



# Curcumin protects remote organs against injury that is caused by intestinal ischemia and reperfusion

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**ABSTRACT.** In addition to several local pathophysiological consequences, intestinal injury that is caused by ischemia and reperfusion can result in the development of lesions in remote organs. Curcumin has therapeutic potential because of its antiinflammatory and antioxidant effects. The present study evaluated the effects of curcumin on oxidative and inflammatory parameters in the liver and kidneys in rats that were subjected to intestinal ischemia and reperfusion. The rats were subjected to 45 min. of ischemia followed by 7 days of reperfusion and treated daily with 60 mg kg<sup>-1</sup> curcumin. The liver and kidneys were collected, weighed, and biochemically analyzed. Intestinal ischemia and reperfusion increased levels of lipid hydroperoxide (LOOH), decreased levels of reduced glutathione (GSH), and increased the enzymatic activity of superoxide dismutase (SOD), glutathione S-transferase (GST), and myeloperoxidase (MPO) in the liver. Intestinal ischemia and reperfusion decreased kidney weight and increased GST activity in the kidneys. Curcumin prevented these changes in the liver and kidneys. Intestinal ischemia and reperfusion mainly affected the liver, promoting inflammation and oxidative stress. The kidneys underwent repair much earlier than the liver, in which they did not present alterations of MPO or main parameters of oxidative stress after 7 days of reperfusion. Treatment with curcumin had beneficial effects, ameliorating or even preventing injury that was caused by intestinal ischemia and reperfusion in the liver and kidneys in rats.

**Keywords:** oxidative stress; myeloperoxidase; antioxidant; liver; kidneys.

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## Introduction

Ischemia and reperfusion (I/R) are pathophysiological sequelae that can be caused by various clinical events (Borges et al., 2018). Several diseases, transplantation surgeries, and accidents can result in intestinal I/R. The incidence of death is high for these patients, particularly for those in critical condition, in which the risk of mortality is 60-80% of cases (Hartmann et al., 2017; Ozban et al., 2015).

Injury that is caused by intestinal I/R is associated with numerous local pathophysiological consequences, such as an increase in neutrophil migration, an increase in cytokine release, and a decrease in the myenteric neuronal population in the ileum in rats (Borges, Souza, Beraldi, Schneider, & Buttow, 2016; Lin et al., 2014). One of the most relevant consequences is the lesion of intestinal cells that negatively affects mucosal integrity, leading to an increase in intestinal epithelial permeability (Borges et al., 2018; Eltzschig & Eckle, 2011; Lin et al., 2014). Several studies have suggested that injury to the intestinal mucosa allows the translocation of bacteria and endotoxins from the intestinal lumen into the bloodstream (Borges et al., 2018; Lin et al., 2014; Tassopoulos, Chalkias, Papalois, Iacovidou, & Xanthos, 2017), which can cause the development of lesions in remote organs (Stringa et al., 2016). However, the consequences of intestinal I/R in remote organs are not yet fully understood.

The consequences of intestinal I/R injury that are associated with bacterial translocation (Hebra et al., 1994; Koch, 2016) are generally known to lead to oxidative stress and systemic inflammation (Fan et al., 2014; Koch, 2016; Wang et al., 2015). Initially, during the period of ischemia, several local inflammatory mediators are generated (Eltzschig & Carmeliet, 2011). The reperfusion period, characterized by the return of blood circulation to ischemic tissue, triggers the formation of free radicals in the intestine (Mallick, Yang,

Winslet, & Seifalian, 2004). The translocation of bacteria and their byproducts, in addition to promoting inflammation in diverse tissues and organs, leads to an increase in reactive species of oxygen (ROS) and reactive nitrogen species (RNS) (Koch, 2016) and consequently oxidative stress in affected tissues and organs.

Therapeutic measures may prevent or alleviate the pathophysiological changes that are promoted by intestinal I/R injury in the intestine (Borges et al., 2016; Tian et al., 2016) and other organs (Fan et al., 2014; Onder et al., 2012). The use of antioxidants has often been adopted as a treatment alternative with good results (Borges et al., 2016; Souza et al., 2015; Onder et al., 2012; Tassopoulos et al., 2017). Several authors reported that curcumin (diferuloylmethane, 1,7-bis[4-hydroxy-3-methoxyphenyl]-1,6-heptadiene-3,5-dione) has antioxidant and antiinflammatory activity and high therapeutic potential (Gupta, Patchva, Koh, & Aggarwal, 2012; He, Yue, Zheng, Zhang, Chen, & Du, 2015). This substance is an orange-yellow component of saffron (*Curcuma longa*) (Okudan, Belviranlı, Gökbel, Oz, & Kumak, 2013). Some intestinal I/R injury studies have reported its beneficial effects on the gut (Karatepe et al., 2009; Atalik et al., 2012; Yucel, Kanter, Pergel, Erboga, & Guzel, 2011). However, studies of the effects of curcumin on remote organs that are affected by intestinal I/R are still limited. Therefore, the present study evaluated the effects of curcumin on oxidative and inflammatory parameters in the liver and kidneys in rats that were subjected to 45 min. of intestinal ischemia followed by 7 days of reperfusion.

## Material and methods

### Ethical conduct

All of the procedures were approved by the Ethical Conduct Committee on the Use of Animals in Experimentation of the *Universidade Estadual de Maringá* (approval no. 056/2014) and conducted in accordance with the ethical principles of the Brazilian Society of Laboratory Animals (SBCAL/COBEA). Wistar rats (*Rattus norvegicus*) were obtained from the Central Vivarium of the *Universidade Estadual de Maringá*. They were housed in the vivarium of the Department of Morphological Sciences under conditions of controlled temperature ( $22^{\circ}\text{C} \pm 2^{\circ}\text{C}$ ) and controlled lighting (12h/12h light/dark cycle). All of the animals received standard chow for rats (NUVILAB, recommended by the National Research Council and National Institutes of Health, Bethesda, MD, USA) and water *ad libitum*.

### Experimental protocol

Thirty male rats (220-260 g) were assigned to five groups ( $n = 6/\text{group}$ ; Table 1): C group (no surgery and vehicle [corn oil] treatment), SC group (sham surgery and vehicle [corn oil] treatment), ST group (sham surgery and curcumin treatment), IRC (I/R surgery and vehicle [corn oil] treatment) and IRT group (I/R surgery and curcumin treatment). The ST and IRT groups were treated orally by gavage with  $60 \text{ mg kg}^{-1}$  curcumin (Sigma-Aldrich, St. Louis, MO, USA) dissolved in corn oil. The C, SC, and IRC groups were treated orally by gavage only with vehicle (corn oil). The treatments began 5 days before surgery and was performed daily for 7 days afterward (reperfusion period).

**Table 1.** Experimental groups.

Groups		Treatment
C	Control not operated	Vehicle
SC	abdominal laparotomy without SMA occlusion	Vehicle
ST	abdominal laparotomy without SMA occlusion	Curcumin
IRC	abdominal laparotomy with SMA occlusion	Vehicle
IRT	abdominal laparotomy with SMA occlusion	Curcumin

S: sham groups which were not subjected to occlusion of the superior mesenteric artery; IR: groups submitted to intestinal ischemia and reperfusion;  
SMA: superior mesenteric artery

### Intestinal ischemia and reperfusion induction

After a 15 hours fast, the rats were intraperitoneally anesthetized with a mixture of  $20 \text{ mg kg}^{-1}$  xylazine (Sespo Indústria e Comércio, Paulínia, SP, Brazil) and  $100 \text{ mg kg}^{-1}$  ketamine (Sespo Indústria e Comércio, Paulínia, SP, Brazil). The SC and ST groups underwent only abdominal laparotomy with exposure of the intestine. The IRC and IRT groups underwent abdominal laparotomy followed by I/R. Saline solution (heated to  $37^{\circ}\text{C}$ ) was used to hydrate and maintain the internal temperature of the animals during the surgical procedure.

As described by Borges et al. (2016), a microvascular clamp was used to occlude the superior mesenteric artery (SMA), and lateral irrigation between the ischemic and non-ischemic regions was blocked by lashings. Mesenteric ischemia was determined by the loss of SMA pulsation and purple coloration of the ileum segment. The clamp was removed after 45 min., and the lashings were removed, thus reestablishing blood flow. During the 7-day reperfusion period, the animals remained in individual cages.

### Euthanasia and sample collection

Euthanasia was performed with sodium thiopental (Cristália - Produtos Químicos Farmacêuticos LTDA, Brazil) at a lethal dose of 120 mg kg<sup>-1</sup> (i.p.). Blood samples (5 mL) were then collected from each animal and centrifuged at 3000 × g for 10 min. The resulting plasma was used to verify aspartate aminotransferase (AST) and alanine aminotransferase (ALT) levels using commercial kits (Analisa®) and a spectrophotometer (Bioplus2000, São Paulo, Brazil) at a wavelength of 340 nm. Afterward, the liver and kidneys of each rat were collected, weighed, washed in sodium phosphate buffer (0.1 M phosphate-buffered saline, pH 7.4), and stored at -80°C until the biochemical assays.

### Biochemical assays

**Subcellular fraction preparation.** To obtain the tissue homogenate, portions of the liver and kidneys were weighed and homogenized separately in 200 mM potassium phosphate buffer (pH 6.5). Part of the homogenate was used to quantify reduced glutathione (GSH) levels, and another part was centrifuged at 9.000 × g for 20 min. The resulting supernatant was used to determine the activity of superoxide dismutase (SOD) and glutathione S-transferase (GST) and measure lipid hydroperoxide (LOOH) levels. Myeloperoxidase (MPO) activity was determined in the precipitate.

**Determination of protein concentration.** Protein concentrations were determined using the BCA protein assay kit (Pierce, BCA protein) according to the manufacturer's instructions, which adopts bovine albumin as the standard.

**Quantification of GSH levels.** The levels of GSH in the liver and kidneys were determined according to Sedlak and Lindsay (1968). Briefly, tissue homogenate plus trichloroacetic acid was centrifuged at 9,700 × g for 15 min. In a 96-well plate, the supernatant and 0.4 M Tris-HCL buffer (pH 8.9) were mixed. The reaction was started by the addition of 5,5'-dithiobis-2-nitrobenzoic acid and read at a wavelength of 412 nm. The individual values were interpolated on a standard GSH curve and are expressed as µg GSH g<sup>-1</sup> tissue.

**Measurement of SOD activity.** This assay is based on the ability of SOD to inhibit the auto-oxidation of pyrogallol (Marklund & Marklund, 1974). Tris-HCL buffer (1 mM) and ethylenediaminetetraacetic acid (EDTA; 5 mM, pH 6.5) were added to each sample, and the reaction was started with 1 mM pyrogallol and incubated at 25°C for 20 min. The reaction was stopped with 1 M hydrochloric acid. The samples were centrifuged at 14.000 × g for 4 min., and the absorbance of each supernatant was read at 405 nm. The amount of protein that inhibited the reaction by 50% was equal to 1 unit (U) of SOD activity. The results are expressed as U of SOD mg<sup>-1</sup> protein.

**Quantification of GST activity.** According to Warholm, Guthenberg, von Bahr, and Mannervik (1985), the supernatant was added to 200 mM potassium phosphate buffer (pH 6.5). After homogenization, the diluted sample and reaction solution (0.1 M potassium phosphate buffer [pH 6.5], 1-chloro-2,4-dinitrobenzene, and GSH) were mixed. The reading was performed at 340 nm. The results are expressed as µmol of GSH min<sup>-1</sup> mg<sup>-1</sup> protein, using the extinction coefficient of 9.6 mM cm<sup>-1</sup>.

**Determination of the LOOH amount.** According to Jiang, Woollard, and Wolff (1991), the supernatant from the samples was homogenized in 90% methanol in a ratio of 1:10 and centrifuged at 10.000 × g at 4°C for 30 min. The resulting supernatant and reaction medium (90% methanol, xylenol orange, 25 mM sulfuric acid, 4 mM butylated hydroxytoluene, and 250 mM FeSO<sub>4</sub> NH<sub>4</sub>) were added and incubated at 25°C for 30 min. The reading was performed at 560 nm. The LOOH concentration was determined based on an extinction coefficient of 4.3 mM cm<sup>-1</sup> and is expressed as mmol LOOH mg<sup>-1</sup> tissue.

**Myeloperoxidase activity assay.** The precipitate of each sample was resuspended in 80 mM potassium phosphate buffer that contained 0.5% hexadecyltrimethylammonium, homogenized, and centrifuged at 11.000 × g at 4°C for 20 min. The supernatant and a solution that contained 0.1 M sodium phosphate buffer (pH 7.4) and

0.017% hydrogen peroxide were added. The reaction was started with 20  $\mu$ L of tetramethylbenzidine. After incubation for 3 min. at 37°C, the reaction was stopped with 1.46 M sodium acetate (pH 3.0). Enzymatic activity was determined at 620 nm. The results are expressed as units of optical density (OD) min<sup>-1</sup>mg<sup>-1</sup> of protein (Smiderle et al., 2014).

### Statistical analysis

The statistical analysis was performed using GraphPad Prism 5.0 software for windows (San Diego, CA, USA). The results are expressed as mean  $\pm$  standard error. The Kolmogorov-Smirnov normality test indicated that all of the data had a normal distribution. One-way analysis of variance (ANOVA) was performed for comparisons between groups, followed by Tukey's post hoc test. Values of  $p < 0.05$  were considered statistically significant.

## Results

### Aspartate aminotransferase and ALT levels

No significant differences in AST or ALT levels were found between groups ( $p > 0.05$ ; Table 2).

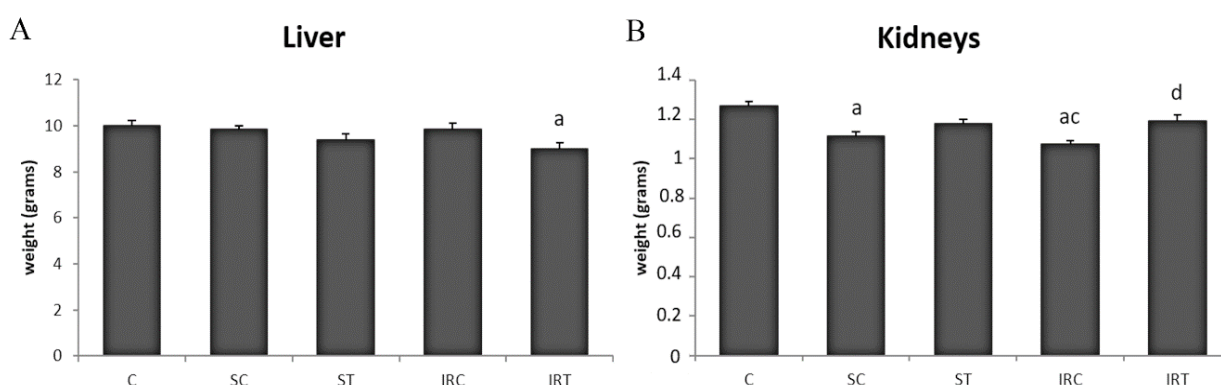
**Table 2.** Levels of Aspartate Aminotransferase (AST) and Alanine Aminotransferase (ALT).

	C	SC	ST	IRC	IRT
AST (U/L)	95.4 $\pm$ 5.5	116.7 $\pm$ 8.0	98.5 $\pm$ 6.8	99.5 $\pm$ 7.9	98.0 $\pm$ 12.4
ALT (U/L)	37.88 $\pm$ 1.8	38.50 $\pm$ 1.1	34.69 $\pm$ 2.4	31.80 $\pm$ 2.1	30.25 $\pm$ 3.3

Results were expressed as mean  $\pm$  standard error. (n = 6). C = control, SC = sham control, ST = sham treated, IRC = ischemic control, and IRT = ischemic treated. There was no statistically significant difference.

### Liver and kidney weights

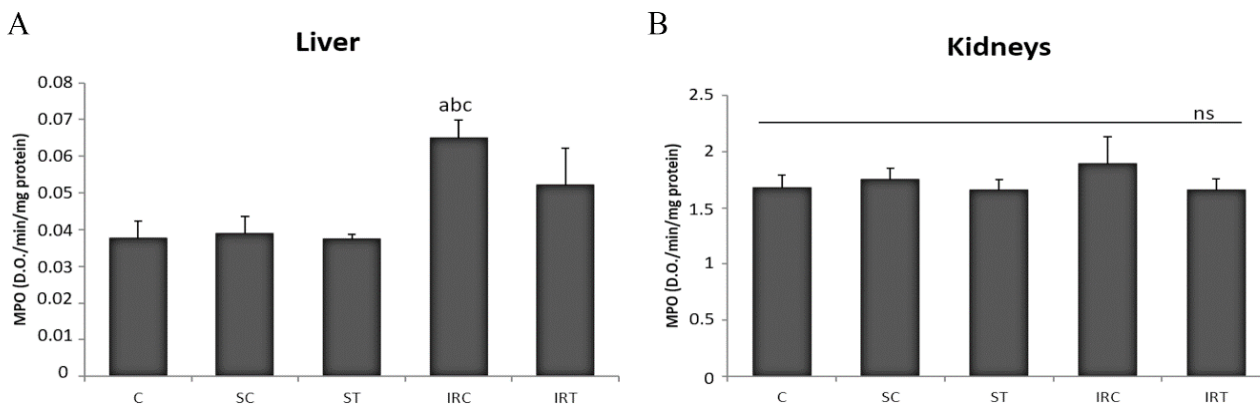
As shown in Figure 1A, no difference in liver weight was found between the IRC group and C group ( $p > 0.05$ ). The IRT group exhibited a significant decrease in liver weight compared with the C group ( $p < 0.05$ ; Figure 1A). The SC and IRC groups exhibited a significant decrease in kidney weight compared with the C group ( $p < 0.001$ ). The IRC group exhibited a decrease in kidney weight compared with the ST group ( $p < 0.05$ ). Kidney weight was not significantly different between the IRT group and C group ( $p > 0.05$ ) but significantly different between the IRT group and IRC group ( $p < 0.01$ ; Figure 1B).



**Figure 1.** (A, B) Weight of the liver (A) and kidneys (B) (in grams). <sup>a</sup>, significant difference from C group; <sup>b</sup>, significant difference from SC group; <sup>c</sup>, significant difference from ST group; <sup>d</sup>, significant difference from IRC group. The data are expressed as mean  $\pm$  standard error (n = 6/group).

### Myeloperoxidase activity

In the liver, the IRC group exhibited a significant increase in MPO activity compared with the C, SC, and ST groups ( $p < 0.05$ ). Myeloperoxidase activity in the liver was not significantly different between the IRT group and IRC group ( $p > 0.05$ ; Figure 2A). No significant difference in MPO activity in the kidneys was observed between groups ( $p > 0.05$ ; Figure 2B).

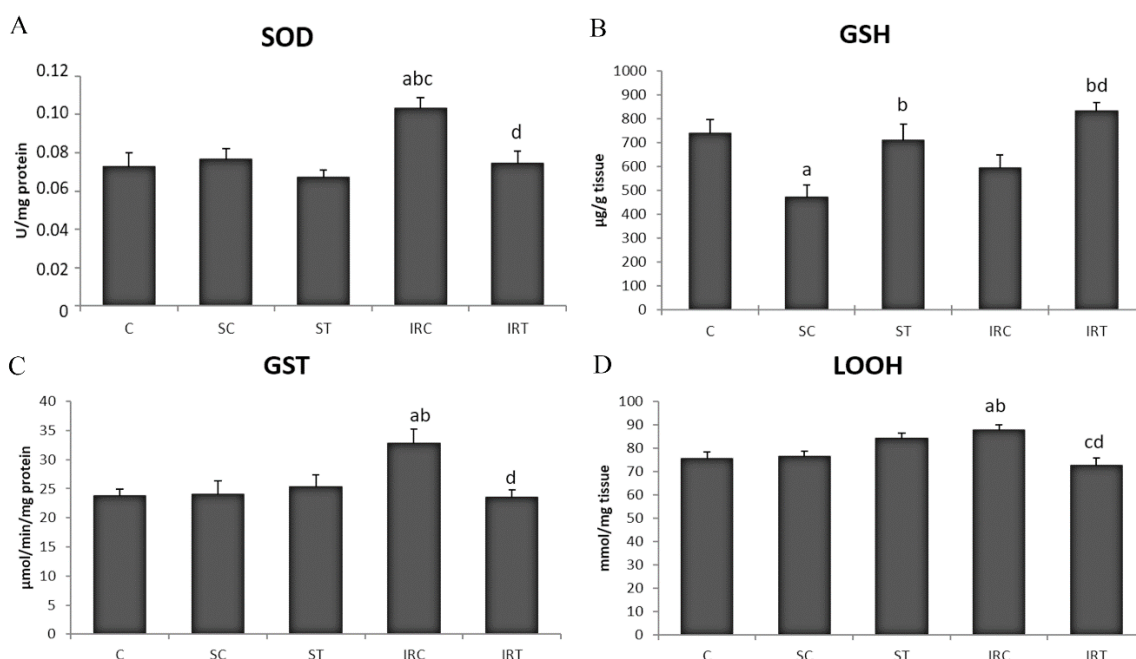


**Figure 2.** (A, B) Enzymatic activity of myeloperoxidase in the liver (A) and kidneys (B) in Wistar rats. <sup>a</sup>, significant difference from C group; <sup>b</sup>, significant difference from SC group; <sup>c</sup>, significant difference from ST group. ns, not significant. The data are expressed as mean ± standard error (n = 6/group).

### Hepatic oxidative parameters

As shown in Figure 3A, SOD activity was significantly higher in the IRC group compared with the C group ( $p < 0.01$ ), ST group ( $p < 0.01$ ), and SC group ( $p < 0.05$ ). The IRT group exhibited a significant difference in SOD activity compared with the IRC group ( $p < 0.05$ ) but not compared with the C group ( $p > 0.05$ ; Figure 3A). Levels of GSH were significantly lower in the SC group compared with the C group ( $p < 0.05$ ), whereas GSH levels were not significantly different between the ST group and C group ( $p > 0.05$ ). The IRT group exhibited a significant increase in GSH levels compared with the SC group ( $p < 0.001$ ). Levels of GSH were not significantly different between the IRC group and C group ( $p > 0.05$ ), whereas GSH levels were significantly higher in the IRT group than in the IRC group ( $p < 0.05$ ; Figure 3B).

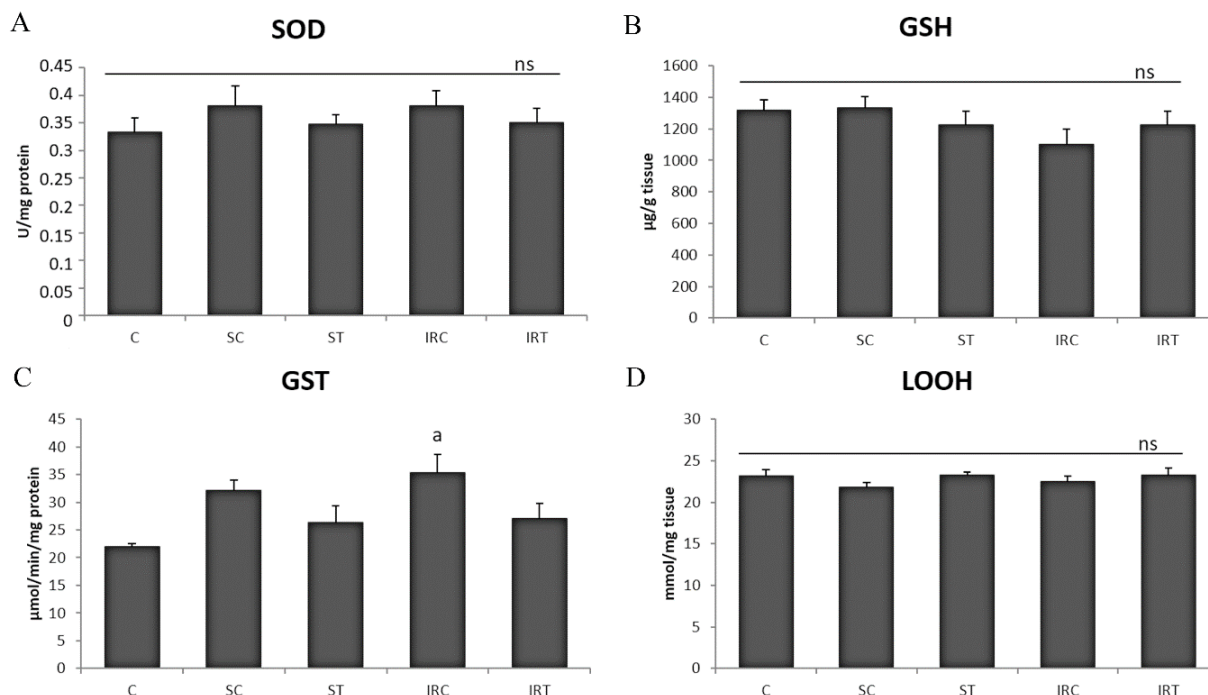
The activity of GST was higher in the IRC group than in the C and SC groups ( $p < 0.05$ ). The activity of GST was significantly different between the IRT group and IRC group ( $p < 0.05$ ) but not between the IRT group and C group ( $p > 0.05$ ; Figure 3C). LOOH levels were not significantly different between the ST and SC groups ( $p > 0.05$ ). The IRC group exhibited a significant increase in LOOH levels compared with the C and SC groups ( $p < 0.05$ ). LOOH levels were not significantly different between the IRT group and C group ( $p > 0.05$ ; Figure 3D).



**Figure 3.** Biochemical liver assays. (A, C) Enzymatic activity of superoxide dismutase (SOD) (A) and glutathione S-transferase (GST) (C). (B, D) Levels of reduced glutathione (GSH) (B) and lipid hydroperoxides (LOOH) (D). <sup>a</sup>, significant difference from C group; <sup>b</sup>, significant difference from SC group; <sup>c</sup>, significant difference from ST group; <sup>d</sup>, significant difference from IRC group. The data are expressed as mean ± standard error (n = 6 group).

### Renal oxidative parameters

Superoxide dismutase activity and GSH and LOOH levels in the kidneys were similar between groups ( $p > 0.05$ ; Figure 4A, B, and D, respectively). The IRC group exhibited a significant increase in GST activity compared with the C group ( $p < 0.01$ ). The activity of GSH was not significantly different between the IRT group and C group ( $p > 0.05$ ) or between the IRT group and IRC group ( $p > 0.05$ ; Figure 4C).



**Figure 4.** Biochemical kidney assays. (A, C) Enzymatic activity of superoxide dismutase (SOD) (A) and glutathione *S*-transferase (GST) (C). Levels of reduced glutathione (GSH) (B) and lipid hydroperoxides (LOOH) (D). <sup>a</sup>, significant difference from C group. ns, not significant. The data are expressed as mean  $\pm$  standard error ( $n = 6$  group).

### Discussion

Several therapeutic substances have been tested and applied for the treatment of I/R-related injury (Borges et al., 2016; Souza et al., 2015; Hartmann et al., 2017; Inan et al., 2013; Ozban et al., 2015; Stringa et al., 2016). Curcumin has been extensively studied in recent years. In addition to antioxidant and antiinflammatory activity, curcumin has been reported to exert antidiabetic, antiangiogenic, antimutagenic, antiinfective, and anticancer effects (Aggarwal, Gupta, & Sung, 2013; He, Yue, Zheng, Zhang, Chen, & Du, 2015; Rahmani, Alsahli, Aly, Khan, & Aldebasi, 2018; Kumar, Houreld, & Abrahamse, 2018). Several studies have shown that curcumin has a protective effect on multiple organs, including the kidneys (Fan et al., 2017; Onder et al., 2012; Tian et al., 2016) and liver (Rahmani et al., 2018). We hypothesized that curcumin may reduce or prevent the increase in neutrophil migration (measured as MPO activity) and prevent oxidative stress in the kidneys and liver in rats that are subjected to I/R.

Intestinal ischemia followed by reperfusion may trigger both local pathophysiological changes and cause damage in remote organs. However, little is known about the ways in which remote organs are affected after intestinal I/R injury or the effects of curcumin thereon. In the present study, we observed an increase in MPO activity in the liver in rats that were subjected to intestinal I/R and treated with vehicle, reflecting an increase in neutrophil migration and oxidative damage. Some authors reported that intestinal I/R injury, in addition to promoting oxidative stress in the ileum in rats (Borges et al., 2018; Souza et al., 2015), also compromises the integrity of the epithelial barrier (Ajamieh & Teoh, 2009; Berg, 1999) and may lead to translocation of bacteria and their byproducts, such as lipopolysaccharide and others endotoxins, from the intestinal lumen to the systemic circulation or portal vein (He, Li, Wang, Zhang, Ke, Yan, & Chen, 2015; Hebra et al., 1994; Koch, 2016). Thus, during intestinal I/R injury, the portal vein, in addition to transporting nutrients, also transports microbial products and bacteria (Wang et al., 2015). The liver receives a greater load of molecules during I/R (Wang et al., 2015), thus resulting in greater damage

compared with more distant organs. In the present study, the beneficial effects of curcumin were more pronounced in hepatic tissues.

The enzymatic activity of MPO in the liver increased by 72% in liver samples in the IRC group relative to the C group. Similar results were also reported by Fan et al. (2014), in which rats were subjected to intestinal ischemia for 1h followed by 2h of reperfusion. The increase in MPO activity is mainly attributable to the infiltration of neutrophils in the tissue, causing liver inflammation (Fan et al., 2014). In addition to bacterial translocation, bacterial products also contribute to the progression of liver injury through the activation of specific pathogen recognition receptors, especially Toll-like receptor 4 (TLR4) (Schnabl & Brenner, 2014; Wang et al., 2015). Even low levels of activated TLR4 are able to activate Kupffer cells. To eliminate liver pathogens, Kupffer cells trigger an inflammatory cascade that results in the release of cytokines (e.g., interleukin-6 [IL-6], IL-1 $\beta$ , and tumor necrosis factor- $\alpha$  [TNF- $\alpha$ ]) (Koch, 2016; Lin et al., 2014) and inflammation (Chassaing, Mesmin, & Gewirtz, 2014; Haque & Barritt, 2016; Koch, 2016). In the present study, oral treatment with curcumin (60 mg kg<sup>-1</sup>) attenuated inflammation in the liver after intestinal I/R injury. The IRT group exhibited improvements in MPO activity. Previous studies substantiated the antiinflammatory potential of curcumin, which has the ability to suppress proinflammatory pathways by blocking both the production and action of inflammatory cytokines, such as TNF- $\alpha$ , IL-1, IL-2, IL-6, IL-8, and IL-12 (Aggarwal et al., 2013; Rahmani et al., 2018; Wojcik, Krawczyk, Wojcik, Cypryk, & Wozniak, 2018).

Inflammation and oxidative stress are closely related phenomena (Wojcik et al., 2018). Oxidative stress is related to disturbances in the balance between oxidants and antioxidants, leading to the aberrant control of redox signaling and molecular damage (Sies & Cadenas, 1985). Superoxide dismutase is an enzyme that inactivates reactive species and protects against oxidative stress. The present results showed that SOD activity in the liver increased by 41% in the IRC group compared with the C group. Curcumin has a chemical structure that is capable of eliminating reactive oxygen and nitrogen species (Simioni et al., 2018), acting as a powerful antioxidant (He, Yue, Zheng, Zhang, Chen, & Du, 2015). Treatment with curcumin in the IRT group promoted the restoration of SOD activity to levels that were similar to the C group, thus demonstrating its therapeutic potential.

Lipid peroxidation is a consequence of the accumulation of reactive species (He, Yue, Zheng, Zhang, Chen, & Du, 2015; Kong, Blennerhassett, Heel, McCauley, & Hall, 1998) that favor the promotion of cellular injury and necrosis (Kong et al., 1998). Hartmann et al. (2017) subjected rats to 30 min. of ischemia followed by 15 min. of reperfusion, and Inan et al. (2013) subjected rats to 2h of ischemia followed by 2h of reperfusion. These two studies reported the occurrence of lipid peroxidation in the liver in rats. Our results indicated that LOOH levels remained high, even after 7 days of reperfusion. The IRC group exhibited a 16% increase in LOOH levels compared with the C group. Rahmani et al. (2018) reported that curcumin prevented the formation of lipid peroxides. In the present study, curcumin treatment in the IRT group resulted in LOOH levels that were similar to the C group.

Reduced glutathione is a critical indicator of antioxidant capacity (Franco & Cidlowski, 2012; Ma et al., 2014), playing a key role in defense mechanisms against oxidative stress, acting as a cofactor of glutathione peroxidase (GPx), and participating in the elimination of lipid hydroperoxides (Karabulut et al., 2006). Ma et al. (2014) subjected rats to 45 min. of intestinal ischemia followed by 90 min. of reperfusion and found low GSH levels in the liver. Similarly, in the present study, GSH levels decreased by 19% in the IRC group compared with the C group, but this change was not statistically significant. The SC group exhibited a significant 36% decrease in GSH levels compared with the C group. Curcumin is able to modulate GSH levels (Simioni et al., 2018), which may explain why GSH levels were similar between the ST and IRT groups and the C group.

Glutathione *S*-transferase is an enzyme that has many functions in the body. In addition to having antioxidant activity and being responsible for the detoxification of xenobiotic compounds (Khurana et al., 2002), GST activity has been related to injury that is caused by I/R (Platz et al., 1997; Schön et al., 1997). We found that GST activity in the liver significantly increased by 38% in the IRC group compared with the C group. Curcumin improves the function of detoxifying enzymes, such as GST (Rahmani et al., 2018). In the present study, the IRT group exhibited GST activity that was similar to the C group.

In the present study, the kidneys presented few alterations of the measured parameters. Significant alterations may have been more evident during an acute period of reperfusion. Kiliç et al. (2012) reported

various changes in the rabbit kidneys, such an increase in LOOH levels and MPO activity and a decrease in SOD activity, during an acute reperfusion time of 3h. In the present study, the effects of intestinal I/R were evaluated after 7 days of reperfusion. We found that MPO and SOD activity and GSH and LOOH levels were not significantly different between groups. However, the weight of the kidneys was reduced by 12% and 15% in the SC and IRC groups, respectively. This led us to infer that the lower kidney weight in these groups resulted from injury that was caused by intestinal I/R during the acute period of injury and that the kidneys recovered from these deleterious alterations of biochemical parameters after 7 days of reperfusion. Importantly, however, curcumin treatment also had beneficial effects on the kidneys, in which kidney weight was similar between the ST and IRT groups and C group. Previous studies indicated that after 24h of reperfusion following intestinal ischemia injury, some parameters, such as histopathological changes in the intestine and liver, were restored (Lindeström & Ekblad, 2004; Marosti et al., 2015; Stringa et al., 2012). Similarly, in the present study, AST and ALT levels were not significantly different between groups. One possibility is that only oxidative and inflammatory damage persisted after 7 days of reperfusion.

As a consequence of oxidative and inflammatory damage that intestinal I/R can trigger, systemic inflammatory response syndrome and multiple organ failure can occur (Pacher, Nivorozhkin, & Szabó, 2006; Stallion et al., 2005). Tian et al. (2016) reported that curcumin pretreatment effectively promoted the maintenance of intestinal barrier integrity and prevented pathological damage that were associated with intestinal I/R injury. Curcumin treatment at a dose of 60 mg kg<sup>-1</sup> started 5 days before surgery and was maintained daily for up to 7 days later. This treatment regimen exerted antiinflammatory, antioxidant, and hepatoprotective effects and possibly renoprotective effects in preventing damage that was caused by intestinal I/R.

## Conclusion

We found that intestinal I/R mainly affected the liver, promoting inflammation and oxidative stress. At a later time of reperfusion (7 days), oxidative and inflammatory damage persisted, and the kidneys appeared to undergo repair much sooner than the liver. Treatment with curcumin had beneficial effects, ameliorating or even preventing injury that was caused by intestinal I/R in the liver and kidneys in rats.

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