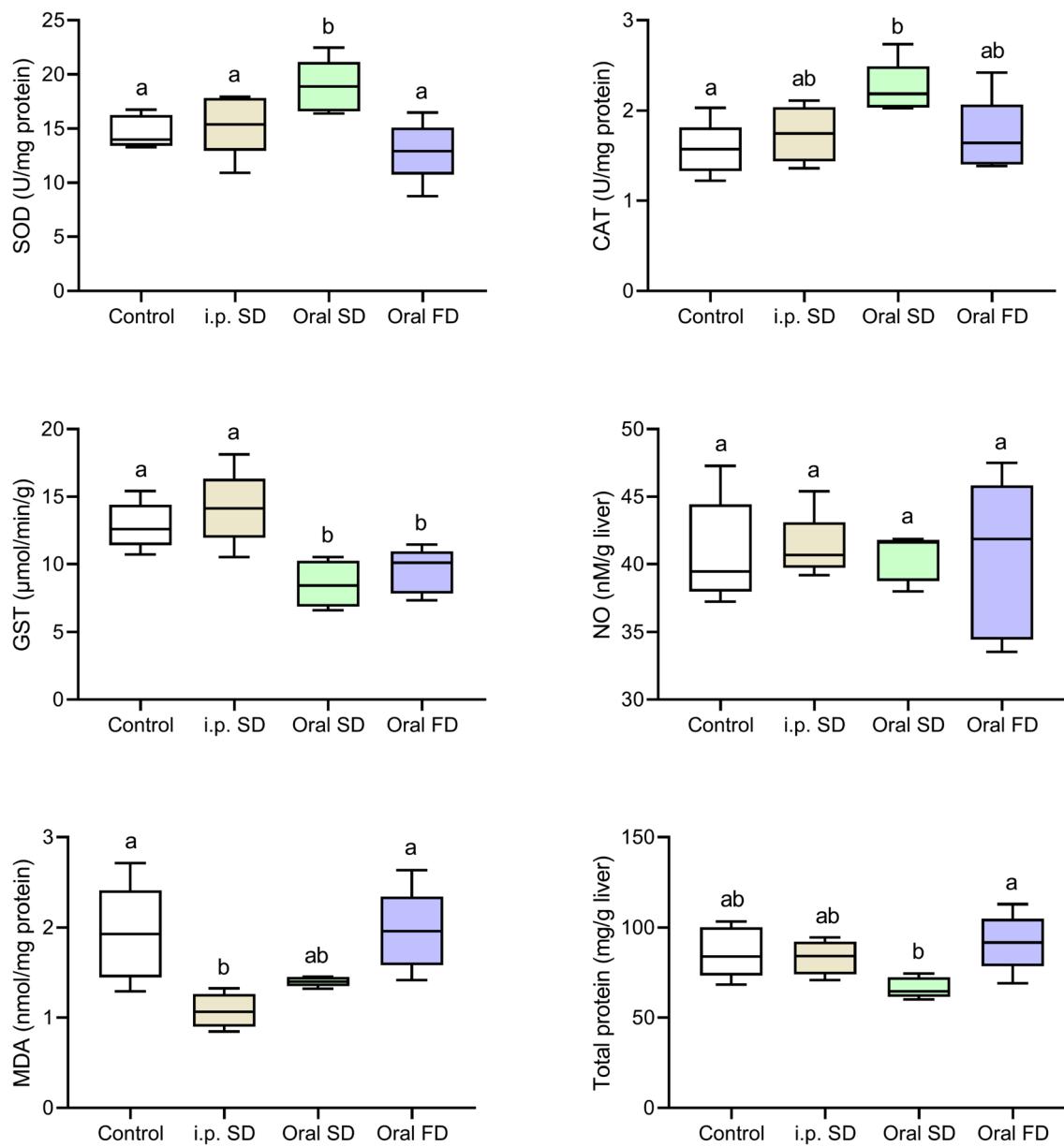


Fig. 2 Activity of antioxidant enzymes, oxidative stress marker and nitric oxide levels of mice exposed to cadmium chloride (CdCl_2). Control = distilled water; i.p. SD = CdCl_2 intraperitoneal single dose; oral SD = CdCl_2 oral single dose; oral FD = CdCl_2 oral fractional dose; SOD =

superoxide dismutase; CAT = catalase; MDA = malondialdehyde; GST = glutathione-S-transferase; NO = nitric oxide. ^{ab}Different letters indicate significant differences by Student test Newman Keuls ($P \leq 0.05$)

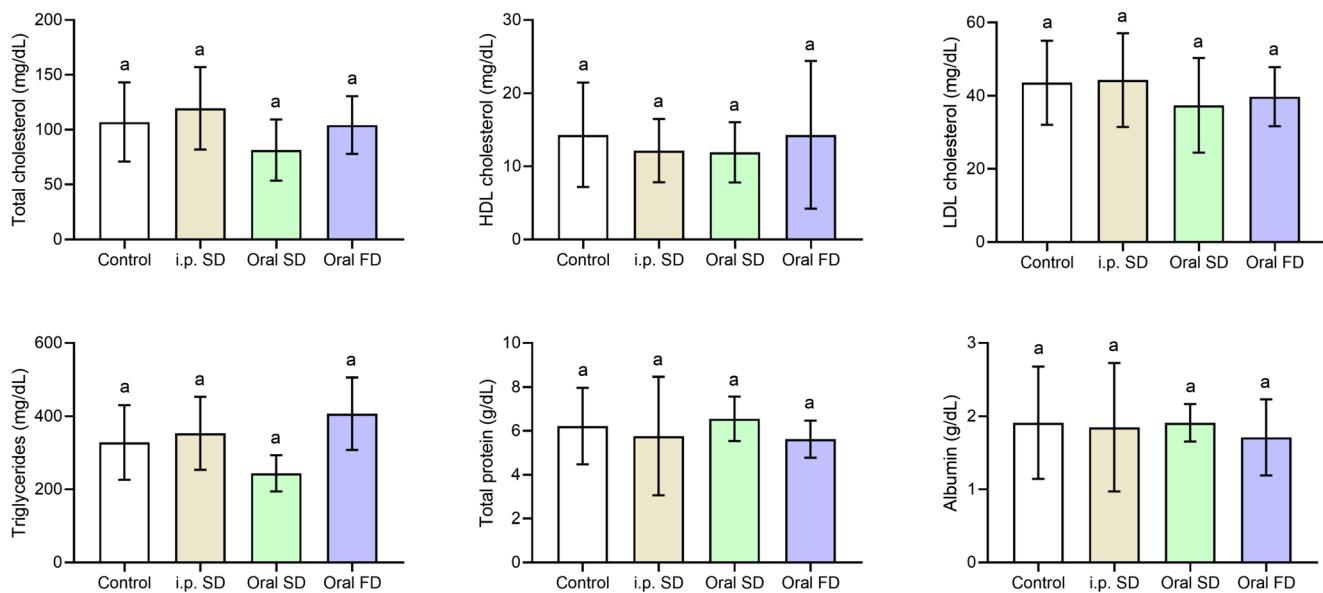


Fig. 3 Serum lipid profile and proteins of mice exposed to cadmium chloride (CdCl_2). Control = distilled water; i.p. SD = CdCl_2 intraperitoneal single dose; oral SD = CdCl_2 oral single dose; oral FD =

CdCl_2 oral fractional dose. ^{ab}Different letters indicate significant differences by Student test Newman Keuls ($P \leq 0.05$)

1.2 mg/kg of CdCl_2 i.p. single dose and evaluated after 7 days [15, 18] and 56 days [18]. However, despite the absence of changes in those parameters, in these studies, CdCl_2 exposure led to tissue disorganization, macrophage infiltration, necrosis, fibrosis, and disruption of antioxidant homeostasis.

Due to its long half-life, Cd exposure can lead to its bioaccumulation in different organs and tissues [23, 35–37]. After being ingested, the Cd is absorbed in the duodenum by the

divalent metal receptor (DMT-1) where the absorption of microminerals occurs [38, 39]. Once absorbed, the circulating Cd is directed to the Port System, it is directed to the liver where it is complexed with metallothioneins to later be distributed to other organs [15, 40]. Therefore, the liver is the first organ where Cd is formed and, consequently, its toxicity occurs [10, 15, 40]. Cadmium in the stomach is known to react with hydrochloric acid and forms of cadmium chloride, which

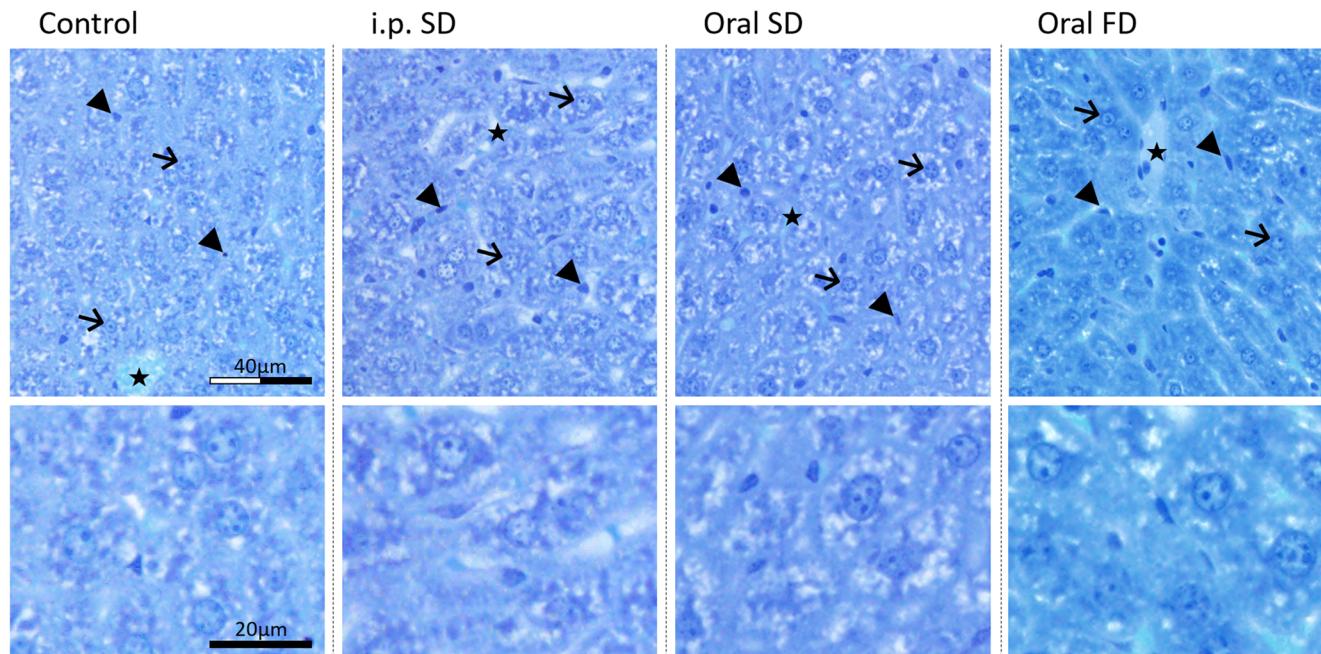


Fig. 4 Representative photomicrography's of the liver tissue from mice exposed to cadmium chloride (CdCl_2). Control = distilled water; i.p. SD = CdCl_2 intraperitoneal single dose; oral SD = CdCl_2 oral single

dose; oral FD = CdCl_2 oral fractional dose. (→) hepatocyte nucleus; (→) cytoplasm; (►) other cells; (★) blood vessels. Toluidine blue/sodium borate. Scale bars = 40 μm and 20 μm (zoom)

Table 2 Liver histomorphometry of mice exposed to cadmium (CdCl_2) i.p. and oral routes

	Control	i.p. SD	Oral SD	Oral FD
Volumetric density (%)				
Mononuclear	5.86 ± 0.55^a	4.39 ± 0.26^b	5.62 ± 0.21^a	5.31 ± 0.43^a
Binuclear	3.20 ± 0.69^a	2.28 ± 0.18^a	1.65 ± 0.40^b	1.44 ± 0.18^b
Cytoplasm	67.79 ± 2.17^a	64.37 ± 4.66^a	52.78 ± 10.87^b	68.45 ± 6.00^a
Blood vessel	20.98 ± 0.99^a	27.26 ± 4.58^a	34.50 ± 5.73^b	23.47 ± 6.05^a
Other Cells	2.16 ± 0.38^a	1.70 ± 0.47^a	1.57 ± 0.28^a	1.32 ± 0.55^a
Volume (mL)				
Mononuclear	0.16 ± 0.01^a	0.11 ± 0.04^b	0.09 ± 0.02^b	0.08 ± 0.02^b
Binuclear	0.09 ± 0.01^a	0.06 ± 0.03^b	0.03 ± 0.01^c	0.02 ± 0.00^c
Cytoplasm	1.91 ± 0.33^a	1.67 ± 0.70^a	0.83 ± 0.25^b	0.98 ± 0.23^b
Blood vessel	0.59 ± 0.06^a	0.68 ± 0.24^a	0.54 ± 0.17^a	0.33 ± 0.09^b
Other Cells	0.06 ± 0.01^a	0.04 ± 0.02^b	0.02 ± 0.01^c	0.02 ± 0.01^c
ND (μm)	5.15 ± 0.50^a	5.69 ± 0.27^a	5.35 ± 0.13^a	5.29 ± 0.31^a

Mean \pm SD; control = distilled water; i.p. SD = CdCl_2 intraperitoneal single dose; oral SD = CdCl_2 oral single dose; oral FD = CdCl_2 oral fractional dose; ND, nuclear diameter of the hepatocyte. ^{a, b, c} Different letters on the same line differ by the test of Student-Newman-Keuls ($P \leq 0.05$)

can induce acute inflammation of the gastrointestinal tract [10]. Thus, after exposure to this metal, hepatic bioaccumulation was observed in all animals. Since no difference in the concentration of hepatic Cd was observed between the evaluated protocols, it is suggested that, regardless of the route of administration, Cd has the same capacity to accumulate in the

liver, and that, even after 7 days of application of the single doses, the levels remained the same as those of the fractional dosage, which indicates that the organ cannot eliminate this metal. In contrast, Matović et al. [24] observed that, in rats exposed to 1.5 mg/kg of Cd via i.p. and 30 mg/kg of oral Cd, both in a single dose, and evaluated after 24 h, i.p. increased the accumulation of Cd in the liver, possibly due to the increased speed of access to blood allowed by this route of administration. However, the difference in the evaluation time after exposure may explain our different results, since, in our study, the evaluation was conducted 7 days after exposure, in single doses. Thus, we believe that, in order to observe the Cd accumulation in oral administration, it is necessary that a longer time elapses after the administration of the metal, to allow its absorption in the intestine before being transported and accumulated.

Exposure to Cd increased hepatic Ca concentration in the fractionated oral route. Ca homeostasis is essential for cell maintenance and survival, since it can act as a second messenger in several signaling pathways [41]. Literature reports reveal that Cd disrupts the function of hepatocyte gap junctions [42, 43], alters Ca homeostasis by activating gene transcription and inducing cell death [43–46], or disrupting the whole body Ca homeostasis [47]. In addition, Cd induces mitochondrial fragmentation dependent on dinamine-related protein, which increases intracellular calcium concentration and promotes liver toxicity [48]. In oral administration of Cd, we verified more aggravated histological changes, with tissue disorganization, decreased hepatocyte density, vacuolization, and degeneration. Similar results were found in the testis of rats exposed to Cd in other studies conducted in our laboratory [13, 22].

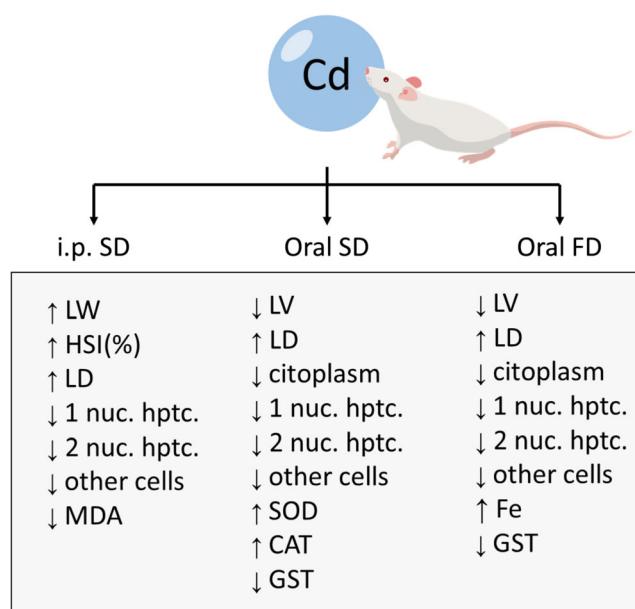


Fig. 5 Effects observed when compared to the control group of different routes of administration in mice exposed to cadmium chloride (CdCl_2). Control = distilled water; i.p. SD = CdCl_2 intraperitoneal single dose; oral SD = CdCl_2 oral single dose; oral FD = CdCl_2 oral fractional dose. ↑ = increased; ↓ = reduced; LW = liver weight; HIS = hepatosomatic index; LD = liver density; nuc. Hptc. = nucleus of hepatocyte; MDA = malondialdehyde; LV = liver volume; SOD = superoxide dismutase; CAT = catalase; GST = glutathione-S-transferase; Fe = iron

Cd toxic action is largely due to its competition with other essential functional elements, such as Cu, Fe, and Ca. It changes the activity of antioxidant enzymes, such as SOD, CAT, and glutathione peroxidase, which modulate oxidative stress [13]. It is known that Cu, together with Zn and Mn, acts as a SOD cofactor, active in the first response to the oxidative damage caused by superoxide [12] and that Cd can increase the generation of ROS and consequently, oxidative damage induction [22]. Since no change was observed in the concentration of these minerals, although Cu concentration was lower in animals that received fractional oral Cd, the SOD activity does not seem to be impaired, acting in the protection of the liver, especially in animals that received oral Cd single dose. In addition, an increased hepatic Zn concentration was observed in these animals, accompanied by increased SOD activity. Matović et al., [24] observed that the Cd bioaccumulation occurred more significantly in i.p. administration than orally, after 24 h of exposure. They also observed reduced SOD activity in both groups exposed to Cd. Their results provide strong evidence that the route of administration plays a crucial role in the intensity of Cd intoxication. The difference between the results observed by the authors and those found in the present study may be due to the different evaluation periods.

The increased Fe concentration observed in animals that received fractioned oral Cd may suggest increased oxidative stress, by the promotion of ROS generation and consequent lipid peroxidation through the Fenton reaction [23, 49, 50]. Fractional exposure to Cd for 15 and 30 days reduces GST and CAT, and for 60 days, reduces MDA, as previously reported [35]. The endogenous Fe concentration decreased after 60 days of administration. However, after 15 and 30 days, the Cd concentration was inversely correlated with endogenous Fe [35]. This suggests that the time of exposure is an important factor in the changes caused by Cd. The bioaccumulation of this metal in the body can also alter the mechanism of Fe release, neutralize lipid peroxidation, and lead to oxidative lipid damage.

Our data reveal that oral routes, especially fractional ones, are more propense to disrupt mineral homeostasis, compared to the i.p., possibly due to the competition of Cd with other elements [47, 51]. However, since no reduction was observed in the concentration of minerals in either form of oral administration, it is considered that, in the investigation of subacute effects, it was not possible to observe the competition of Cd with other minerals in oral administration.

One of the functions of the liver is lipid metabolism. Liver disorders can develop due to changes in the balance of fatty acids and triglycerides [52]. However, exposure to Cd in either route did not affect lipid metabolism in the liver, similarly to the findings of the study conducted by Cupertino et al. [15], who reported no lipid changes after exposure to Cd either.

The lower volume of mononuclear and binuclear hepatocytes found in the i.p., fragmented oral and oral single dose exposure to Cd indicate cell death. The reduced cytoplasm, found in the fragmented oral route and a single dose, in addition to the reduced volume of hepatocytes, in these same groups, indicate cell shrinkage, a morphological mark of cell death due to apoptosis [53]. Indeed, Cd can induce apoptotic cell death [11, 16, 26], but caspase-induced apoptosis is generally accompanied by nucleus and cell fragmentation [53], which were not observed in our study. Indeed, Cd-induced apoptosis may not be necessarily linked to caspase activation, and mitochondrial mechanisms may be more important in early apoptotic cell death, with decreased glutathione levels and elevation of calcium concentration [54–56].

The volumetric proportion of the tissue components (%) is considered a localized analysis, and therefore, in some cases, the results are not significant, whereas the volumetry and the global analysis of the organ, that takes into account the volume and mass, as a whole [57]. Thus, the results of volumetry demonstrate that the oral routes (single and fractional dose) presented greater intensity in tissue disorganization. Furthermore, it was observed exactly in the groups with reduced total volume of the organ and increased density, which may indicate its reduced function [58]. In their study, Predes et al. [18] report reduced proportion of hepatocytes and increased proportion of sinusoids after the experimental period of Cd exposure. In addition, Cupertino et al. [15] reported that the percentage of sinusoid capillaries, blood vessels, and binucleated hepatocytes increased in the liver from which they received CdCl₂ i.p..

It is known that human beings are exposed to Cd through oral and respiratory routes [24]. In this study, it is observed that the route by which human beings are exposed to Cd (orally) presented a greater intensity of hepatic changes, compared to the route generally used in studies (intraperitoneal). Thus, it seems that, in oral route, regardless of the form of administration (SD or FD), Cd induces greater liver toxicity. Therefore, when hepatocytes suffer consecutive attacks, their regeneration function is impaired, causing the formation of disorganized arrangements, instead of the normal organization of the hepatic lobes, which indicates possible organ damage [59], and may have reflected in the greatest change observed in the fractionated oral route.

Conclusion

Our data indicate that, even if administered subacutely, the different routes of administration lead to different effects on cadmium liver poisoning. Prolonged exposure times and higher doses could cause more aggravated effects. Besides, further studies under these conditions are needed to clarify such possible variations.

Acknowledgments The authors thank Bioclin Laboratories for kindly providing biochemical kits used in this work, and Enedina Sacramento for the English proofreading; this work was supported by Universidade Federal de Viçosa (UFV), Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES), and Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), Brazil.

Data Availability The data used to support the findings of this study are available from the corresponding author upon request.

Compliance with Ethical Standards

Conflict of Interest The authors declare that they have no conflict of interest.

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