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Oxidative Stress Markers and Histological Analysis in Diverse Organs from Rats Treated with a Hepatotoxic Dose of Cr(VI): Effect of Curcumin

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Abstract Hexavalent chromium [Cr(VI)] compounds are extremely toxic and carcinogenic. Despite the vast quantity of reports about Cr(VI) toxicity, the information regarding its effects when it is intraperitoneally (i.p.) administered is still limited. In contrast, it has been shown that curcumin prevents hepatotoxicity induced by a single intraperitoneal injection of 15 mg/kg body weight (b.w.) of potassium dichromate ($K_2Cr_2O_7$). This study aims to evaluate oxidative stress markers, the activity of antioxidant enzymes, and the potential histological injury in brain, heart, lung, kidney, spleen, pancreas, stomach, and intestine from rats treated with a hepatotoxic dose of $K_2Cr_2O_7$ (15 mg/kg b.w.), and the effect of curcumin pretreatment. Rats were divided into four groups: control, curcumin, $K_2Cr_2O_7$, and curcumin+$K_2Cr_2O_7$. At the end of the treatment, plasma and ascites fluid were collected and target organs were dissected out for biochemical and histological analysis. $K_2Cr_2O_7$ induced hepatotoxicity but failed to induce in all the other studied organs either oxidative or histological injury, since levels of malondialdehyde (MDA), glutathione (GSH), and the activity of superoxide dismutase (SOD), catalase (CAT), and related GSH enzymes were unchanged. As expected, curcumin was safe. Lack of $K_2Cr_2O_7$-induced toxicity in those target organs could be due to the following: (1) route of administration, (2) absorption through the portal circulation, (3) lower dose than needed, (4) short time of exposure, or (5) repeated doses are required to produce damage. Thus, the intraperitoneal injection of 15 mg/kg of $K_2Cr_2O_7$, that is able to induce hepatotoxicity, was unable to induce histological and oxidative damage in other target organs.

Keywords Curcumin · Antioxidants · Chromium · Oxidative stress · Histological studies

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Introduction

Chromium has been identified as a potential environmental and occupational poison and hexavalent chromium [Cr(VI)] compounds were among of the earliest chemicals to be classified as human carcinogens [1]. Cr(VI) compounds such as potassium dichromate ($K_2Cr_2O_7$), sodium chromate, or chromic acid, are widely used in leather, electroplating, welding, painting, chrome plating, and dye-producing industries [2]. Elevated levels of chromium in blood, urine, and some tissues, have been found in workers occupationally exposed to Cr(VI) [3]. Health effects of Cr(VI) compounds may vary with route of exposure. Respiratory exposure has been associated with

lung cancer and nasal and sinus cancer [4]. While accidental or intentional ingestion of extremely high doses of Cr(VI) compounds produces severe respiratory, cardiovascular, gastrointestinal, hematological, hepatic, renal, and neurological effects that can result in death [5]. Reproductive and developmental effects have been also reported [6].

Experimental evidences suggest that most herbs and spices possess a wide range of biological and pharmacological activities including antioxidant properties that may protect tissues against oxidative stress-induced damage [7]. Curcumin is a hydrophobic polyphenol derived from the rhizome of the herb *Curcuma longa*, which exhibits antioxidant, antimicrobial, anti-inflammatory, and anticarcinogenic properties, and it has been characterized as a safe natural product by different international regulatory agencies [8]. Curcumin may protect cells from oxidative stress since the presence of the phenolic, β-diketone, as well as the methoxy groups that contribute to the free-radical scavenging activity of curcumin by donating electrons and neutralizing free radicals [9]. Also, curcumin protects cells indirectly by inducing the nuclear factor (erythroid-derived 2)-like 2 (Nrf2), which upregulates the expression of phase II enzymes, including superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), glutathione reductase (GR), and glutathione-S-transferase (GST), among others [10].

Several studies have demonstrated the high benefit of curcumin in the treatment of hepatic disorders, such as drug-induced hepatotoxicity, alcoholic liver disease, non-alcoholic liver disease, hepatitis B and C, and hepatocarcinoma [11]. Recently, it was shown that curcumin, administered by gavage at a dose of 400 mg/kg body weight (b.w.), successfully prevented the Cr(VI)-induced liver injury in rats injected intraperitoneally (i.p.) with $K_2Cr_2O_7$ (15 mg/kg b.w.) [12, 13]. In that study, curcumin reduced hepatocyte damage, ameliorated oxidative stress, maintained the activity of antioxidant enzymes, and protected against mitochondrial dysfunction. However, despite the enormous quantity of information about Cr(VI) toxicity, the information regarding the Cr(VI)-induced effects when it is i.p. administered is still limited. Thus, this work was designed to evaluate the effect of a single intraperitoneal injection of $K_2Cr_2O_7$ (15 mg/kg b.w.) on the potential histological injury, oxidative stress and the activity of antioxidant enzymes in brain, heart, lung, kidney, spleen, pancreas, stomach, and intestine of rats as well as the effect of curcumin pretreatment. Furthermore, liver toxicity was evaluated in these rats by the measurement of the following injury markers in plasma: lactate dehydrogenase (LDH), aspartate aminotransferase (AST), alanine aminotransferase (ALT), total proteins and albumin, as well as by the levels of the oxidative stress marker malondialdehyde (MDA). In addition, the ascites fluid accumulation was also determined since it is a consequence of the Cr(VI)-induced hepatotoxicity for this route of administration [14, 15].

Materials and Methods

Reagents

Curcumin, $K_2Cr_2O_7$, bovine serum albumin, bromocresol green, butylated hydroxytoluene (BHT), 1-methyl-2-phenylindole, tetramethoxypropane, xanthine, xanthine oxidase, nitroblue tetrazolium (NBT), glutathione (GSH), glutathione disulfide (GSSG), GR, GST, 1-chloro-2,4-dinitrobenzene (CDNB), dimethyl sulfoxide (DMSO), NADPH, N-(2-hydroxyethyl)piperazine-N′-(2-ethanesulfonic acid) (HEPES), nicotinamide adenine dinucleotide (NADH), ethylene glycol tetraacetic acid (EGTA), 3-(N-morpholino) propanesulfonic acid (MOPS), and paraformaldehyde were purchased from Sigma-Aldrich (St. Louis, MO, USA). Monochlorobimane was purchased from Fluka (Schnelldorf, Germany). Potassium phosphate monobasic (KH_2PO_4), sodium phosphate dibasic (Na_2HPO_4), trichloroacetic acid (TCA), hydrogen peroxide (H_2O_2), methanol, high-performance liquid chromatography (HPLC)-grade acetonitrile, and ethyl acetate were acquired from J.T. Baker (Xalostoc Edo. Mex, México). Commercial kits to measure the plasma activity of LDH, AST, and ALT were from ELITech Diagnostic (Sées, France). All other reagents and chemicals used were of the highest grade of purity commercially available.

Experimental Design

Wistar male rats (150–200 g) housed under standard conditions (12-h light/12-h dark, 22±2 °C) and fed ad libitum were randomly divided into four groups (n=5/group): (1) Control, received a single intraperitoneal injection of isotonic saline solution. (2) Curcumin was suspended in 0.5 % carboxymethylcellulose and was given by oral gavage at dose of 400 mg/kg b.w. daily for 10 days. (3) $K_2Cr_2O_7$, rats received a single intraperitoneal injection of $K_2Cr_2O_7$ 15 mg/kg b.w. on day 10. (4) CUR-$K_2Cr_2O_7$, curcumin was given daily for 10 days and $K_2Cr_2O_7$ was injected on day 10; rats were sacrificed 24 h later. Animals were weighed daily. At the end of the study, animals were anesthetized i.p. with sodium pentobarbital (60 mg/kg b.w.), ascites fluid was collected and measured with a 3–5-ml syringe from the opened abdominal cavity and blood was obtained via abdominal aorta for the measurement of the activity of LDH, AST, and ALT, and the levels of MDA, total protein, and albumin. Brain, heart, lungs, kidneys, spleen, pancreas, stomach, and intestine were dissected out, cleaned, and weighed. Tissue samples for histological analyses and for the measurement of both oxidative damage

markers and activity of antioxidant enzymes were obtained. All the experimental protocols were approved by the Local Ethical Committee (FQ/CICUAL/036/12), according to the Official Mexican Guidelines for the use and care of laboratory animals (NOM-062-ZOO-1999) and for disposal of biological residues (NOM-087-SEMARNAT-SSA1-2002).

Hepatic Injury Markers

The activity of LDH, AST, and ALT was measured using commercial kits according to instructions by the manufacturer. Total protein concentration was measured in plasma according to Lowry et al. [16]. Albumin concentration was determined by the method of Doumas et al. [17].

Histological Analysis

Organ slices of 0.5-cm width were fixed by immersion in 4 % paraformaldehyde, dehydrated, and embedded in paraffin. Thin sections of 3–5 μm were stained with hematoxylin and eosin and were examined under light microscope Leica (Cambridge, UK).

Preparation of Homogenates

Organs were homogenized in a Brinkmann Polytron Model PT 2000 (Westbury, NY, USA) for 10 s in cold 50-mM potassium phosphate buffer, pH 7.3, and 0.5 M BHT. The homogenates were centrifuged at 3000*g* and 4 °C for 10 min and the supernatant was separated to measure oxidative stress markers and the activity of antioxidant enzymes. Protein concentration was measured according to Lowry et al. [16].

Markers of Oxidative Damage

Markers of oxidative damage were measured as previously described [18]. MDA, an important toxic byproduct of lipid peroxidation, was measured by the reaction with 1-methyl-2-phenylindole. A standard curve of tetramethoxypropane was used and optical density was measured at 586 nm. GSH content was evaluated by following the formation of fluorescent adducts between GSH and monochlorobimane, in a reaction catalyzed by GST. A standard curve of GSH was used and the fluorescence measured at excitation and emission wavelengths 385 and 478 nm, respectively, using a Synergy HT multimode microplate reader.

Activity of Antioxidant Enzymes

Activity of antioxidant enzymes was measured as previously described [19]. SOD activity was evaluated spectrophotometrically at 560 nm by a previously reported method, based on the NBT reduction to formazan. The amount of protein that inhibits NBT reduction to 50 % of maximum was defined as one unit of SOD activity. CAT activity was assayed by a method based on the decomposition of H_2O_2 by CAT contained in the samples at 240 nm. One unit of CAT is defined as the amount of enzyme that reduces 1 mmol of H_2O_2 per minute. GPx activity was evaluated in an assay mixture containing H_2O_2, GSH, GR, and NADPH. When GPx reduces H_2O_2, GSH is oxidized to GSSG that is additionally reduced to GSH by GR using NADPH, which is measured at 340 nm. GR activity was assayed by using GSSG as substrate and measuring the disappearance of NADPH at 340 nm. One unit of GPx or GR is defined as the amount of enzyme that oxidizes 1 μmol of NADPH per minute. GST activity was assayed in a mixture containing GSH and CDNB. One unit of GST was defined as the amount of enzyme that conjugated 1 nmol of CDNB with GSH per minute.

Statistical Analysis

Results were expressed as means±standard error of the mean (SEM). Data were analyzed by one-way ANOVA followed by Bonferroni's multiple-comparisons test using Prism 5.0 software (GraphPad, San Diego, CA, USA). A p value of <0.05 was considered statistically significant.

Results

Effect of $K_2Cr_2O_7$ and Curcumin Exposure on Body and Organs Weight as Well as Plasma MDA and Activity of LDH, AST, ALT, and Ascites Accumulation

The treatment with $K_2Cr_2O_7$ (15 mg/kg b.w.) and with curcumin (400 mg/kg b.w.) did not modify the body weight values (final and gain) in any group studied (Table 1). Also, the weight of brain, heart, lungs, liver, kidney, spleen, pancreas, stomach, and intestine was not affected (Table 1). The treatment with $K_2Cr_2O_7$ increased plasma MDA levels by ~88 % in comparison with control and by ~42 % versus CUR-$K_2Cr_2O_7$, although these changes were not significant ($p>0.05$). In addition, $K_2Cr_2O_7$ increased plasma activity of LDH, AST, and ALT as well as ascites accumulation, while reduced total protein (~22 %) and albumin (~18 %), though these changes were not significant ($p>0.05$) (Table 1). Curcumin prevented the increased activities of plasma enzymes but it was unable to prevent ascites accumulation.

Effect of $K_2Cr_2O_7$ and Curcumin Exposure on Brain Tissue

Our results show that at 24 h, neither the intraperitoneal acute treatment with $K_2Cr_2O_7$ nor oral curcumin

Table 1 Body and organ weight, plasma MDA content, LDH, AST, and ALT activities as well as the ascites fluid accumulation in the four groups of rats studied

	Control	CUR	$K_2Cr_2O_7$	CUR+$K_2Cr_2O_7$
Body weight (g)				
Initial	168.8±1.7	169.4±3.6	166.0±1.9	168.2±1.9
Final	227.0±6.5	224.2±3.6	220.0±5.7	228.2±2.7
Gain	58.2±5.9	54.8±2.3	54.0±5.0	60.0±2.0
Organ weight (g)				
Brain	2.39±0.08	2.34±0.08	2.23±0.03	2.21±0.08
Heart	1.11±0.02	1.16±0.05	1.08±0.03	1.14±0.02
Lungs	1.29±0.15	1.03±0.03	1.06±0.09	0.96±0.04
Liver	8.87±0.41	9.17±0.34	9.25±0.45	9.15±0.37
Kidney*	1.05±0.03	0.99±0.03	0.97±0.02	1.02±0.03
Spleen	0.81±0.07	0.96±0.07	0.85±0.04	1.01±0.05
Pancreas	0.69±0.04	0.68±0.02	0.88±0.11	0.83±0.06
Stomach	2.17±0.13	2.22±0.05	2.27±0.10	2.30±0.10
Intestine (~5 cm duodenum)	0.92±0.03	1.07±0.05	0.92±0.09	1.01±0.08
Oxidative stress marker				
Plasma MDA (nmol MDA/mg protein)	0.25±0.03	0.23±0.03	0.47±0.10	0.33±0.06
Hepatic injury markers				
LDH (U/l)	181.1±14.8	172.9±13.6	335.6±24.7[a]	224.9±22.8[b]
AST (U/l)	77.5±3.4	73.1±4.3	269.0±32.4[a]	143.9±29.9[b]
ALT (U/l)	46.9±3.8	49.1±5.6	149.0±18.1[a]	85.1±7.6[b]
Ascites (ml)	0.2±0.1	0.4±0.1	2.9±0.4[a]	2.2±0.4[a]
Total protein (mg/ml)	7.7±0.8	7.0±0.4	6.0±0.6	6.8±0.5
Albumin (mg/ml)	3.3±0.3	3.4±0.2	2.7±0.4	3.1±0.3

CUR curcumin, *MDA* malondialdehyde, *LDH* lactate dehydrogenase, *AST* aspartate aminotransferase, *ALT* alanine aminotransferase

*The weight of one kidney is reported. Values are mean ± SEM, $n=5$

[a] $p<0.05$ versus control

[b] $p<0.05$ versus $K_2Cr_2O_7$

pretreatment induced oxidative stress. The activity of antioxidant enzymes SOD, CAT, GR, and GST was unchanged in all groups while GPx showed a non-significant reduction in its activity of ~30 % in $K_2Cr_2O_7$ group and ~14 % versus CUR-$K_2Cr_2O_7$ (Table 2). Consistently, there were no histological alterations found on the cerebral cortex (Fig. 1).

Effect of $K_2Cr_2O_7$ and Curcumin Exposure on Cardiac Tissue

Animals exposed to $K_2Cr_2O_7$ and curcumin did not show oxidative stress (Table 3) or histological injury (Fig. 2) in the cardiac tissue. The activity of SOD and GPx was slightly reduced in both $K_2Cr_2O_7$ and CUR-$K_2Cr_2O_7$ groups; however, these differences were not statistically significant ($p>0.05$).

Table 2 Oxidative stress markers and activity of antioxidant enzymes in the brain of rats of the four groups studied

	Control	CUR	$K_2Cr_2O_7$	CUR+$K_2Cr_2O_7$
Oxidative stress markers				
MDA (nmol MDA/mg protein)	1.21±0.03	1.26±0.24	1.16±0.18	1.19±0.19
GSH (mmol GSH/mg protein)	0.176±0.017	0.144±0.017	0.155±0.027	0.194±0.023
Antioxidant enzymes				
SOD (U/mg protein)	206.9±18.3	243.7±43.7	241.8±24.6	252.4±26.0
CAT (U/mg protein)	12.1±0.7	14.3±3.3	14.9±2.3	13.9±2.5
GPx (U/mg protein)	63.2±5.6	67.2±8.8	44.0±11.5	51.0±4.9
GR (U/mg protein)	30.2±3.7	26.6±1.9	23.0±2.7	32.6±4.3
GST (U/mg protein)	31.1±2.4	44.4±2.4	37.6±2.2	40.6±5.6

Values are mean±SEM, $n=4$–5, $p<0.05$

CUR curcumin, *MDA* malondialdehyde, *GSH* glutathione, *SOD* superoxide dismutase, *CAT* catalase, *GPx* glutathione peroxidase, *GR* glutathione reductase, *GST* glutathione-*S*-transferase

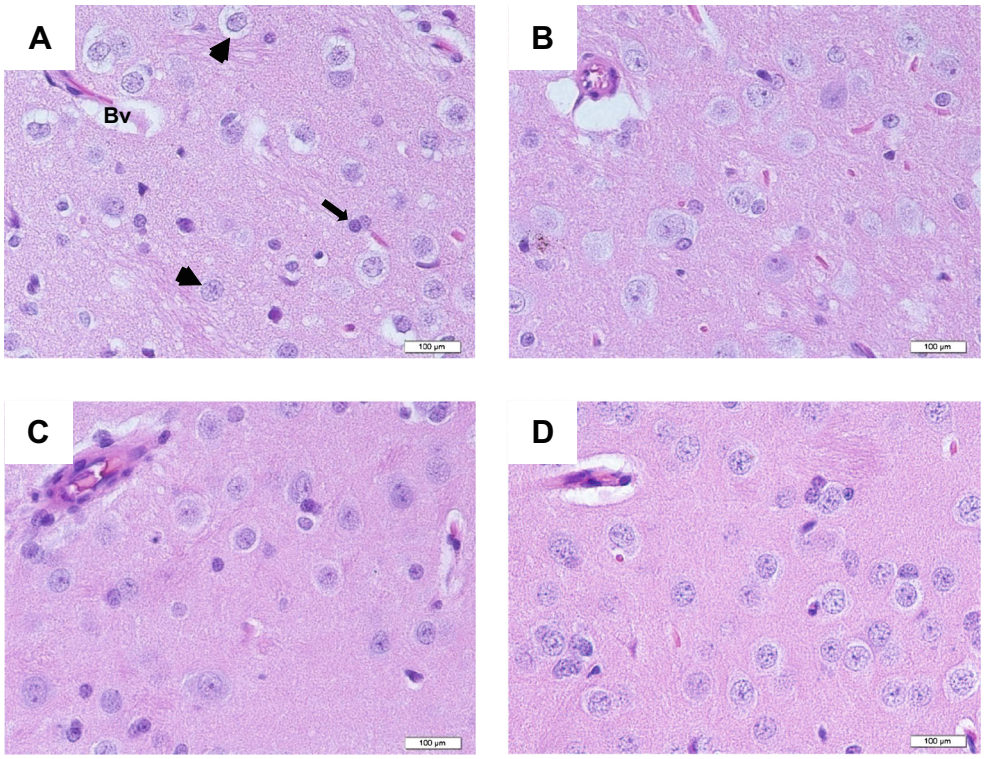

Fig. 1 Representative histological figures of cerebral cortex showing normal architecture in the groups studied. **a** Control. **b** Curcumin. **c** $K_2Cr_2O_7$. **d** Curcumin+$K_2Cr_2O_7$. Neurons (*arrowheads*), glial cells (*arrow*), and blood vessels (Bv). H&E stain, ×400

Effect of $K_2Cr_2O_7$ and Curcumin Exposure on Pulmonary Tissue

We identified that rats administered with $K_2Cr_2O_7$ or curcumin did not present important alterations in the oxidative stress markers, though the $K_2Cr_2O_7$ group showed a non-significant increase of ~40 % in MDA content compared with control and of ~65 % versus CUR-$K_2Cr_2O_7$ (Table 4). Moreover, the activity of antioxidant enzymes was not altered by the exposure to those agents (Table 4). Consistently with the above findings, pulmonary tissue did not present histological alterations (Fig. 3).

Effects of $K_2Cr_2O_7$ and Curcumin Exposure on Renal Tissue

In our rats, the results showed that intraperitoneal administration of $K_2Cr_2O_7$ (15 mg/kg b.w.) or orally with curcumin (400 mg/kg b.w.) did not induce alterations in the oxidative

Table 3 Oxidative stress markers and activity of antioxidant enzymes in the heart of rats of the four groups studied

	Control	CUR	$K_2Cr_2O_7$	CUR+$K_2Cr_2O_7$
Oxidative stress markers				
MDA (nmol MDA/mg protein)	0.33±0.02	0.25±0.03	0.33±0.09	0.38±0.07
GSH (mmol GSH/mg protein)	0.091±0.007	0.099±0.021	0.110±0.011	0.105±0.007
Antioxidant enzymes				
SOD (U/mg protein)	121.5±7.7	117.3±14.4	94.0±8.3	97.7±6.7
CAT (U/mg protein)	13.1±1.5	10.6±0.9	10.2±1.3	10.0±1.0
GPx (U/mg protein)	389.5±18.8	331.3±35.9	284.2±41.0	270.8±18.2
GR (U/mg protein)	5.2±0.2	4.8±0.5	4.1±0.4	4.7±0.5
GST (U/mg protein)	17.0±1.4	16.0±1.6	16.0±1.3	14.6±0.7

Values are mean±SEM, n=4–5, $p<0.05$

CUR curcumin, *MDA* malondialdehyde, *GSH* glutathione, *SOD* superoxide dismutase, *CAT* catalase, *GPx* glutathione peroxidase, *GR* glutathione reductase, *GST* glutathione-*S*-transferase

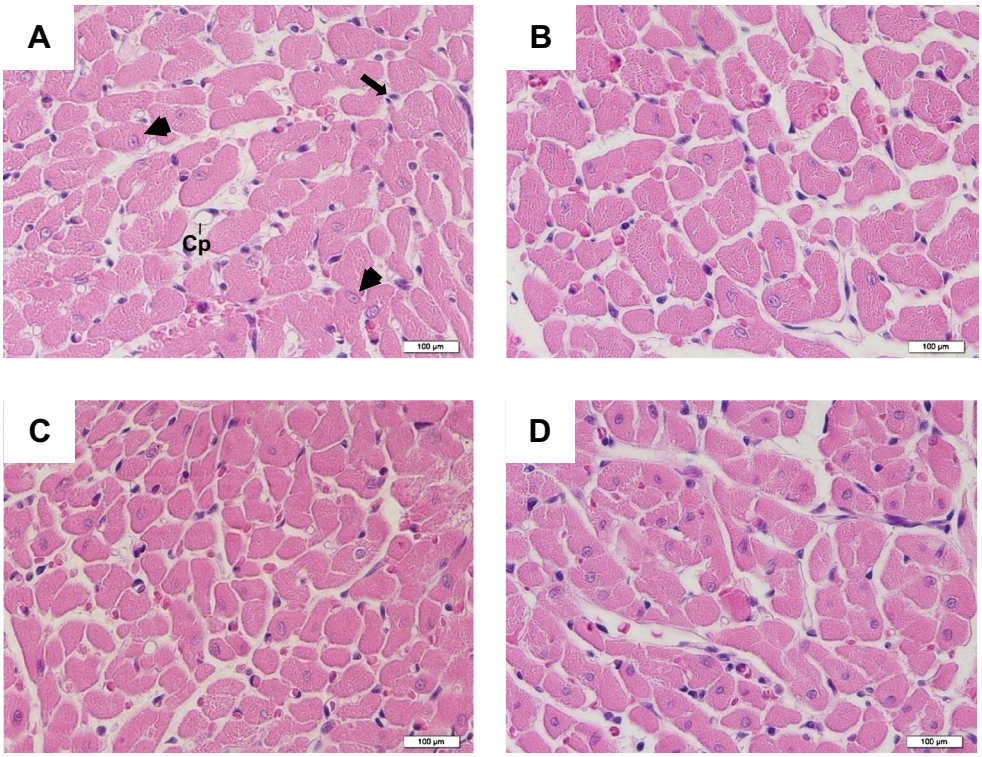

Fig. 2 Representative micrographs showing transverse sections of striated muscle tissue of heart from the groups studied. **a** Control. **b** Curcumin. **c** $K_2Cr_2O_7$. **d** Curcumin+$K_2Cr_2O_7$. There are not histological abnormalities in any of the studied groups. Cardiomyocytes(*arrowheads*), fibroblasts (*arrow*), and capillary (Cp). H&E stain, ×400

stress markers at 24 h (Table 5). In consequence, there was no evident histological damage on renal cortex (Fig. 4). On the other hand, it was found that coadministration of $K_2Cr_2O_7$ and curcumin increased significantly the GSH content and GPx, GR, and GST activities in relation to control or $K_2Cr_2O_7$ groups (Table 5). Activity of CAT and GR was slightly reduced in a non-significant way in $K_2Cr_2O_7$ group (32 and 23 %, respectively, Table 5).

Effects of $K_2Cr_2O_7$ and Curcumin Exposure on Splenic Tissue

The treatments did not induce any effect on oxidative stress markers (Table 6) or splenic histological architecture (Fig. 5). The activity of CAT was reduced by ~25 % in the $K_2Cr_2O_7$ group ($p>0.05$) and the activity of SOD, GPx, GR, and GST was unchanged (Table 6).

Table 4 Oxidative stress markers and activity of antioxidant enzymes in the lung of rats of the four groups studied

	Control	CUR	$K_2Cr_2O_7$	CUR+$K_2Cr_2O_7$
Oxidative stress markers				
MDA (nmol MDA/mg protein)	0.27±0.04	0.28±0.06	0.38±0.07	0.23±0.04
GSH (mmol GSH/mg protein)	0.097±0.014	0.108±0.017	0.091±0.013	0.111±0.014
Antioxidant enzymes				
SOD (U/mg protein)	126.8±6.8	113.4±16.8	115.7±5.7	110.5±3.8
CAT (U/mg protein)	117.6±13.0	105.8±4.8	93.8±7.8	128.9±12.0
GPx (U/mg protein)	54.5±3.0	56.0±8.7	43.8±6.7	66.7±9.6
GR (U/mg protein)	3.6±0.4	3.2±0.4	2.9±0.3	3.2±0.1
GST (U/mg protein)	14.3±1.0	12.8±2.3	15.0±1.3	14.5±1.3

Values are mean±SEM, $n=4–5$, $p<0.05$

CUR curcumin, *MDA* malondialdehyde, *GSH* glutathione, *SOD* superoxide dismutase, *CAT* catalase, *GPx* glutathione peroxidase, *GR* glutathione reductase, *GST* glutathione-*S*-transferase

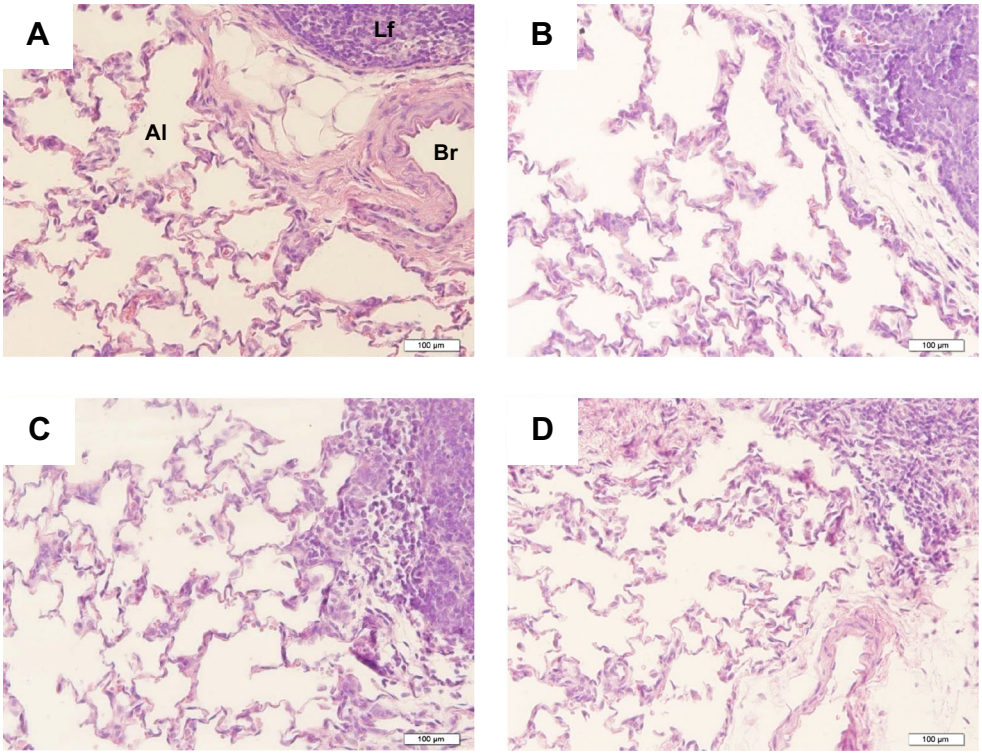

Fig. 3 Representative lung sections showing normal histology in the groups: **a** control, **b** curcumin, **c** $K_2Cr_2O_7$, and **d** Curcumin+$K_2Cr_2O_7$. Alveolus (*Al*), bronchioli (*Br*), and bronchial-associated lymphoid tissue (*Lf*). H&E stain, ×200

Effects of $K_2Cr_2O_7$ and Curcumin Exposure on Pancreatic Tissue

The intraperitoneal administration of $K_2Cr_2O_7$ did not induce alterations in the oxidative stress markers or in the activity of antioxidant enzymes (Table 7). Consistently, there were no histological alterations in pancreas (Fig. 6). In addition, curcumin had no deleterious effects on pancreas.

Effects of $K_2Cr_2O_7$ and Curcumin Exposure on Gastric Tissue

$K_2Cr_2O_7$ (15 mg/kg b.w.) injected i.p. to rats produced a non-significant increase ~21 % in the MDA content compared with control group and of ~44 % versus CUR-$K_2Cr_2O_7$ (Table 8). SOD activity was slightly reduced in $K_2Cr_2O_7$ group (22 % compared with control, $p>0.05$, Table 8). Moreover, there were no histological abnormalities in gastric mucosa (Fig. 7).

Table 5 Oxidative stress markers and activity of antioxidant enzymes in the kidney of rats of the four groups studied

	Control	CUR	$K_2Cr_2O_7$	CUR+$K_2Cr_2O_7$
Oxidative stress markers				
MDA (nmol MDA/mg protein)	0.39±0.04	0.34±0.04	0.46±0.03	0.35±0.02
GSH (mmol GSH/mg protein)	0.122±0.015	0.128±0.016	0.112±0.012	0.174±0.010[b]
Antioxidant enzymes				
SOD (U/mg protein)	233.5±17.2	227.8±31.2	196.2±17.4	242.7±20.8
CAT (U/mg protein)	358.8±38.7	381.1±30.3	244.9±31.7	346.5±49.3
GPx (U/mg protein)	341.8±46.4	340.3±31.6	364.8±40.7	553.0±63.2[a,b]
GR (U/mg protein)	173.0±19.3	159.0±11.5	132.6±16.8	240.3±18.6[b]
GST (U/mg protein)	50.5±4.6	51.8±2.5	61.4±4.3	78.8±6.3[a]

Values are mean±SEM, n=4–5

CUR curcumin, *MDA* malondialdehyde, *GSH* glutathione, *SOD* superoxide dismutase, *CAT* catalase, *GPx* glutathione peroxidase, *GR* glutathione reductase, *GST* glutathione-*S*-transferase

[a] $p<0.05$ versus control

[b] $p<0.05$ versus $K_2Cr_2O_7$

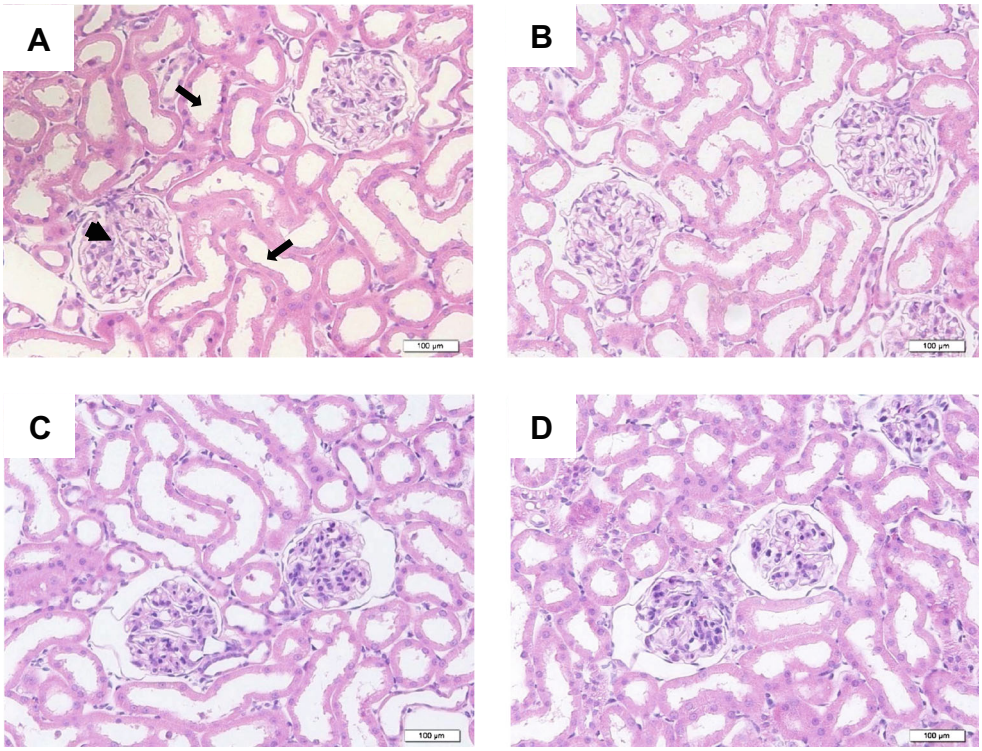

Fig. 4 Representative micrographs of renal tissue showing normal histology of the kidney cortex from the studied groups. **a** Control. **b** curcumin. **c** $K_2Cr_2O_7$. **d** Curcumin+$K_2Cr_2O_7$. Glomeruli (*arrowhead*), convoluted tubules (*arrows*). H&E stain, ×400

Effects of $K_2Cr_2O_7$ and Curcumin Exposure on Intestinal Tissue

The intraperitoneal injection of $K_2Cr_2O_7$ did not induce oxidative stress, alterations in the activity of antioxidant enzymes (Table 9) or histological damage to duodenal mucosa (Fig. 8).

Discussion

In this work, the effect of a single dose of $K_2Cr_2O_7$ (15 mg/kg b.w.) administered i.p. to rats on the potential damage induced in brain, heart, lungs, kidneys, spleen, pancreas, stomach, and intestine that are targets of Cr(VI) toxicity as well as the curcumin pretreatment (400 mg/kg b.w.) was evaluated. $K_2Cr_2O_7$ i.p. administered leads to liver dysfunction [15, 20] since the hepatic portal vein carries Cr(VI) to the liver making it susceptible to the first and persistent exposition [21]. Moreover, Bosgelmez et al. [20] revealed that a single intraperitoneal Cr(VI) administration caused a significant chromium accumulation in liver tissue.

Once Cr(VI) enters the body, it can efficiently penetrate cellular membranes through channels for isoelectric and isostructural anions, such as SO_4^{2-} and HPO_4^{2-} [22]. Inside

Table 6 Oxidative stress markers and activity of antioxidant enzymes in the spleen of rats of the four groups studied

	Control	CUR	$K_2Cr_2O_7$	CUR+$K_2Cr_2O_7$
Oxidative stress markers				
MDA (nmol MDA/mg protein)	0.033±0.009	0.033±0.006	0.032±0.006	0.038±0.008
GSH (mmol GSH/mg protein)	0.013±0.001	0.018±0.002	0.015±0.002	0.015±0.001
Antioxidant enzymes				
SOD (U/mg protein)	101.6±8.7	103.9±6.5	92. 7±9.4	117.1±5.8
CAT (U/mg protein)	121.3±5.3	112.6±11.2	90.3±7.2	108.5±8.6
GPx (U/mg protein)	148.3±13.5	154.8±13.0	128.8±8.2	135.0±4.1
GR (U/mg protein)	59.0±5.4	58.9±4.0	57.1±1.4	54.8±2.4
GST (U/mg protein)	11.8±0.7	11.9±0.7	10.3±0.5	10.5±0.4

Values are mean±SEM, n=4–5, $p<0.05$

CUR curcumin, *MDA* malondialdehyde, *GSH* glutathione, *SOD* superoxide dismutase, *CAT* catalase, *GPx* glutathione peroxidase, *GR* glutathione reductase, *GST* glutathione-*S*-transferase

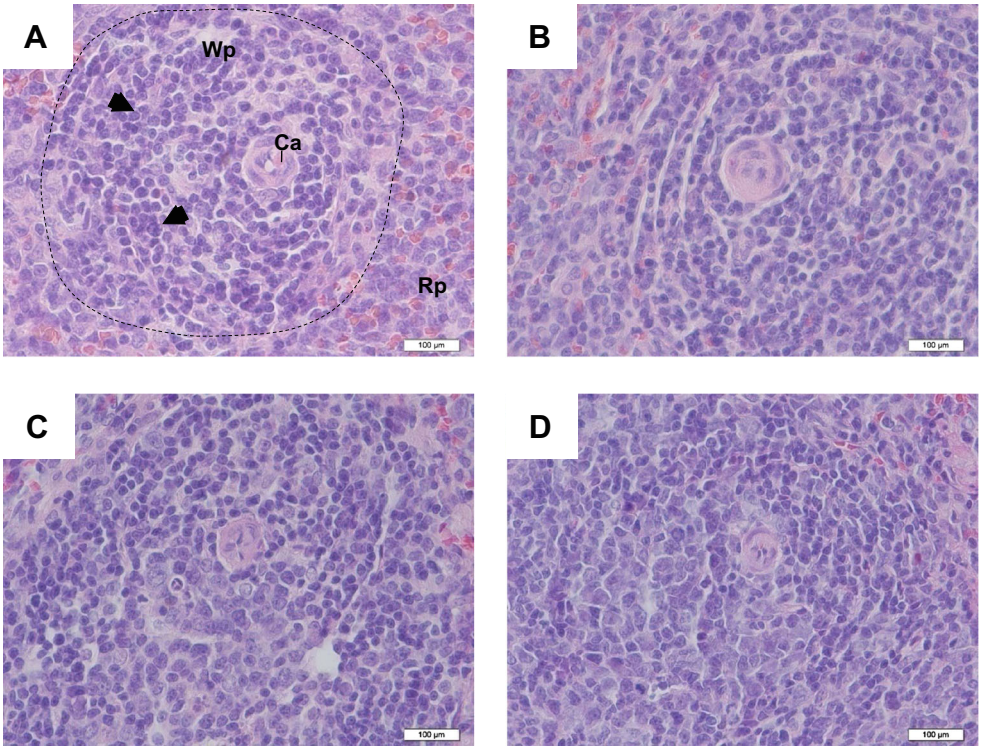

Fig. 5 Representative micrographs showing normal spleen histology from the studied groups. **a** Control. **b** Curcumin. **c** $K_2Cr_2O_7$. **d** Curcumin+$K_2Cr_2O_7$. Lymphocytes (*arrowheads*) conforming the white pulp (*Wp*) delimited by a *dotted line*, follicular artery (*Ca*), and red pulp (*Rp*). H&E stain, ×400

cells, Cr(VI) is reduced through reactive intermediates Cr(V), Cr(IV) to the more stable Cr(III) by cellular reductants such as GSH, cysteine, ascorbic acid, riboflavin, and NADPH-dependent flavoenzymes [23]. In fact, the Cr(VI)/(V), Cr(V)/(IV), and Cr(III)/(II) redox couples have been shown to serve as cyclical electron donors in a Fenton-like reaction, which generates reactive oxygen species (ROS) such as hydroxyl radical ($HO^{\bullet}$), superoxide radical ($O_2^{\bullet-}$), or H_2O_2 [24, 25]. The resulting excessive production of ROS may lead to oxidative damage to deoxyribonucleic acid (DNA), lipids, and proteins. It has been reported that Cr(VI) compounds may induce injury to brain [26], heart [27], lungs [28], kidneys [29, 30], spleen [31], pancreas [32], gastrointestinal system [33], and other vital organs [34] depending on dose level, schemes of treatment, and route of administration [35, 36].

Elevations in serum enzyme levels are taken as relevant indicators of cell damage or cell death [37]. LDH is found within the cytoplasm of essentially all cells; it is a highly sensitive but nonspecific biomarker [38]. AST is widely distributed in cells throughout the body and is found in the liver, heart, skeletal muscle, kidneys, brain, and pancreas [39]. ALT is widely distributed but the largest pool of ALT is in the cytosol of hepatic parenchymal cells. AST and ALT are very important markers of hepatic injury [40]. Thus, the increase in

Table 7 Oxidative stress markers and activity of antioxidant enzymes in the pancreas of rats of the four groups studied

	Control	CUR	$K_2Cr_2O_7$	CUR+$K_2Cr_2O_7$
Oxidative stress markers				
MDA (nmol MDA/mg protein)	0.018±0.005	0.020±0.004	0.014±0.002	0.015±0.003
GSH (mmol GSH/mg protein)	0.057±0.002	0.056±0.010	0.054±0.003	0.065±0.007
Antioxidant enzymes				
SOD (U/mg protein)	92.8±18.3	87.7±12.4	89.4±8.3	86.7±14.2
CAT (U/mg protein)	50.2±7.4	47.1±7.6	40.0±3.9	50.5±12.0
GPx (U/mg protein)	118.8±12.5	125.0±14.7	90.6±5.4	89.7±3.1
GR (U/mg protein)	50.2±5. 6	58.2±9.5	45.7±4.2	53.6±4.8
GST (U/mg protein)	11.7±0.9	10.8±1.1	10.3±1.1	10.6±1.4

Values are mean±SEM, n=4–5, $p<0.05$

CUR curcumin, *MDA* malondialdehyde, *GSH* glutathione, *SOD* superoxide dismutase, *CAT* catalase, *GPx* glutathione peroxidase, *GR* glutathione reductase, *GST* glutathione-*S*-transferase

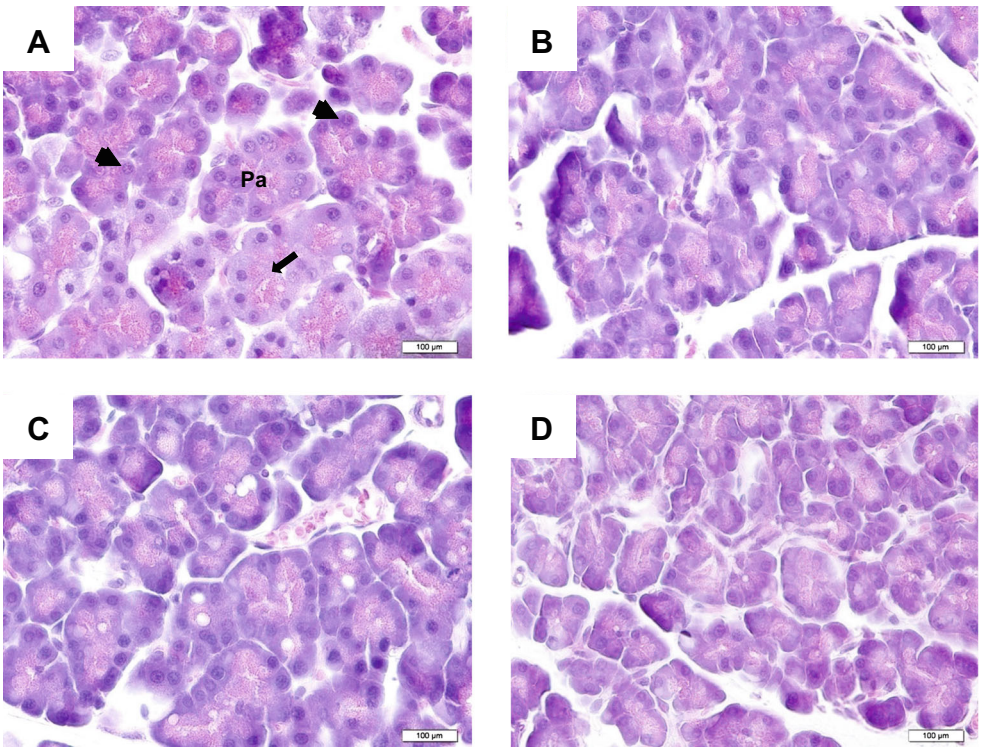

Fig. 6 Representative histological sections of exocrine pancreas showing normal architecture in the groups studied. **a** Control. **b** Curcumin. **c** $K_2Cr_2O_7$. **d** Curcumin+$K_2Cr_2O_7$. Pancreatic acinus (*Pa*), acinar cells (*arrowheads*), secretory granules (*arrow*), H&E stain, ×400

the activity of ALT, AST, and LDH in rats treated with Cr(VI) at the dose of 15 mg/kg was associated with hepatotoxicity. In comparison with previously published data [12], the activity ALT was similar while those of AST and LDH were higher by 50 and 37 %, respectively. However, as it has been shown, curcumin is able to prevent this damage. In this regard, it is worth mentioning that in order to get a significant increase in AST and ALT markers when $K_2Cr_2O_7$ is subcutaneously injected is necessary a dose higher than 30 mg/kg [41]. Moreover, one of the most serious outcomes of liver injury induced by Cr(VI) is ascites, the pathological accumulation of fluid in the peritoneal cavity that associated with a postsinusoidal block of hepatic blood flow [14]. In contrast, the increase in Cr(VI)-induced ascites fluid accumulation was not attenuated by curcumin and it is probable that this antioxidant was unable to resolve the postsinusoidal block associated with the Cr(VI)-induced congestion and hemorrhage in the hepatic sinusoids at this exposure time. Although in a previous work, it was demonstrated that curcumin ameliorates the ascites production induced by this poison at 48 h [13]. On the other hand, serum

Table 8 Oxidative stress markers and activity of antioxidant enzymes in the stomach of rats of the four groups studied

	Control	CUR	$K_2Cr_2O_7$	CUR+$K_2Cr_2O_7$
Oxidative stress markers				
MDA (nmol MDA/mg protein)	0.110±0.030	0.105±0.022	0.134±0.026	0.093±0.014
GSH (mmol GSH/mg protein)	0.048±0.014	0.072±0.012	0.049±0.013	0.055±0.015
Antioxidant enzymes				
SOD (U/mg protein)	189.1±23.4	197.1±7.2	148.0±11.1	158.7±29.0
CAT (U/mg protein)	14.2±1.8	12.7±2.7	13.5±1.9	14.0±1.4
GPx (U/mg protein)	46.3±5.2	52.7±1.9	43.0±5.8	44.0±7.9
GR (U/mg protein)	14.7±1.6	18.6±1.1	16.0±1.5	14.8±1.5
GST (U/mg protein)	2.3±0.3	2.1±0.1	2.2±0.3	2.8±0.3

Values are mean±SEM, n=4–5, $p<0.05$

CUR curcumin, *MDA* malondialdehyde, *GSH* glutathione, *SOD* superoxide dismutase, *CAT* catalase, *GPx* glutathione peroxidase, *GR* glutathione reductase, *GST* glutathione-*S*-transferase

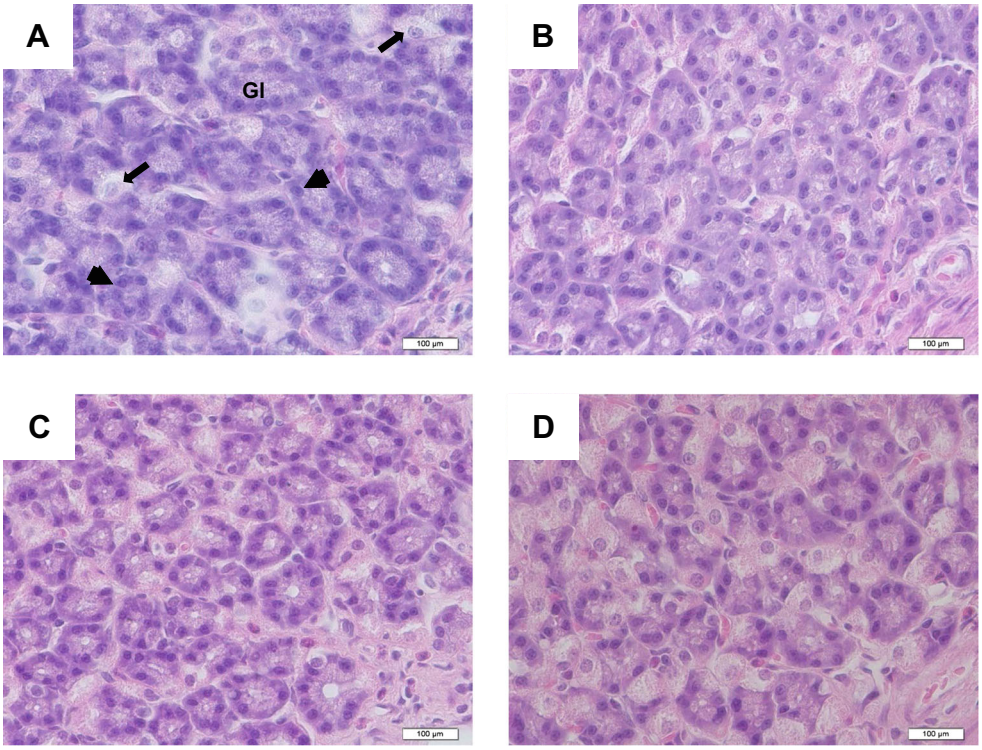

Fig. 7 Representative gastric tissue showing normal histology of mucosa from the studied groups. **a** Control. **b** Curcumin. **c** $K_2Cr_2O_7$. **d** Curcumin+$K_2Cr_2O_7$. Gastric glands (*GI*), chief cells (*arrowheads*), and parietal cells (*arrows*). H&E stain, ×400

protein content is helpful in identifying hepatotoxicity since the majority of plasma proteins like albumin are produced in the liver, and when this organ is injured, its protein synthetic capacity decreased [37]. Thus, in our experiment, Cr(VI) induced a slight reduction in total protein and albumin concentrations that it was not different from control groups at 24 h. Recently, Balakrishnan et al. [42] found in female rats that Cr(VI) affects liver function and diminishes serum total proteins after 14 days of a single subcutaneous injection of $K_2Cr_2O_7$ (10 mg/kg).

In addition, it has been reported that Cr(VI) compounds may induce damage to brain, heart, lungs, kidneys, spleen, pancreas, stomach, and intestine using several doses and schemes of treatment. Other previous in vivo or in vitro studies have shown the protective effects of curcumin against oxidative damage in brain [43], heart [44], lungs [45], kidneys [46], spleen [47], pancreas [48], stomach [49], and intestine [50].

In this regard, our study suggests that, at 24 h of treatment, the single i.p. $K_2Cr_2O_7$ (15 mg/kg) administration did not alter

Table 9 Oxidative stress markers and activity of antioxidant enzymes in the intestine of rats of the four groups studied

	Control	CUR	$K_2Cr_2O_7$	CUR+$K_2Cr_2O_7$
Oxidative stress markers				
MDA (nmol MDA/mg protein)	0.40±0.05	0.43±0.03	0.45±0.07	0.35±0.04
GSH (mmol GSH/mg protein)	0.029±0.007	0.034±0.009	0.023±0.005	0.032±0.010
Antioxidant enzymes				
SOD (U/mg protein)	134.9±10.6	116.4±14.7	105.9±12.0	118.7±5.5
CAT (U/mg protein)	17.3±3.7	18.7±1.1	19.0±2.7	17.9±3.5
GPx (U/mg protein)	44.8±6.1	52.3±8.4	43.8±7.2	55.0±5.0
GR (U/mg protein)	4.3±0.4	3.9±0.7	4.1±0.8	4.2±0.8
GST (U/mg protein)	8.5±1.2	10.3±0.9	11.0±1.1	11.2±2.3

Values are mean±SEM, n=4–5, $p<0.05$

CUR curcumin, *MDA* malondialdehyde, *GSH* glutathione, *SOD* superoxide dismutase, *CAT* catalase, *GPx* glutathione peroxidase, *GR* glutathione reductase, *GST* glutathione-*S*-transferase

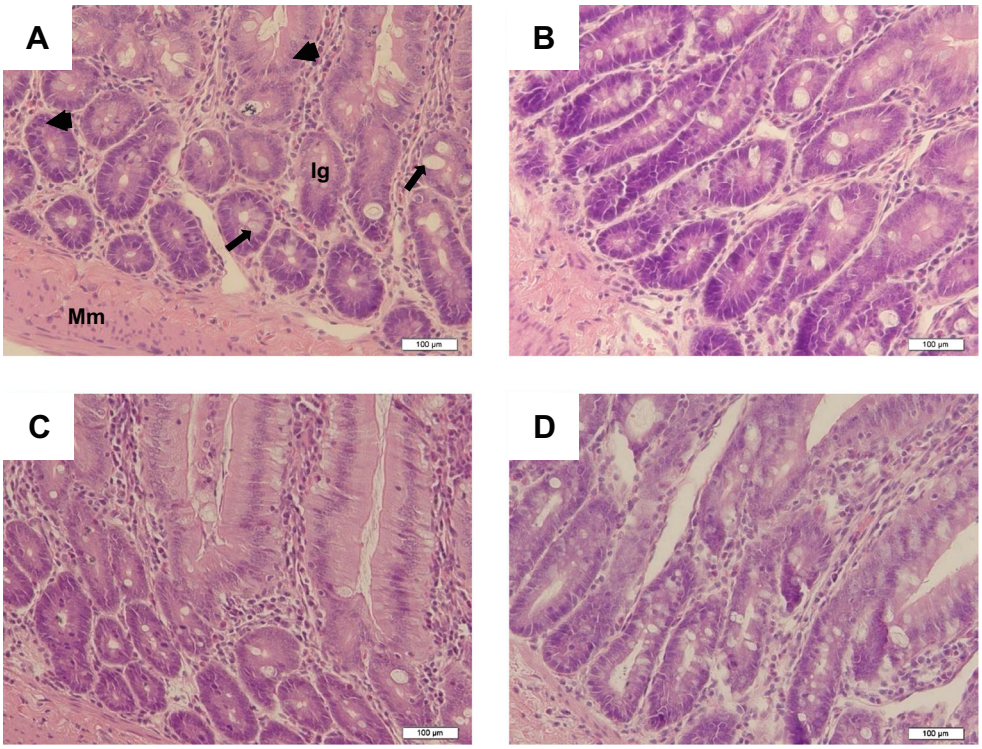

Fig. 8 Representative histological sections of intestinal mucosa showing normal architecture in the studied groups. **a** Control. **b** Curcumin. **c** $K_2Cr_2O_7$. **d** Curcumin+$K_2Cr_2O_7$. Intestinal gland (Ig), absorptive cells (*arrowheads*), goblet cells (*arrow*), and muscularis mucosae (Mm). H&E stain, ×400

body weight, organs weight, oxidative status, or the tissue architecture of brain, heart, lungs, kidneys, spleen, pancreas, stomach, and intestine of rats exposed to this agent, at least at 24 h. In some organs, a slight but not significant increase in oxidative stress parameters and a reduction in the activity of antioxidant enzyme were found. These findings may be explained, in all probability, to the fact that the dose and/or the time of exposure were not enough to cause further damage to these organs. Another possibility is that endogenous antioxidant systems (enzymatic and nonenzymatic) act synergistically for protecting cells against the potential oxidative injury induced by Cr(VI). This may be achieved by reducing ROS levels in several ways: SOD dismutates $O_2^{\bullet-}$ to H_2O_2, CAT transforms H_2O_2 to H_2O, GR regenerates GSH and GPx, using GSH, detoxifies H_2O_2 and peroxyl radicals. Also, GSH may scavenge $HO^{\bullet}$ and regenerate ascorbic acid and α-tocopherol to their active forms. In kidney, curcumin increased the GSH content and the activity of related GSH enzymes as a response to the Cr(VI)-induced lipid peroxidation. In particular, peroxyl radicals seem to have been the predominant ROS, because the activity of GPx increased and that of CAT remains essentially unchanged. On the other hand, curcumin pretreatment alone or in combination with Cr(VI) has no harmful consequences on these tissues.

Diaz-Mayans et al. [51] observed neurotoxicity in rats injected i.p. with sodium chromate at 2 mg/kg b.w. during 14 days. Bagchi et al. [52] identified oxidative stress and DNA single-strand breaks in brain of rats treated with a daily dose of sodium dichromate (2.5 mg/kg b.w. orally) in water for 120 days, while Soudani et al. [53] recognized oxidative stress in brain and cerebellum in animals administered with $K_2Cr_2O_7$ (700 ppm) during 21 days. In an epidemiologic study of boilermakers, Magari et al. [54] suggested an association between exposure to chromium and significant alterations in cardiac autonomic function. Soudani et al. [55] showed that $K_2Cr_2O_7$ treatment induce oxidative stress and abnormal ultrastructural changes such as myonecrosis, vacuolization, hemorrhage, and fibrosis in the cardiac tissue of rats that received 700 ppm in drinking water during 21 days. Tsapakos et al. [56] observed low levels of strand break in rat lung after intraperitoneal injection of sodium dichromate (20 mg/kg b.w.) 1 h after injection; however, no strand breaks remained by 24 or 36 h. Moreover, animals exposed intranasallly [28] or intratracheally [57] to particulate Cr(VI), presented injury, inflammation, and a significant elevation of the mutation frequency in the lung. Chorvatovicova et al. [58] injected $K_2Cr_2O_7$ (12 mg/kg b.w.) i.p to rats six times over 2 weeks and they found a reduction on ascorbic acid levels in lungs and kidneys, and an increase in hepatic MDA.

Nephrotoxicity has been reported in humans and experimental animals following exposure to Cr(VI) [59]. However, Kim and Na [36] have shown that nephrotoxicity and hepatotoxicity of Cr(VI) depends on the route of administration. Thus, the subcutaneous injection of Cr(VI) produces renal damage and affect some other organs than liver because of the compound-availability in the systemic circulation. In

contrast, intraperitoneal injection generates liver injury since Cr(VI) is directly acquired through portal vein and accumulated in liver. Additionally, Dartsch et al. [60] showed that proximal tubule OK cells were 10 times more susceptible than human hepatocellular carcinoma HepG2 cells to the toxicity induced by Cr(VI) on 24 h. This fact could be useful to understand why it is necessary to administer higher doses of Cr(VI)-compounds subcutaneously for inducing hepatic damage.

The subcutaneous injection of sodium dichromate produces a higher degree of nephrotoxicity than when it is administered i.p. to rats [36]. Compelling evidence have shown that a single subcutaneous injection of $K_2Cr_2O_7$ (15 mg/kg b.w.) to rats induced tubule interstitial damage [61–63]. In the study by Fatima and Mahmood [64], a single intraperitoneal dose of $K_2Cr_2O_7$ (15 mg/kg b.w.) induced an impairment of renal function and a decrease in the activities of brush border membranes, antioxidant enzymes, and phosphate transporter in rats at 48 h. Patlolla et al. [15] administered $K_2Cr_2O_7$ i.p. to rats at the doses of 2.5, 5, 7.5, and 10 mg/kg b.w. at 24-h intervals, and they showed that liver and kidney could be damaged by Cr(VI)-induced oxidative stress. das Neves et al. [65] found that the injection of potassium chromate (30 mg/kg b.w.) induced the enlargement of the capsule and depletion of the red pulp cells in the spleen, accompanied by an increase in macrophages. Dey and Roy [31] injected rats i.p. with Cr(VI) as chromium trioxide at a dose of 0.8 mg/100 g b.w. per day during 28 days, and they found an increase on GSH levels and CAT activity as a compensatory mechanism against ROS. Tarasub et al. [66] observed in rats administered with $K_2Cr_2O_7$ (5, 10, and 20 mg/kg b.w.) by the oral route that the histology of spleen remains unchanged, but there was a significant increase in percentage of chromosome aberrations in a dose-dependent manner. The acute administration of Cr(VI) induces pancreatic injury and extensive oxidative damage [32] in rats administered subcutaneously a single dose of $K_2Cr_2O_7$ (50 mg/kg b.w.). Solis-Heredia et al. [41] observed in rats injected subcutaneously with $K_2Cr_2O_7$ (20–50 mg/kg b.w.) that the endocrine cells were more resistant to $K_2Cr_2O_7$ toxicity than exocrine cells apparently because of Cr(VI)-mediated metallothionein induction protect them against Cr(VI)-induced toxicity.

Cr(VI) compounds, which may be found in the diet, can interact directly with DNA of gastric mucosa [67] and modify the expression of genes involved in cancer induction [68], but its carcinogenic potential when orally ingested remains controversial [69, 70]. De Flora et al. [71] administered sodium dichromate to mice for 9 consecutive months, at doses corresponding to 5 and 20 mg Cr(VI)/l. Under these conditions, Cr(VI) failed to enhance the frequency of DNA–protein crosslinks and did not cause oxidative DNA damage in the stomach and duodenum. The extracellular reduction of Cr(VI) to Cr(III), which occurs primarily in the stomach, is considered a mechanism of detoxification [72]. Acute oral administration of Cr(VI) resulted in epithelial cell injury and the decrease in antioxidant activities associated with oxidative stress in the intestine [73–75]. Upreti et al. [76] and Shrivastava et al. [77] showed that the resident intestinal microflora have a significant role in detoxification and elimination of the harmful chromium from the body by converting toxic Cr(VI) to a less toxic Cr(III).

According to the evidence, the lack of systemic toxicity after intraperitoneal poisoning with Cr(VI) could be explained by the following: (1) toxicity depending on the route of administration; (2) compounds administered i.p. are absorbed primarily through the portal circulation and, therefore, it must pass through the liver where it is accumulated before reaching other organs; (3) the dose we used in this experiment was too low in order to induce oxidative stress and injury in the evaluated organs; (4) the time of exposure was not enough to detect an increase in oxidative stress markers, alterations on the activity of antioxidant enzyme, or histopathological changes; or (5) a single intraperitoneal injection was not enough so that repeated doses are required to produce systemic damage.

As it was shown, the intraperitoneal administration of Cr(VI) could be an excellent tool for developing models to understand in a better way the mechanisms by which this kind of agents can cause toxicity if the necessary conditions are fulfilled. We suggest in future studies to deepen in the mechanisms associated with ascites fluid accumulation induced by Cr(VI) since the information is scarce. On the other hand, the security and efficacy of curcumin, as well as their antioxidant properties, bring this promising natural product to the forefront of therapeutic agents for environmental and occupational toxins. Despite the protective effects of curcumin against Cr(VI)-induced nephrotoxicity [46], hepatotoxicity [12], and toxicity in male reproductive system [78, 79], the number of studies is limited so that further investigation is necessary in order to propose it as a potential therapeutic agent against oxidative damage generated by Cr(VI) exposure. In conclusion, the intraperitoneal injection of 15 mg/kg of $K_2Cr_2O_7$, that is able to induce hepatotoxicity, was unable to induce histological and oxidative damage in diverse target organs because, in all probability, is preferably accumulated in liver.

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

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