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# Effects of chronic exposure to hexavalent chromium in water on oxidative stress parameters in Wistar rats

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**ABSTRACT.** Hexavalent chromium [Cr (VI)] is a metal utilized in different industries and consequently disposed in the environment. It is a toxic substance and its reduction to trivalent Cr [Cr (III)] generates intermediates, which are responsible for the oxidation of molecules, and cause the oxidative stress. Therefore, the aim of this study was to evaluate if Cr (VI) could induce oxidative stress in Wistar rats. In this study, Wistar rats were chronically exposed to 25 and 50 ppm of potassium dichromate in drinking water for 30 days. The levels of Cr were evaluated in the blood and tissues (liver, kidneys, and lungs). Oxidative stress was determined in the liver, kidneys, and lungs and was evaluated by DFCH, TBA-RS and carbonyl test. Antioxidant enzymes were evaluated through catalase (CAT), superoxide dismutase (SOD) and glutathione peroxidase (GPx) activities. Regarding the results, Cr concentration was significantly elevated in all tissues, however, it was lower in the lungs. In relation to the oxidative stress parameters, there was a significant increase of DCFH levels in the kidneys and carbonyls in liver and kidneys. Regarding the antioxidant enzymes, SOD was decreased in all organs and GPx was diminished in the kidneys. These data indicated that Cr (VI) could induce oxidative stress in the kidneys and liver due to an imbalance between oxidative and antioxidant parameters. The lungs were little affected, possibly by the lowest chromium accumulation.

**Keywords:** antioxidant enzymes; environmental contamination; hexavalent chromium; oxidative stress.

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## Introduction

Chromium (Cr) is a hazardous metal, and the trivalent Cr [Cr (III)] and hexavalent Cr [Cr (VI)] are the most important and utilized in industries, such as electroplating, tannery, steel production, among others (Unceta, Séby, Malherbe, & Donard, 2010) due to their stability in the environment (Lushchak, 2011). The usage of this metal in the industries entails in its occupational exposure on workers (Proctor, Suh, Campleman, & Thompson, 2014), as well as the exposure on living beings in the aquatic system after its release in the environment (Palaniappan & Karthikeyan, 2009).

Lacerda et al. (2018) performed a study with chrome plating workers and confirmed the occurrence of occupational exposure. Significant elevated Cr concentrations were found in the blood of the exposed group (2.02 ± 0.20 μg $L^{-1}$) in relation to control group (1.73 ± 0.16 μg $L^{-1}$). This result reflects in how Cr can be spread in a working environment.

Most of the tanning industries located in India discharge the wastewater into the environment without any previous treatment (Srinath, Verma, Ramteke, & Garg, 2002), and concentrations of Cr (VI) in these places were found between the range of 20 ppm (Srivastava, Singh, Prakash, & Srivastava, 2013) and 1500 ppm (Chandra & Kulshreshtha, 2004). However, independently of the Cr source, after a long period being exposed to it, Cr may accumulate in the tissue of aquatic organisms and consequently cause their suffering due to its toxic effects (Palaniappan & Karthikeyan, 2009).

In Brazil, the Resolution no. 357/2005 (Conselho Nacional do Meio Ambiente [CONAMA], 2005) of the Environment National Council (CONAMA) establishes conditions about the water quality in aquatic systems, in which the maximum permissible limit of total Cr is 0.05 mg $L^{-1}$. Regarding the release of Cr (VI)

in industrial effluents, the Resolution no. 430/2011 (Conselho Nacional do Meio Ambiente [CONAMA], 2011) of CONAMA allows the maximum concentration of 0.01 mg $L^{-1}$.

Cr (VI) is structurally similar to sulfate, which allows its entrance in the cell through sulfate channels (Zhitkovich, 2005). Once Cr (VI) is inside the cell, it is promptly reduced to Cr (III), which causes the generation of reactive oxygen species (ROS) (O'Brien, Ceryak, & Patierno, 2003). This reduction results in a massive generation of ROS and consequently, it is one of the most important damages induced by Cr (VI) exposure (Patlolla, Barnes, Yedjou, Velma, & Tchounwou, 2009).

Concentrations of Cr (VI) in the environment are recurrent, and they range from small to large concentrations. Concentration values of up to 1500 mg $L^{-1}$ (Chandra & Kulshreshtha, 2004) were found in India. The disposal of Cr from industries is responsible for the enlargement of this concentration (Zhitkovich, 2011), increasing the exposure of humans and organisms that rely on the aquatic environment to survive. Therefore, the aim of the present study was to analyze the potential of Cr (VI) to induce oxidative stress, as well as its capability of accumulation in different tissues, through the exposure of Cr (VI) in water using Wistar rats as the animal model.

## Material and methods

### Experimental animals and treatment

Twenty-six healthy adult male Wistar rats (4-6 weeks of age) were used in this study. They were obtained from the Feevale University rats' breeding laboratory. The animals were maintained in standard laboratory conditions (12 hours light/dark cycle, temperature 22 ± 2ºC) with free access to 20% (w $w^{-1}$) protein commercial chow (SUPRA, Porto Alegre, RS, Brazil) and water *ad libitum*. This exposure route was chosen to be more realistic to the environmental conditions. Moreover, they were randomly separated into three groups (4 - 5 rats per cage) of nine (Group I and II) and 8 animals (Group III).

Potassium dichromate ($K_2Cr_2O_7$) was diluted in distilled water and provided to the animals for 30 days: Group I (control group) – 9 rats exposed to potable water; Group II – 9 rats exposed to 25 ppm (25 mg $L^{-1}$) of $K_2Cr_2O_7$ in the water (Srivastava et al., 2013); Group III – 8 rats exposed to 50 ppm (50 mg $L^{-1}$) of $K_2Cr_2O_7$ in the water (not described in the literature).

All cages had regular water changes and the intake was measured three times a week. Also, the animals were weighted twice a week. After 30 days of chronic exposure to Cr VI, the animals were euthanized and beheaded, after a fasting of 6 hours. Posterior to this procedure, the blood was collected, and the tissues (liver, kidneys, and lungs) were immediately removed and kept under -70ºC.

### Determination of Cr in blood and tissues

The determination of Cr was accomplished using a graphite furnace atomic absorption spectrophotometer (Bosnak, Bradshaw, Hergenreder, & Kingston, 1993). The Cr concentration was determined according to Shinn, Dauba, Grenouillet, Guenard, and Lek (2009) with modifications. Each sample, weighing 0.25 g, was immersed in 10% $NH_4NO_3$, using a ratio of 2 mL $g^{-1}$. To determine the water content, the samples were dried at 110ºC until no weight loss occurred. Then, the samples were calcined (500ºC) for 2 hours, and the ashes formed were mixed with 1-2 mL of 65% $HNO_3$ using a ratio of 2 mL $g^{-1}$, followed by drying at 500°C for 2 hours. After this last procedure, the tissues were treated with 10 mL of 0.1% Triton and 1% $HNO_3$.

### Tissue preparation

For the determination of the oxidative stress parameters, we analyzed liver, kidneys and lung. The tissues were separated and then homogenized using a Potter-Elvehjem glass with (1:10 w $v^{-1}$) 20 mM sodium phosphate ($Na_3PO_4$) and 140 mM potassium chloride (KCl) buffer (pH 7.4). Then, the samples were subsequently centrifuged at 800 x *g* for 10 minutes at 4ºC (De Franceschi et al., 2013).

### Oxidative stress parameters

#### Determination of carbonyls

Carbonyl levels verify the oxidation of proteins, which was determined according to Reznick and Packer (1994). To perform this methodology, 200 µL of the samples' supernatant was mixed with 400 µL of 10 mM

2,4-dinitrophenylhydrazine (DNPH) or 400 µL of 2 mM hydrochloric acid (HCl) followed by incubation at room temperature during one hour in a dark environment. After incubation, 500 µL of 20% trichloroacetic acid (TCA) was added to the sample, which was then centrifuged at 10.000 x *g* for 3 minutes. Next, the protein pellet was washed twice with 1 mL of ethyl acetate/ethanol 1:1 (v $v^{-1}$), suspended with 600 µL of 6 mM guanidine and kept during 15 minutes at 60ºC. The samples were centrifuged at 10.000 x *g* for 3 minutes, then they were read at 370 nm in a spectrophotometer. The results were reported as nmol of carbonyls $mg^{-1}$ of protein.

**Determination of thiobarbituric acid reactive substances**

The method determines the levels of malondialdehyde (MDA), a product of lipid peroxidation. The method used to determine the levels of thiobarbituric acid reactive substances (TBA-RS) was described by Ohkawa, Ohishi, and Yagi (1979), with slight modifications of De Franceschi et al. (2013). Briefly, the tubes contained 200 µL of tissue supernatant, 50 µL of 8.1% sodium dodecyl sulphate (SDS), 375 µL of 20% acetic acid in aqueous solution (v $v^{-1}$) pH 3.5, and 375 µL of 0.8% thiobarbituric acid (TBA). The solution was homogenized and then maintained in boiled water for one hour and posteriorly cooled on water for 5 minutes. Then, they were centrifuged at 750 x *g* for 10 minutes. A pink solution was obtained, and TBA-RS was read by spectrophotometer at 532 nm. The results were expressed as nmol of TBA-RS $mg^{-1}$ of protein.

**Determination of 2'7'-dihydrodichlorofluorescein oxidation**

This test verifies the production of reactive oxygen and nitrogen compounds. For the determination of this parameter, we used the reduced 2'7'-dihydrodichlorofluorescein diacetate (DCF-DA), according to LeBel, Ischiropoulos, and Bondy (1992). Thirty µL of the sample was added with 30 µL of 20 mM $Na_3PO_4$ buffer with 140 mM KCl (pH 7.4), and 240 µL of 100 µM reduced 2'7'-dichlorofluorescein diacetate ($H_2$DCF-DA) solution, followed by incubation during 30 minutes at 37ºC in a dark environment. The fluorescence was measured using excitation wavelengths of 480 and emission wavelengths of 535 nm. The levels of the reactive compounds were expressed as nmol of DCF $mg^{-1}$ of protein.

**Determination of catalase activity**

The assay was performed according to Aebi (1984), through the measurement of the absorbance decrease of $H_2O_2$ at 240 nm. The reaction was composed of 20 mm $H_2O_2$, 0.1% Triton X-100, 10 mM potassium phosphate ($KH_2PO_4$) buffer (pH 7.0) and 10 µL of tissue supernatant. One unit of catalase (CAT) is proportional to1 µmol of $H_2O_2$ consumed per minute and the activity was calculated as units of CAT $mg^{-1}$ of protein.

**Determination of superoxide dismutase activity**

The determination of superoxide dismutase (SOD) activity was accomplished according to Marklund (1985). This method is based upon the autoxidation of pyrogallol at 420 nm, which depends on superoxide radicals. The reaction contained 50 nm Tris buffer with 1 mM ethylenediamine tetraacetic acid (EDTA) (pH 8.2), 10 mM HCl, 9.6 mM pyrogallol in 10 mM HCl, 30 µM CAT and a buffer containing CAT enzyme. For the calibration curve, 10 U of SOD was used as standard. The activity was expressed as units of SOD $mg^{-1}$ of protein.

**Determination of glutathione peroxidase activity**

The glutathione peroxidase (GPx) activity method was described by Wendel (1981) using *tert*-butyl-hydroperoxide as substrate. The enzymatic activity was determined through the monitoring of the disappearance of nicotinamide adenine dinucleotide phosphate (NADPH) at 340 nm. The test was performed by mixing 100 mM potassium phosphate ($K_3PO_4$) buffer 1 $mM^{-1}$ EDTA (pH 7.7), 2 mM glutathione, 0.15 U $mL^{-1}$ glutathione reductase, 0.4 mM azide, 0.5 mM *tert*-butyl hydroperoxide, 0.1 mM NADPH and 10 µL of tissue supernatant. One unit of GPx consumes 1 µmol of NADPH per minute. The specific activity was reported as µmol of NADPH consumed per minute $mg^{-1}$ of protein.

**Determination of protein**

The quantification of proteins was performed according to Lowry, Rosebrough, Farr, and Randall (1951), using albumin from bovine serum as standard.

### Ethical aspects

The experimental procedures were carried out according to 'Principles of laboratory animal care' (NIH Publication no. 80-23, revised 1996) and approved by the Ethics Committee for Animal Research of the Feevale University (nº 01.14.030).

### Statistical analysis

Data were expressed as mean ± SD. The results obtained were analyzed by One-way ANOVA, followed by Tukey post-test when the F value was significant, and the *p*-value < 0.05. The data were analyzed by the Statistical Package for the Social Sciences Software (SPSS) version 17.0.

## Results

The animals exposed to Cr (VI) in water neither suffered any apparent alterations nor were evidenced any deaths. Groups II and III (exposed to $K_2Cr_2O_7$) had 30% lower weight gain than Group I (Control group) ($p < 0.01$). The average water intake was significantly greater in the control group than in the exposed ones ($p < 0.05$). The concentration of Cr in the exposed groups was increased in all the studied tissues (Table 1).

**Table 1.** Concentration of Cr VI in the animals' tissues.

| Tissue | Group I (n = 9) | Group II (n = 9) | Group III (n = 8) |
|---|---|---|---|
| Blood (μg $dL^{-1}$) | 0.01 ± 0.01 | 841.9 ± 164.3* | 1166.8 ± 151.8* |
| Lung (μg $g^{-1}$) | 0.21 ± 0.07 | 1.34 ± 0.25* | 1.72 ± 0.44* |
| Liver (μg $g^{-1}$) | 0.03 ± 0.02 | 5.77 ± 2.84* | 10.09 ± 4.12* |
| Kidney (μg $g^{-1}$) | 2.3 ± 1.1 | 157.5 ± 16.4* | 178.3 ± 21* |

Data are expressed as mean ± standard deviation. Group I - Control with n = 9; Group II - exposed to 25 ppm of Cr VI with n = 9; Group III - exposed to 50 ppm of Cr VI with n = 8. *p < 0.01 compared to the control group (One-way ANOVA followed by Tukey test).

The determination of the liver oxidative stress parameters demonstrated a significant increase in carbonyls and the decrease of SOD activity in the exposed groups (Group II and III) in comparison to the control group (Group I) (Table 2).

**Table 2.** Oxidative and antioxidant parameters in the liver of Wistar rats.

| Parameter | Group I | Group II | Group III |
|---|---|---|---|
| Oxidative | | | |
| Carbonyl (mmol of carbonyl $mg^{-1}$ of protein) | 2.03 ± 0.46 | 2.53 ± 0.43* | 2.51 ± 0.2* |
| TBA-RS (mmol TBA-RS $mg^{-1}$ of protein) | 0.51 ± 0.12 | 0.54 ± 0.12 | 0.62 ± 0.15 |
| DCFH (μmol of DCFH $mg^{-1}$ of protein) | 11.61 ± 1.21 | 11.33 ± 1.71 | 11.32 ± 0.7 |
| Antioxidant | | | |
| GPx (U GPx $mg^{-1}$ of protein) | 2.3 ± 0.23 | 2.31 ± 0.24 | 2.3 ± 0.22 |
| SOD (U SOD $mg^{-1}$ of protein) | 128.03 ± 12.3 | 103.32 ± 17* | 109.99 ± 17.77* |
| CAT (U CAT $mg^{-1}$ of protein) | 298.54 ± 63.84 | 307.12 ± 46.88 | 340.88 ± 34.87 |
| SOD/CAT ratio (arbitrary units) | 0.44 ± 0.06 | 0.33 ± 0.05** | 0.33 ± 0.07** |

Data are expressed as mean ± standard deviation. Group I - Control with n = 9; Group II - exposed to 25 ppm of Cr VI with n = 9; Group III - exposed to 50 ppm of Cr VI with n = 8. TBA-RS = Thiobarbituric acid reactive substances; DCFH = 2'7'- Dihydrodichlorofluorescein; GPx = Glutathione peroxidase; SOD = Superoxide dismutase; CAT = Catalase. *p < 0.05; **p < 0.01 compared to the control group (One-way ANOVA followed by Tukey test).

Regarding the oxidative stress parameters, there was more significant alterations in the kidneys' tissues, than in liver and lungs. Two oxidative parameters (carbonyl and DCFH) were higher in the exposed groups (Group II and III) than in the control group (Group I). In relation to the antioxidant parameters, there was a decrease in two enzymes activities (GPx and SOD) (Table 3).

**Table 3.** Oxidative and antioxidant parameters in the kidney of Wistar rats.

| Parameter | Group I | Group II | Group III |
|---|---|---|---|
| Oxidative | | | |
| Carbonyl (mmol of carbonyl $mg^{-1}$ of protein) | 1.62 ± 0.11 | 1.76 ± 0.16 | 1.91 ± 0.18** |
| TBA-RS (mmol TBA-RS $mg^{-1}$ of protein) | 1.14 ± 0.10 | 1.11 ± 0.30 | 1.08 ± 0.10 |
| DCFH (μmol of DCFH $mg^{-1}$ of protein) | 1.62 ± 0.50 | 1.76 ± 0.60* | 1.91 ± 0.53* |

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| Parameter | Group I | Group II | Group III |
|---|---|---|---|
| Antioxidant | | | |
| GPx (U GPx $mg^{-1}$ of protein) | 4.40 ± 0,66 | 3.46 ± 0.66** | 3.58 ± 0.40** |
| SOD (U SOD $mg^{-1}$ of protein) | 35.98 ± 3.16 | 31.83 ± 4.10 | 30.78 ± 4.10* |
| CAT (U CAT $mg^{-1}$ of protein) | 81.50 ± 5.70 | 77.77 ± 8.25 | 77.32 ± 5.04 |
| SOD/CAT ratio (arbitrary units) | 0.44 ± 0.06 | 0.44 ± 0.03 | 0.38 ± 0.04** |

Data are expressed as mean ± standard deviation. Group I - Control with n = 9; Group II - exposed to 25 ppm of Cr VI with n = 9; Group III - exposed to 50 ppm of Cr VI with n = 8. TBA-RS = Thiobarbituric acid reactive substances; DCFH = 2'7'- Dihydrodichlorofluorescein; GPx = Glutathione peroxidase; SOD = Superoxide dismutase; CAT = Catalase. *p < 0.05; **p < 0.01 compared to the control group (One-way ANOVA followed by Tukey test).

SOD activity was the only analyzed parameter that was altered in the lungs' tissue of the exposed groups (Table 4).

**Table 4.** Oxidative and antioxidant parameters in the lung of Wistar rats.

| Parameter | Group I | Group II | Group III |
|---|---|---|---|
| Oxidative | | | |
| Carbonyl (mmol of carbonyl $mg^{-1}$ of protein) | 1.22 ± 0,28 | 0.90 ± 0.40 | 1.25 ± 0.39 |
| TBA-RS (mmol TBA-RS $mg^{-1}$ of protein) | 0.51 ± 0.12 | 0.54 ± 0.11 | 0.62 ± 0.09 |
| DCFH (µmol of DCFH $mg^{-1}$ of protein) | 6.82 ± 2.57 | 7.56 ± 0.90 | 7.13 ± 0.90 |
| Antioxidant | | | |
| GPx (U GPx $mg^{-1}$ of protein) | 1.49 ± 0.70 | 1.22 ± 0.61 | 1.47 ± 0.90 |
| SOD (U SOD $mg^{-1}$ of protein) | 32.21 ± 4.35 | 26.29 ± 4.15* | 24.65 ± 4.36** |
| CAT (U CAT $mg^{-1}$ of protein) | 0.87 ± 0.08 | 0.82 ± 0.08 | 0.85 ± 0.12 |
| SOD/CAT ratio (arbitrary units) | 1.61 ± 0.40 | 1.63 ± 0.40 | 1.48 ± 0.57 |

Data are expressed as mean ± standard deviation. Group I - Control with n = 9; Group II - exposed to 25 ppm of Cr VI with n = 9; Group III - exposed to 50 ppm of Cr VI with n = 8. TBA-RS = Thiobarbituric acid reactive substances; DCFH = 2'7'- Dihydrodichlorofluorescein; GPx = Glutathione peroxidase; SOD = Superoxide dismutase; CAT = Catalase. *p < 0.05; **p < 0.01 compared to the control group (One-way ANOVA followed by Tukey test).

## Discussion

The present study was undertaken to assess the potential of Cr (VI) in altering the oxidative stress parameters of the liver, kidneys, and lungs of Wistar rats treated with different concentrations of $K_2Cr_2O_7$ in the water. The accumulation of Cr in the tissues was also evaluated.

Oral exposure demonstrated a difference in the average water intake, which was greater on the control group in comparison to the treatment groups exposed to Cr (VI). The possible alterations in the odor and taste in water may have caused some resistance to the animals in drinking it. A previous study reports that the higher the concentration of Cr, the lower the water consumption among the exposed animals (Thompson et al., 2012). This reduction in water intake, as well as in its taste, may have contributed to the significant decrease in weight gain among the exposed groups when compared to the control group.

Cr concentrations were significantly higher in all analyzed tissues. The kidneys had the most altered tissue in comparison to the liver (average of 22.5 times greater) and lungs (average of 110.6 times greater). Cr is absorbed and then distributed to organs through blood (Ahmad & Mahmood, 2012), which explains its high concentration in this tissue and demonstrates its bioavailability. The liver is the first organ to receive Cr after its absorption and even so, the concentration was not as high as in the kidneys, which indicates the possibility that the liver did not retain Cr (VI) (Sutherland, Zhitkovich, Kluz, & Costa, 2000), probably because it is the main organ to biotransform xenobiotics (Patlolla et al., 2009). The increase of Cr (VI) concentration in the water of exposed groups did not duplicate the Cr concentration in the tissues as well as the alterations in the oxidative stress parameters. One of the reasons may be due to the lower ingestion of water in Group III in comparison to Group II, probably because of the water's taste.

The fact that the kidneys are responsible for the excretion of Cr (VI) (Filler et al., 2017) may explain its high concentration in this organ. Kidneys demonstrated to be the most susceptible organ to alterations related to oxidative stress caused by the exposure of Cr (VI), when compared to liver and lungs. The renal tissue obtained a significantly higher production of ROS as shown by DCFH levels in both exposed groups. This happened because the kidney depends on osmotic pressure and the concentration gradient contributes to its susceptibility (Patlolla et al., 2009). In addition, the fact of being responsible for excreting Cr (VI) (Parveen, Khan, & Siddiqui, 2009) may have collaborated to a higher oxidative effect in this tissue.

The proteins are one of the main targets of ROS and their consequent oxidation can cause alterations in their structures and affect the metabolic functions (Cabiscol, Tamarit, & Ros, 2000). Therefore, the carbonyl test was undertaken to verify the oxidation of proteins and it demonstrated a significant increase in this parameter in the kidneys and liver. The fact that proteins are the main molecules affected by ROS and secondary products of oxidative stress (Dalle-Donne, Rossi, Colombo, Giustarini, & Milzani, 2006) may have contributed to the increased levels of carbonyls in both organs.

The results of the present study are in agreement with other findings (Parveen et al., 2009; Balakrishnan et al., 2013) performed in the kidneys and liver with intraperitoneal and subcutaneous injections, whereupon they demonstrated increased carbonyl levels after Cr (VI) exposure. Despite the distinct exposure routes used in these studies and in the present research, it is evident that Cr (VI) can increase carbonyl levels in the liver and kidneys. In our study, the values of carbonyl in lungs did not increase significantly. The Cr (VI) concentration in the drinking water may have been the main factor related to the low increase in the lungs, since another research with the same methodology of exposure and a higher Cr (VI) concentration demonstrated a significant increase in the carbonyl levels (Soudani et al., 2013).

Lipid peroxidation, verified by TBA-RS test, was not altered in any of the analyzed tissues. Previous studies confirm that an up-regulation of lipid peroxidation and oxidation of proteins can interfere in the antioxidant system by down-regulating some enzymes, such as SOD, CAT, and GPx (Navya, Phani Kumar, G., Chandrasekhar, & Anilakumar, 2018). Even though the TBA-RS levels were not enhanced in the present study, it was evidenced a decrease in the activity of the evaluated antioxidant enzymes, mainly in the kidneys.

These results are congruent with the research accomplished by García-Niño et al. (2015), who analyzed the effects of Cr (VI) in the kidneys and confirmed the study of Tagliari, Vargas, Zimiani, and Cecchini (2004), in which the rats' livers were investigated and, in both studies, there was no increase in the levels of MDA. However, these studies were accomplished with acute exposures and intraperitoneal injections of Cr (VI), differently from our study, in which we used chronic exposure and contamination of water with Cr (VI) in an animal model. These studies also suggest the exposure route and the duration of the treatments are some of the factors that increase lipid peroxidation levels. Although the exposure period was 30 days, the exposure route may have contributed to a compensatory effect of the oxidative levels, such as TBA-RS.

The enzymatic activities of SOD, CAT, and GPx are responsible for reducing oxidative levels in the organism (Davies, 2000). SOD catalyzes the dismutation of superoxide radical ($O_2^{\bullet}$) in $H_2O_2$ and oxygen ($O_2$) (Soudani et al., 2013), after, CAT and GPx catalyze the resulting $H_2O_2$ in water and $O_2$ (Davies, 2000). Through the enzymes antioxidants actions that work independently, cooperatively, and synergistically, the integrity of the tissues is maintained (Soudani et al., 2013). Therefore, in the present study, SOD was decreased significantly in all analyzed tissues and the GPx was altered considerably in the kidneys. CAT enzymatic activities did not evidence significant differences in any of the examined organs, and one of the possible motives was the decreased SOD enzymatic activities, which provides the substrate ($H_2O_2$) for CAT.

Another reason is the fact that CAT is produced in the peroxisomes of the liver and kidneys (Patlolla et al., 2009). Once the kidneys demonstrated indications of oxidative stress, which could be nephrotoxic, there might have been a dysfunction in this organelle (Nordgren & Frasen, 2014), consequently diminishing the production of CAT in the kidneys and probably also in the liver.

SOD/CAT ratio fairly decreased in the liver and kidneys of the exposed groups (II and III) in comparison to the control group. This imbalance between SOD and CAT indicates the generation of ROS (Halliwell & Gutteridge, 1990). These alterations may be explained by the administration of Cr (VI) in the drinking water, which might have suppressed the production or action of the antioxidant enzymes (Boşgelmez & Güvendík, 2004).

The lungs did not have enough alterations to characterize an induction of oxidative stress, probably due to the exposure route utilized in the present research. Although this tissue did not express many alterations, studies accomplished with oral route exposure to Cr (VI), along with a further evaluation of oxidative stress parameters in the lungs are not very frequent. An exposure of a greater concentration of Cr (VI) could enable a different result, as expressed in Soudani et al. (2013) study. In the examined lungs of our research, only SOD activity was altered, and one of the reasons may be explained by the lowest Cr concentration in this tissue in comparison to the other analyzed tissues. Furthermore, the SOD is the first antioxidant to defend the organism against ROS production, with a key role in balancing the concentration of ROS and

protecting against oxidative damage (Lu, Wang, & Liu, 2015). Although there were no alterations in the oxidative parameters, they might have been avoided by the SOD activity.

Long-term exposures are related to lung cancer, mainly among workers of different industries, such as chrome plating, chromate production, and stainless-steel welding. The workers are exposed mainly through inhalation of Cr (VI) (Stout et al., 2009). Different studies accomplished with tanneries, cement, and chrome-plating workers had related the exposure of Cr (VI) to various skin and lung diseases as well as alterations in oxidative stress parameters (Caglieri et al., 2006; Ambreen, Khan, Bhadauria, & Kumar, 2014; Elhosary, Maklad, Soliman, El-Ashmawy, & Oreby, 2014).

It should be noted that most of the studies above-mentioned (Tagliari et al., 2004; Parveen et al., 2009; Balakrishnan et al., 2013; García-Niño et al., 2015) were performed with acute exposures and by injections, either intraperitoneal or subcutaneous. Our study, however, was accomplished by chronic treatment and oral exposure with water *ad libitum*, and it obtained significant results that corroborate to studies with another exposure route. The oral route reproduces a possible environmental exposure, e.g., industrial contaminations, since many different concentrations of Cr (VI) were obtained in aquatic systems. Therefore, the use of water for rats' treatment in this research demonstrated to be a great exposure route to Cr (VI), since high concentrations of this metal were obtained in the analyzed tissues.

As a result, the chronic Cr (VI) exposure to Wistar rats in drinking water caused an alteration in pro-oxidant parameters (DCFH and carbonyls) and antioxidant enzymes (SOD and GPx). This imbalance between oxidants and antioxidants enzymes characterizes an oxidative stress. Also, this research demonstrated that the highest Cr (VI) concentration applied to the animals was the one that caused the most alterations in the oxidative stress parameters, as well as the highest accumulation capacity in all analyzed tissues.

## Conclusion

In the present study, the Cr (VI) was able to induce the oxidative stress in the liver and kidneys, being the latest most susceptible tissue to Cr (VI) toxicity. The treatment period confirmed the Cr accumulation capacity, since all the analyzed tissues expressed high concentrations of this metal. Therefore, the results supported the capacity of Cr (VI) to induce oxidative stress in two of the analyzed tissues, to accumulate in all of them and, consequently, showed possible damages to these organs.

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