Influence of water pH in the hepato- and nephrotoxicity of chronic cadmium poisoning in Wistar rats

Influência do pH da água na hepatotoxicidade e nefrotoxicidade da intoxicação crônica por cádmio em ratos Wistar

Influencia del pH del agua en la hepato y nefrotoxicidad de la intoxicación crónica por cadmio en ratas Wistar

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Abstract
Introduction: Cadmium is a heavy metal found in the environment that is used industrially; however, it also causes hepato- and nephrotoxic effects. Objective: To evaluate the effect of drinking water pH on the hepato- and nephrotoxicity caused by chronic cadmium poisoning. Material and Methods: We used 90 adult, male Wistar albino rats divided into 6 groups (n = 15): GC5 received a solution of cadmium chloride in drinking water with an acidic pH (pH 5.0); GC7 received a solution of cadmium chloride (400 mg/L) in drinking water with a neutral pH (pH 7.0 water); GC8 received a solution of cadmium chloride in water with an alkaline pH (pH 8.0); GWC5 received drinking water with an acidic pH (pH 5.0); GWC7 received drinking water with a neutral pH (pH 7.0); GWC8 received drinking water with an alkaline pH (pH 8.0). The animals were euthanized 6 months after the start of the experiment. We performed tests for hepatic and renal function and conducted liver and renal histopathology. Results: Water with an acidic pH caused alterations in ALP, ALT and urea in animals exposed to cadmium (P<0.05). In the liver, the majority of animals from the GC7 (57.1%) and GC5 (53.3%) groups showed diffuse microvesicular steatosis, while other groups showed no steatosis (P>0.05). In the kidney, the majority of animals from the GC7 (78.6%) and GWC5 (71.4%) groups showed tubular hydropic degeneration; however, these data were only statistically different from the GWC7 group (P<0.05). Conclusion: Exposure to cadmium in water with an acidic pH led to higher elevations of serum ALP, AST and urea, suggesting that the pH of drinking water influences the hepato- and nephrotoxic effects of this heavy metal.
Keywords: Cadmium; Acidification; Kidney; Liver; Toxicity.

Resumo
Introdução: O cádmio é um metal pesado encontrado no meio ambiente e utilizado industrialmente; entretanto, também causa efeitos hepato e nefrotóxicos. Objetivo: Avaliar o efeito do pH da água de beber sobre a hepatotoxicidade e nefrotoxicidade causadas por intoxicação crônica por cádmio. Material e Métodos: Foram utilizados 90 ratos Wistar albinos adultos, machos, divididos em 6 grupos (n = 15): GC5 recebeu solução de cloreto de

cádmio em água potável com pH ácido (pH 5,0); GC7 recebeu solução de cloreto de cádmio (400 mg / L) em água potável com pH neutro (água pH 7,0); GC8 recebeu solução de cloreto de cádmio em água com pH alcalino (pH 8,0); GWC5 recebeu água potável com pH ácido (pH 5,0); GWC7 recebeu água potável com pH neutro (pH 7,0); O GWC8 recebeu água potável com pH alcalino (pH 8,0). Os animais foram sacrificados 6 meses após o início do experimento. Realizamos testes de função hepática e renal e conduzimos histopatologia hepática e renal. Resultados: Água com pH ácido causou alterações na ALP, ALT e uréia em animais expostos ao cádmio (P<0,05). No fígado, a maioria dos animais dos grupos GC7 (57,1%) e GC5 (53,3%) apresentou esteatose microvesicular difusa, enquanto os demais grupos não apresentaram esteatose (P> 0,05). No rim, a maioria dos animais dos grupos GC7 (78,6%) e GWC5 (71,4%) apresentou degeneração hidrópica tubular; no entanto, esses dados foram apenas estatisticamente diferentes do grupo GWC7 (P <0,05). Conclusão: A exposição ao cádmio em água com pH ácido levou a maiores elevações de ALP, AST e ureia séricas, sugerindo que o pH da água potável influencia os efeitos hepato e nefrotóxicos deste metal pesado.

Palavras-chave: Cádmio; Acidificação; Rim; Fígado; Toxicidade.

Resumen

Introducción: El cadmio es un metal pesado que se encuentra en el medio ambiente y se utiliza industrialmente; sin embargo, también causa efectos hepato y nefrotóxicos. Objetivo: Evaluar el efecto del pH del agua potable sobre la hepato y nefrotoxicidad causada por intoxicación crónica por cadmio. Material y métodos: Se utilizaron 90 ratas albinas Wistar macho adultas divididas en 6 grupos (n = 15): GC5 recibió una solución de cloruro de cadmio en agua potable con un pH ácido (pH 5,0); GC7 recibió una solución de cloruro de cadmio (400 mg / L) en agua potable con pH neutro (agua pH 7.0); GC8 recibió una solución de cloruro de cadmio en agua con un pH alcalino (pH 8.0); GWC5 recibió agua potable con un pH ácido (pH 5,0); GWC7 recibió agua potable con pH neutro (pH 7,0); GWC8 recibió agua potable con pH alcalino (pH 8.0). Los animales se sacrificaron 6 meses después del inicio del experimento. Realizamos pruebas de función hepática y renal y realizamos histopatología hepática y renal. Resultados: El agua con pH ácido provocó alteraciones en ALP, ALT y urea en animales expuestos a cadmio (P<0.05). En el hígado, la mayoría de los animales de los grupos GC7 (57,1%) y GC5 (53,3%) mostraron esteatosis microvesicular difusa, mientras que otros grupos no mostraron esteatosis (P> 0,05). En el riñón, la mayoría de los animales de los grupos GC7 (78,6%) y GWC5 (71,4%) mostraron degeneración hidrópica tubular; sin embargo, estos datos solo fueron estadísticamente diferentes del grupo GWC7 (P <0.05). Conclusión: La exposición al cadmio en agua con un pH ácido condujo a elevaciones más altas de ALP, AST y urea séricos, lo que sugiere que el pH del agua potable influye en los efectos hepato y nefrotóxicos de este metal pesado.

Palabras clave: Cadmio; Acidificación; Riñón; Hígado; Toxicidad.

1. Introduction

Cadmium (Cd) is a heavy metal that was discovered in approximately 1815 within ores containing carbonate and zinc. Cd is one of the most abundant non-essential elements found in the environment, and it is also a component of cigarette smoke and widely used in industrial applications (Genchi et al., 2020; WHO, 1992; Yuan et al., 2014).

This mineral is known to be one of the most toxic environmental and industrial pollutants (El-Refaiy & Eissa, 2013). Cd is commercially generated as a by-product of the industrial exploitation of ores and the smelting of zinc and lead. Compounds containing cadmium are used as stabilizers in various products, including polymers of vinyl chloride (PVC), pigments, nickel cadmium and rechargeable batteries (Järup & Akesson, 2009), and dental products (Menezes et al., 2009). Furthermore, Cd can be found as a contaminant in phosphate-based fertilizers (Järup & Akesson, 2009).

Cd enters the body mainly by inhalation and ingestion (Souza & Santos, 2010), and chronic exposure to heavy metals is associated with the development of multiple toxic effects, including lung, liver or kidney disease and problems with the cardiovascular system and bone (Castro-González & Méndez-Armenta, 2008).

The target organs of cadmium are the testicles, liver and kidneys (Yuan et al., 2014). Cadmium produces extensive liver damage after acute and chronic exposure in animals (Liu et al., 2004). During chronic exposures (a situation that occurs in dietary exposure), Cd accumulates mainly in the kidneys (WHO, 1992). Renal dysfunction occurs when the content of cadmium in the renal cortex reaches a critical threshold of 200 mg/Kg, and osteomalacia can develop in severe cases. The excretion of absorbed cadmium occurs mainly via urine; however, this process is insufficient and slow, which explains the long half-life of Cd in the body and the occurrence of toxic effects even after a partial or total reduction of exposure (Castro-

González & Méndez-Armenta, 2008).

Cd can contaminate water, soil and plants, thus leading to human exposure. A better understanding of the influence of drinking water pH as a vehicle for chronic cadmium poisoning is important for the prevention of renal and hepatic toxicity. We evaluated changes in the liver and kidney of rats exposed to Cd in drinking water with an acidic, neutral or alkaline pH. To our knowledge, this is the first study evaluating the influence of drinking water pH on the hepatotoxicity and nephrotoxicity induced by cadmium poisoning.

The aim of this study was to evaluate the effect of drinking water pH on the hepato- and nephrotoxicity caused by chronic cadmium poisoning.

2. Methodology

2.1 Animals and treatments

The study was conducted in accordance with the ethical principles of the Universal Declaration of Animal Rights of the United Nations Educational, Scientific and Cultural Organization (UNESCO) and the study protocol was approved by the Ethics Committee on the Use of Animals, under Protocol 1180, from the University of Western São Paulo, UNOESTE, Brazil.

For our study, we used 90 male adult Wistar rats (*Rattus norvegicus albinus*) that weighed between 200-250 g. The rats were divided into groups of four in large rectangular cages (measuring 49x34x16 cm) suitable for the accommodation of up to five adult rats. Animals were maintained under a controlled temperature of $25\pm2°C$ and humidity of $50\pm15\%$ and a normal photoperiod (12-12 h light-dark cycle). Allocation concealment, management strategy, and treatment of the groups of animals were performed to reduce bias in the study (Köche, 2011; Ma et al., 2017).

Cadmium exposure was completed using cadmium chloride ($CdCl_2$ - Sigma Chemical Company, St. Louis, MO, USA) with a minimum hydration of 98% and water content of approximately 2.5 mol/mol. For six months, cadmium chloride was added daily to the drinking water of the animals at a concentration of 400 mg/L (adapted from Motta et al. (2004). The water was acidified with hydrochloric acid or made alkaline with sodium hydroxide. The drinking water was changed three times a week to maintain the pH. Any wastewater containing cadmium was sent to the central reservoir of the University of Western São Paulo (UNOESTE) and neutralized for disposal. Any remaining water from each cage was measured and recorded at each solution change to estimate the average intake for each animal.

The animals were divided into 6 groups (n = 15): GC5 received a solution of cadmium (400 mg/L) chloride in drinking water with an acidic pH (pH 5.0); GC7 received a solution of cadmium (400 mg/L) chloride in drinking water with a neutral pH (pH 7.0); GC8 received a solution of cadmium (400 mg/L) chloride in water with an alkaline pH (pH 8.0); GSC5 received drinking water with an acidic pH (pH 5.0); GSC7 received drinking water with a neutral pH (pH 7.0); and GSC8 received drinking water with an alkaline pH (pH 8.0). The diet was formulated to meet the nutritional needs of the rats (National Research Council, 1995) and is shown in Table 1. Animals from all groups received water and a solid diet *ad libitum*.

Anesthesia and euthanasia were performed with intraperitoneal injections of thiopental sodium (Syntec, USA) at doses of 40 mg/Kg and 100 mg/Kg body weight, respectively (Paiva et al., 2005). Animals from all groups were euthanized 6 months after the start of the experiment.

Table 1 - Composition (%) of the diet administered to Wistar rats.

Ingredients	Standard diet
Corn bran	82.95
Soy oil	7.00
L-Cysteine	0.30
Cellulose	5.00
Sodium Chloride	0.25
Vitamin mix*	1.00
Mineral mix**	3.50

* Vitamin mix/Kg: Nicotinic acid 30 mg; Pantothenate 15 mg; Pyridoxine 6 mg; Thiamine 5 mg; Riboflavin 6 mg; Folic acid 2 mg; Biotin 0.2 mg; Vitamin B12 25 mg; Vitamin E 75 IU; Vitamin A 4000 IU; Vitamin D3 1000 IU; Vitamin K 900 mg; Choline 1000 mg. ** Mineral mix mg/Kg: Calcium 5000; Phosphorus 1,561; Potassium 3600; Sulfur 300; Sodium 1019; Chlorine 1.574; Magnesium 507; Iron 35; Zinc 30; Manganese 10; Copper 6; Iodine 0.2; Molybdenum 0.15; Selenium 0.15. Source: Authors.

2.2 Biochemical analysis

Once a proper depth of anesthesia was achieved, animals underwent an intracardiac puncture with a vacutainer (BD Vacutainer™, Becton, Dickinson and Company, USA) to collect 10 mL of blood for the determination of urea, creatinine, AST (aspartate aminotransferase), ALT (alanine aminotransferase), alkaline phosphatase (ALP), GGT (gamma-glutamyl transpeptidase), albumin, glucose, total and direct bilirubin. All serum biochemistry was performed using automated equipment (COBAS C111, Roche Diagnostics, Indianapolis, USA) (Hasan et al., 2018).

2.3 Histopathology

After euthanasia, the liver and kidneys of each animal were collected. Portions of these organs were fixed in 10% formalin (Chemical Kinetics, São Paulo, Brazil) for 24 hours and subjected to standard histological processing and paraffin embedding (Dynamic Analytical Reagents, São Paulo, Brazil). Serial sections with a thickness of 5 μm were obtained using a LEICA microtome RM2265 (Leica Biosystems Nussoch GmbH, Germany) and stained with hematoxylin-eosin (HE) (Younan et al., 2019).

Histopathological analysis was performed by two independently experienced observers, blinded to the treatment groups, using a standard optical microscope (NIKON Labophot, Japan). The following scoring system was used for liver: interstitial inflammatory infiltrate (0 = absent 1 = mild, 2 = moderate, 3 = severe) (Mori et al, 2009); inflammatory cell type present (polymorphonuclear and/or mononuclear); tissue congestion (0 = absent, 1 = mild, 2 = moderate, 3 = severe); necrosis (0 = absent, 1 = present: focal); vascular necrosis (0 = absent, 1 = present); cholestasis (0 = absent, 1 = present); and presence and type of steatosis (0 = absent, 1 = present: microvesicular and/or macrovesicular). The following scoring system was used for kidney: interstitial inflammatory infiltrate (0 = absent, 1 = mild, 2 = moderate, 3 = severe) (Mori et al, 2009); type of inflammatory cells present (polymorphonuclear and/or mononuclear); tubular changes (0 = absent, 1 = hydropic degeneration, 2 = tubular necrosis); glomerular injury (0 = absent, 1 = presence of sclerosis); necrosis of the arteriolar wall (0 = absent, 1 = focal necrosis, 2 = diffuse necrosis); cylinders (0 = absent, 1 = present); and nephrocalcinosis (0 = absent, 1 = present) (Bano & Najam, 2019).

2.4 Statistical analysis

To compare the effects of pH and detect differences between the experimental and control groups a Kruskal-Wallis test was used. Treatment differences were evaluated by the Student-Newman-Keuls method. All analyses were performed

using SPSS software for Windows v.13.0 (Field, 2018). The results are presented as the median (25th and 75th quartiles), and the significance level was set at 5%.

3. Results

Five animals died during the study (one rat each from groups GC7, GC8 and GSC5 and two rats from group GSC8). The cause of death for the animals from group GC7 and GC8 was acute pulmonary edema, a complication associated with cadmium exposure (Järup & Akesson, 2009). The cause of death for the rats from groups GSC8 and GSC5 was unable to be determined.

The average water intake per animal per day was 55 mL for group GC7, 57 mL for group GC5, 52 mL for group GC8, 60 ml for group GSC5, 70 mL for group GSC8 and 73 mL for group GSC7. No statistically significant differences were found between the groups (P>0.05).

No gross changes in the liver and kidneys of animals were observed.

3.1 Biochemical analysis

The results of the biochemical analysis for liver and kidney function are summarized in Table 2.

3.2 Histopathology

3.2.1 Liver

Animals from all groups showed mild hepatic congestion. Only one animal each from the GC5 and GSC7 groups showed mild lymphocytic infiltrates among hepatocytes (P=0.9984). Only 2 animals from the GSC8 group showed intracellular cholestasis. No animal showed vascular necrosis (P>0.05) (Table 3).

In the GC5 group, 26.6% of the animals showed focal hepatocyte necrosis, while the other groups showed no change (P=0.7606) (Table 3 and Figure 1).

A pattern of microvesicular steatosis was observed in the majority of animals. The GC7 group showed diffuse steatosis (57.1%); in addition, three rats had focal steatosis and three showed no signs of steatosis. A subset of animals from group GC5 (53.3%) also showed diffuse steatosis. The majority of animals in groups GC8 (64.3%), GSC5 (78.6%), GSC7 (60%) and GSC8 (84.6%) showed no steatosis (P>0.05). There was no statistically significant difference in the presence of steatosis between the animals of groups GC7 and GC5 (p = 0.3336), groups GC5 and GC8 (P=0.1322) or groups GC5 and GSC7 (P=0.1654) (Table 3 and Figure 1).

3.2.2 Kidney

No animal showed glomerular changes, vascular necrosis or the presence of cylinders (Table 3). No nephrocalcinosis or stones in the renal pelvis were observed.

Two animals from group GC7 showed mild congestion, while the others within the group showed moderate congestion (P=0.0681) (Table 3).

In cases of observed inflammation, there was a predominance of lymphocytes in the interstitium. The majority of animals from group GC7 (85.7%) showed mild inflammation. A total of 80% of the animals from group GC5 and 57.1% of animals from group GSC5 showed mild lymphocytic infiltrates. Animals from group GC8 showed moderate infiltration in 40% of cases and mild infiltration in 35.7% of cases. The majority of animals from groups GSC8 (84.6%) and GSC7 (80%) showed no inflammatory infiltrates (P <0.00001) (Table 4 and Figure 2).

Table 2 - Biochemical parameters (evidence of hepatic and renal function) in Wistar rats[#].

Parameters (unit)	Groups[*]					
	GC5	GC7	GC8	GSC5	GSC7	GSC8
ALP (U.L^{-1})	107.6 (105.4 – 110.5)[c]	99.5 (96.6 – 102.5)[b]	102.1 (100.8 – 104.4)[b]	94.9 (92.4 – 95.6)[a]	92.7 (91.9 – 94.3)[a]	92.1 (90.8 – 94.4)[a]
ALT (U.L^{-1})	68.0 (65.1 – 69.9)[c]	60.5 (57.0 – 64.4)[b]	61.2 (59.8 – 62.2)[b]	52.1 (51.0 – 53.5)[a]	51.7 (48.8 – 54.3)[a]	51.3 (49.9 – 53.3)[a]
AST (U.L^{-1})	151.0 (150.8 – 158.5)[b]	152.6 (150.8 – 157.5)[b]	155.8 (150.8 – 158.5)[b]	132.7 (130.2 – 136.0)[a]	131.0 (129.6 – 135.8)[a]	130.6 (128.9 – 135.2)[a]
GGT (U.L^{-1})	9.4 (9,3 – 9.6)[b]	9.0 (8.9 – 9.3)[b]	9.2 (8.7 – 9.5)[b]	5.0 (4.5 – 5.4)[a]	5.1 (4.6 – 5.2)[a]	5.0 (4.5 – 5.1)[a]
Total bilirubin (mg.dL^{-1})	1.3 (0.9 – 1.1)[b]	1.2 (0.9 – 1.1)[b]	1.2 (0.9 – 1.2)[b]	1.0 (1.2 – 1.4)[a]	1.1 (1.2 – 1.3)[a]	1.0 (1.2 – 1.3)[a]
Direct bilirubin (mg.dL^{-1})	0.6 (0.6 – 0.7)[b]	0.5 (0.5 – 0.6)[b]	0.6 (0.5 – 0.6)[b]	0.3 (0.3 – 0.4)[a]	0.3 (0.3 – 0.3)[a]	0.3 (0.3 – 0.4)[a]
Albumin (g.dL^{-1})	2.1 (2.1 – 2.2)[a]	2.6 (2.0 – 2.3)[a]	2.1 (2.3 – 2.7)[a]	4.4 (4.0 – 4.6)[b]	4.5 (4.2 – 5.4)[b]	4.5 (4.3 – 4.8)[b]
Glucose (mg.dL^{-1})	135.0 (129.5 – 142.0)[b]	123.2 (116.3 – 127.7)[b]	122.8 (116.2 – 129.9)[b]	105.3 (102.1 – 106.4)[a]	109.2 (103.3 – 111.0)[a]	106.7 (104.7 – 109.2)[a]
Urea (mg.dL^{-1})	65.2 (63.1 – 67.4)[c]	58.9 (55.9 – 60.0)[b]	58.7 (56.7 59.5)[b]	41.0 (39.3 – 42.1)[a]	38.7 (37.7 – 42.3)[a]	42.0 (39.5 – 43.4)[a]
Creatinine (mg.dL^{-1})	0,8 (0.7 – 0.8)[b]	0.8 (0.7 – 0.8)[b]	0.7 (0.7 – 0.8)[b]	0.6 (0.6 – 0.6)[a]	0.6 (0.5 – 0.6)[a]	0.6 (0.6 – 0.6)[a]

[#]Values are expressed as the median (interquartile range). Treatment differences were evaluated by the Student-Newman-Keuls method. Results with different superscripts differ significantly in the same line ($P<0.05$).

[*] GC5 received a solution of cadmium (400 mg/L) chloride in drinking water with an acidic pH (pH 5.0); GC7 received a solution of cadmium (400 mg/L) chloride in drinking water with a neutral pH (pH 7.0); GC8 received a solution of cadmium (400 mg/L) chloride in water with an alkaline pH (pH 8.0); GSC5 received drinking water with an acidic pH (pH 5.0); GSC7 received drinking water with a neutral pH (pH 7.0); and GSC8 received drinking water with an alkaline pH (pH 8.0).

Source: Authors.

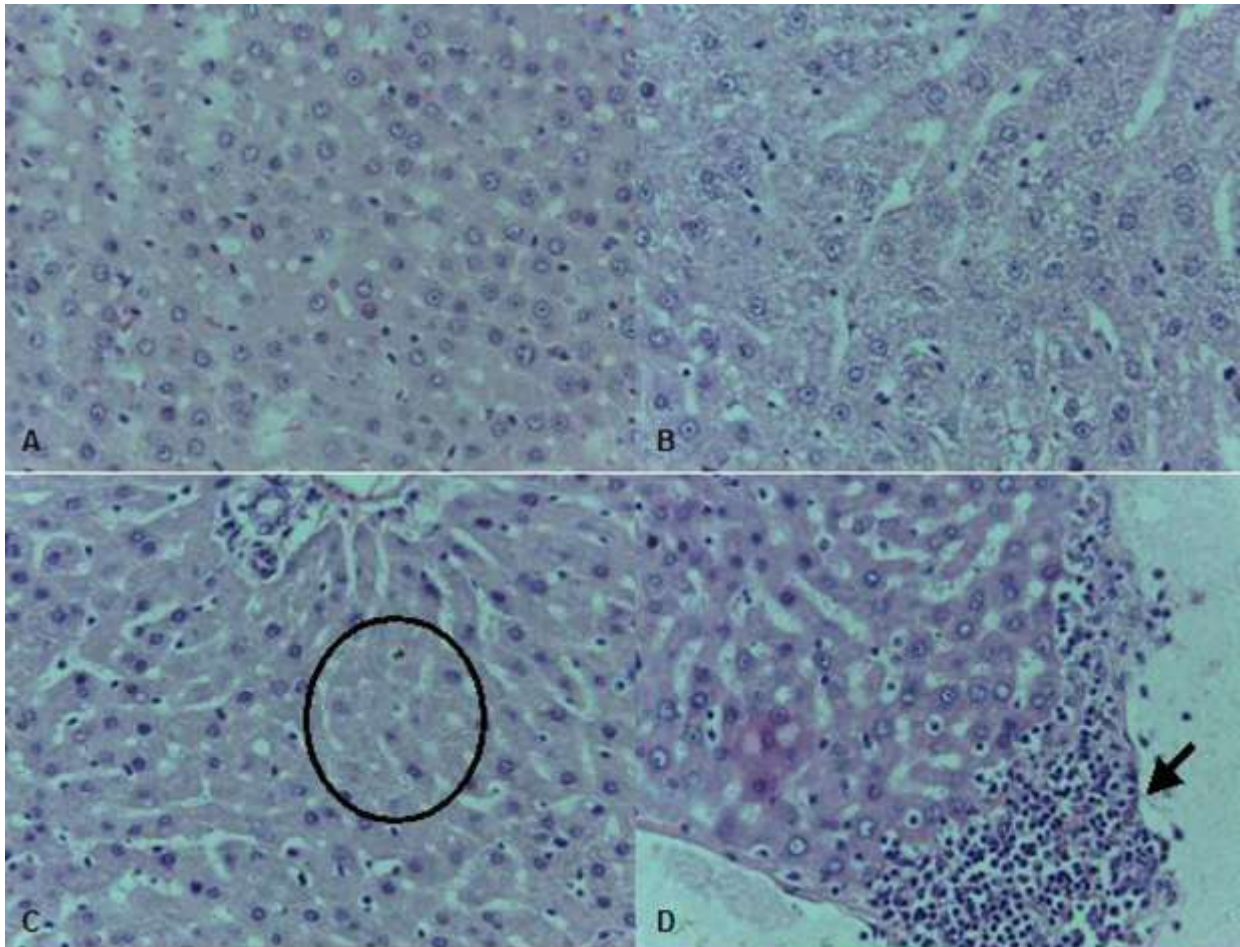**Table 3 -** Mean scores from the histopathological analysis of the liver[#].

Parameters	Groups					
	GC5	GC7	GC8	GSC5	GSC7	GSC8
Congestion	1[a]	1[a]	1[a]	1[a]	1[a]	1[a]
Intensity of inflammation	0[a]	0[a]	0[a]	0[a]	0[a]	0[a]
Hepatocyte necrosis	0,3[a]	0[a]	0[a]	0[a]	0[a]	0[a]
Cholestasis	0[a]	0[a]	0[a]	0[a]	0[a]	0,2[a]
Steatosis	1,1[a, b]	1,4[a]	0,4[b, c]	0,2[c]	0,5[b, c]	0,3[c]
Vascular necrosis	0[a]	0[a]	0[a]	0[a]	0[a]	0[a]

[#]Values are expressed as the median (interquartile range). Treatment differences were evaluated by the Student-Newman-Keuls method. Results with different superscripts differ significantly in the same line ($P<0.05$).
* GC5 received a solution of cadmium (400 mg/L) chloride in drinking water with an acidic pH (pH 5.0); GC7 received a solution of cadmium (400 mg/L) chloride in drinking water with a neutral pH (pH 7.0); GC8 received a solution of cadmium (400 mg/L) chloride in water with an alkaline pH (pH 8.0); GSC5 received drinking water with an acidic pH (pH 5.0); GSC7 received drinking water with a neutral pH (pH 7.0); and GSC8 received drinking water with an alkaline pH (pH 8.0).
Source: Authors.

Figure 1 - Photomicroscopy of the liver. A – Normal liver from an animal in group GC7. B - Diffuse microvesicular steatosis was observed in an animal from group GC7. C - Localized hepatocellular necrosis (circle) from an animal of GC5 group was observed. Hematoxylin-eosin, magnification of 200x. D - Focal lymphocytic infiltrates (arrow) are shown from an animal in group GC5. Hematoxylin-eosin, magnification of 400x.

Source: Authors.

Table 4 - Mean scores from the histopathological analysis of the kidney[#].

Parameters	Groups					
	GC5	GC7	GC8	GSC5	GSC7	GSC8
Congestion	2[a]	1,9[a]	2[a]	2[a]	2[a]	2[a]
Intensity of inflammation	0,9[b]	1,7[a]	1,1[a, b]	0,6[b, c]	0,4[c]	0,3[c]
Alteration of renal tubule	0,4[a]	0,9[a]	0,5[a]	0,7[a]	0[b]	0,2[a, b]
Glomerulus changes	0[a]	0[a]	0[a]	0[a]	0[a]	0[a]
Vascular necrosis	0[a]	0[a]	0[a]	0[a]	0[a]	0[a]
Presence of cylinders	0[a]	0[a]	0[a]	0[a]	0[a]	0[a]

#Values are expressed as the median (interquartile range). Treatment differences were evaluated by the Student-Newman-Keuls method. Results with different superscripts differ significantly in the same line ($P<0.05$).
* GC5 received a solution of cadmium (400 mg/L) chloride in drinking water with an acidic pH (pH 5.0); GC7 received a solution of cadmium (400 mg/L) chloride in drinking water with a neutral pH (pH 7.0); GC8 received a solution of cadmium (400 mg/L) chloride in water with an alkaline pH (pH 8.0); GSC5 received drinking water with an acidic pH (pH 5.0); GSC7 received drinking water with a neutral pH (pH 7.0); and GSC8 received drinking water with an alkaline pH (pH 8.0).
Source: Authors.

One animal from group GC7 showed acute tubular necrosis, while group GSC7 showed no tubular changes. A total of 78.6% of group GC7, 40% of group GC5, 50% of group GC8, 71.4% of group GSC5 and 38.4% of group GSC8 showed tubular hydropic degeneration (P=0.0018) (Table 4 and Figure 2).

Figure 2 - Photomicroscopy of the kidney. A - Normal kidney of an animal in group GC7. B - Acute tubular necrosis (arrow) was found in an animal from GC7 group. C - Tubular hydropic degeneration is shown from an animal of group GC5. D - Mild lymphocytic infiltrates (arrow) and tubular hydropic degeneration were observed in an animal from group GC8. Hematoxylin-eosin, magnification of 400x.
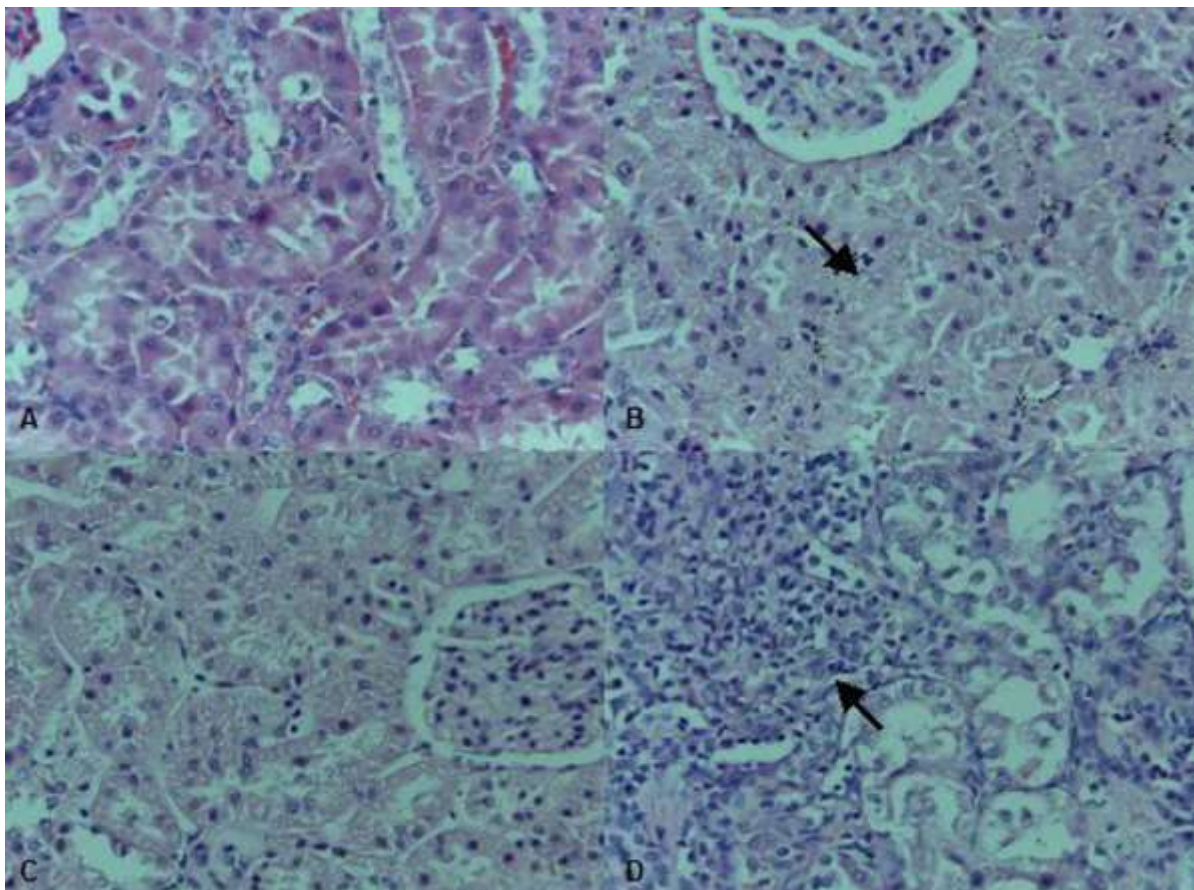
Source: Authors.

4. Discussion

Cadmium is present in many foods at highly variable levels; thus, ingestion is the major route of human exposure to this heavy metal (Järup & Akesson, 2009; Thomas et al., 2013). According to the World Health Organization, the maximum daily intake of cadmium should be 1 µg.kg^{-1} of body weight (WHO, 1992). In the present study, the animals were exposed to 400 mg of cadmium/liter of drinking water, which is well above the permissible daily intake. This level of exposure simulates cases of large scale environmental contamination and a 180-d period of chronic intoxication.

The availability of heavy metals to plants has an inverse relationship with soil pH. The negative effect of liming on the phytoavailability of metals is primarily due to an increased cation exchange capacity and the formation of hydroxides and carbonates of low solubility (Cunha et al., 2008). In the current study, we assessed if pH influences the absorption and toxicity of cadmium.

Once the liver and the kidneys of poisoned individual are affected, the patient often dies from respiratory arrest and cardiovascular collapse (Said Aki et al., 2019). In this study, two animals exposed to cadmium died from acute pulmonary edema, which is a complication associated with cadmium poisoning (Järup & Akesson, 2009).

After the acute administration of cadmium, hepatic congestion, ischemia and hypoxia occur very quickly. The resulting hypoxia leads to the ischemic infiltration of neutrophils and Kupffer cell activation and inflammation, which can contribute to hepatocellular necrosis and apoptosis (Liu et al., 2004). An increase in the level of transaminases in the blood, and the blurring of trabecular structures in the lobes, vacuolar degeneration, necrosis of individual hepatocytes and mononuclear infiltrates have also been observed (Brzóska et al., 2003). In the study by El-Refaiy and Eissa (2013), animals that ingested cadmium at a concentration of 3 mg of Cd/kg of body weight showed severe liver injury, including fatty changes, focal necrosis, pyknotic nuclei, karyolysis and a decreased number of bile ducts. In our study, a reduction of bile ducts and vascular necrosis was not observed in any of the groups. Focal inflammatory infiltrates were observed in only a subset of cases. There was mild congestion in animals from all groups, regardless of exposure to cadmium or the drinking water pH. Focal necrosis of hepatocytes was observed in 26.6% of animals from group GC5. This result is likely due to a chronic exposure at levels lower than those used by El-Refaiy and Eissa (2013). Animals from the GC5 and GC7 groups showed diffuse microvesicular steatosis, while the majority of animals from the other groups showed no steatosis (P>0.05). Although cholestasis was observed rarely in animals from group GSC8, exposure to cadmium (independent of the pH) caused increased levels of GGT, suggesting canalicular injuries that could not be observed microscopically.

In the study by Zhang et al. (2013), rats exposed to 50 mg/L of cadmium showed no change in AST levels; however, ALT was greatly increased when compared with the control group. In our current study, ALP, AST and ALT (markers of liver damage) were increased. The increase in AST observed in the present study was likely was due to chronic exposure to cadmium. In addition, a decreased liver function in animals exposed to cadmium was evidences by an increase in the levels of total and direct bilirubin.

The increase in ALP and AST was larger in animals exposed to cadmium in water with an acidic pH. Animals exposed to cadmium in an acidic environment showed necrosis and an enhanced enzyme elevation. Thus, despite no significant microscopic liver injuries (absence of inflammation and small number of cases with hepatocellular necrosis), a loss of liver function in animals exposed to cadmium was found. Our results differ from that of Zhang et al. (2013) because different cadmium concentrations (400 mg.L^{-1}) and exposure times were used.

Studies show that high doses of cadmium may increase levels of cholesterol, triglycerides and glucose (Mladenović et al., 2014); however, chronic oral exposure to low doses of cadmium does not affect these markers (Lovásová et al., 2013). In the present study, animals exposed to cadmium (independent of the pH) show increased glucose levels that were most likely due to the exposure to high concentrations of cadmium.

At a renal concentration of cadmium greater than 10 μg.g⁻¹, ultrastructural alterations (lesioning of the brush border microvilli and swollen mitochondria in the cells of the proximal convoluted tubules) are observed. Necrosis was found at a concentration of 30 μg.g⁻¹ (Brzóska et al., 2003). Evidence suggests that cadmium-induced nephrotoxicity is mediated by the complex metallothionein cadmium (Cd-MT), which is synthesized in the liver, released into the circulation, and taken up by the kidney proximal tubule cells. The Cd-TM complex is filtered by the glomerulus and absorbed by the proximal tubular cells. During transport through the kidney, this complex causes lesions mainly in the cortical region, and causes a progressive loss of organ function once it reaches the proximal tubule (El-Refaiy & Eissa, 2013).

In the study by Castro-Silva and Fregoneze (2010), cadmium reduced water intake in dehydrated rats and rats with water intake induced by the pharmacological stimulation of central cholinergic pathways. These data show that cadmium blocks neurochemical pathways that are essential to the generation of thirst. These authors also observed that the salt intake in dehydrated animals was blocked by acute cadmium administration in the central nervous system; therefore, acute cadmium exposure exhibits a natriuretic and caliuretic action. In the present study, we observed a decreased water intake in the groups exposed to cadmium, and there was no statistically significant difference between the groups not exposed to cadmium. This finding may explain the increase in urea in the animals exposed to cadmium.

Cadmium exposure is associated with increased urinary calcium excretion. Hypercalciuria is recognized as an important risk factor for kidney stone formation. There is an increased prevalence of kidney stones among individuals occupationally exposed to cadmium (Thomas et al., 2013). In this study, no nephrocalcinosis or calculi in the renal pelvis was observed, despite exposure to a high concentration of cadmium.

Despite the conflicting results from clinical observational studies, experimental studies show clear evidence for renal damage after exposure to cadmium at low doses. Chronic exposure results in proximal tubular damage with mitochondrial dysfunction. The main mechanism of cadmium-induced renal injury is oxidative stress (Chung et al., 2014).

The morphological changes in the kidney after exposure to cadmium at low doses develop slowly. According to a previous study involving experimental animals continuously exposed to low levels of cadmium for 1 year, a mild tubular atrophy and interstitial fibrosis was observed only after 12 months of exposure (Khalil-Manesh et al., 1993). Other studies show that the kidneys of animals exposed to cadmium showed proximal tubular degeneration, apoptosis, atrophy, interstitial inflammation and glomerular edema (El-Refaiy & Eissa, 2013; Gonick, 2008; Sabolić et al., 2001). The study of Zhang et al. (2013) showed tubular changes without glomerular differences. In the current study, animals exposed to cadmium showed increased levels of urea and creatinine. Relatively, the urea levels showed a greater increase then creatinine when compared with control groups. In addition, urea levels were higher in animals exposed to cadmium in water with an acidic pH. High levels of serum creatinine occur after a 50-60% reduction in the glomerular filtration rate (Shemesh et al., 1985). In the current study, we used a concentration and exposure period that was larger than Zhang et al. (2013); however, we did not observe glomerular changes, vascular necrosis or the presence of cylinders. The differences observed between this study and others can be explained by the different concentrations of cadmium and the time of exposure.

Heavy metals, such as cadmium, play a crucial role in the induction of apoptosis and may alter the balance of cellular homeostasis to increase cell mortality (Gao et al., 2013). In this study, we observed only a subset of animals with renal and hepatic necrosis, despite the high level of cadmium exposure.

5. Conclusion

Cadmium exposure in acidic drinking water led to a higher elevation of serum ALP, AST and urea, suggesting that an acidic pH maximizes the hepatic and renal toxicity of cadmium. Cadmium exposure in alkaline drinking water decreased steatosis formation, suggesting that an alkaline pH may confer protection against the liver toxicity of cadmium. Further studies

should be conducted to demonstrate the effects of exposure to other organs and thus implement public policies to mitigate the effects of exposure to this heavy metal.

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