Research, Society and Development, v. 9, n. 11, e1519119660, 2020
(CC BY 4.0) | ISSN 2525-3409 | DOI: http://dx.doi.org/10.33448/rsd-v9i11.9660

Remote organs respond differently to curcumin treatment after intestinal ischemia/reperfusion injury

Órgãos remotos respondem de maneira diferente ao tratamento com curcumina após lesão de isquemia/reperfusão intestinal

Los órganos remotos responden de manera diferente al tratamiento con curcumina después de una lesión por isquemia/reperfusión intestinal

Received: 10/26/2020 | Reviewed: 10/30/2020 | Accept: 11/04/2020 | Published: 11/08/2020

Elouisa Bringhentti
ORCID: https://orcid.org/0000-0003-3249-3199
Universidade Estadual de Maringá, Brasil
E-mail: elouisa_bringhentti@hotmail.com
Stephanie Carvalho Borges
ORCID: https://orcid.org/0000-0002-2534-143X
Universidade Estadual de Maringá, Brasil
E-mail: stephaniecarvalhoborges@hotmail.com
Camila Quaglio Neves
ORCID: https://orcid.org/0000-0002-8466-4984
Universidade Estadual de Maringá, Brasil
E-mail: camila_neves_76@hotmail.com
Nilza Cristina Buttow
ORCID: https://orcid.org/0000-0002-7228-7990
Universidade Estadual de Maringá, Brasil
E-mail: ncbuttow@uem.br

Abstract

We aimed investigate the effects of 45 min of ischemia followed by 72 h of intestinal reperfusion (IR) in the ileum, liver, lungs, and kidneys in *Wistar* rats and the responses of these organs to curcumin treatment. Ischemia was induced by occluding the superior mesenteric artery. Rats were treated orally with 40 mg/kg curcumin. We analyzed oxidative stress and inflammation in the ileum, liver, lungs, and kidneys. Intestinal IR led to a reduction

of reduced glutathione levels in the intestine, lungs, and kidneys and increased lipid hydroperoxide levels in all organs. An increase in the enzymatic activity of catalase was observed in all organs, and an increase in superoxide dismutase activity was observed in the ileum and lungs. Glutathione *s*-transferase levels increased only in the kidneys. Myeloperoxidase increased in all four organs, and n-acetyl-glycosaminidase increased only in the ileum and lungs. Curcumin prevented all of the changes in the ileum and liver. In the lungs, curcumin had no effect on n-acetyl-glycosaminidase. Curcumin did not prevent the changes in reduced glutathione, lipid hydroperoxides, or myeloperoxidase in the kidneys. Intestinal IR caused oxidative stress and inflammation in the ileum, lungs, and kidneys and to a lesser degree in the liver. Because of its systemic distribution, curcumin prevented changes mainly in the ileum, lungs, and liver and to a lesser degree in the kidneys.

Keywords: Oxidative stress; Inflammation; Superior Mesenteric Artery.

Resumo

Nosso objetivo foi investigar os efeitos de 45 min de isquemia seguidos por 72 h de reperfusão intestinal (IR) no íleo, fígado, pulmões e rins em ratos *Wistar* e as respostas desses órgãos ao tratamento com curcumina. A isquemia foi induzida pela oclusão da artéria mesentérica superior. Os ratos foram tratados por via oral com 40 mg/kg de curcumina. Analisamos o estresse oxidativo e a inflamação no íleo, fígado, pulmões e rins. A IR intestinal levou a uma redução dos níveis reduzidos de glutationa no intestino, pulmões e rins e aumentou os níveis de hidroperóxidos lipídicos em todos os órgãos. Um aumento na atividade enzimática da catalase foi observado em todos os órgãos, e um aumento na atividade da superóxido dismutase foi observado no íleo e nos pulmões. Os níveis de glutationa s-transferase aumentaram apenas nos rins. A mieloperoxidase aumentou em todos os quatro órgãos e a n-acetil-glicosaminidase aumentou apenas no íleo e nos pulmões. A curcumina evitou todas as alterações no íleo e no fígado. Nos pulmões, a curcumina não teve efeito sobre a n-acetil-glicosaminidase. A curcumina não preveniu as mudanças na redução da glutationa, hidroperóxidos lipídicos ou mieloperoxidase nos rins. A IR intestinal causou estresse oxidativo e inflamação no íleo, nos pulmões e nos rins e em menor grau no fígado. Devido à sua distribuição sistêmica, a curcumina evitou alterações principalmente no íleo, pulmões e fígado e, em menor grau, nos rins.

Palavras-chave: Estresse oxidativo; Inflamação; Artéria Mesentérica Superior.

Resumen

Nuestro objetivo fue investigar los efectos de 45 min de isquemia seguidos de 72 h de reperfusión intestinal (IR) en el íleon, hígado, pulmones y riñones en ratas Wistar y las respuestas de estos órganos al tratamiento con curcumina. La isquemia se indujo ocluyendo la arteria mesentérica superior. Las ratas se trataron con 40 mg / kg de curcumina. Analizamos el estrés oxidativo y la inflamación. La IR intestinal produjo una reducción de los niveles de glutatión en el intestino, los pulmones y los riñones y un aumento de los niveles de hidroperóxido de lípidos en todos los órganos. Se observó un aumento de la actividad enzimática de la catalasa en todos los órganos y un aumento de la actividad de la superóxido dismutasa en el íleon y los pulmones. Los niveles de glutatión s-transferasa aumentaron solo en los riñones. La mieloperoxidasa aumentó en los cuatro órganos y la n-acetil-glicosaminidasa aumentó sólo en el íleon y los pulmones. La curcumina previno todos los cambios en el íleon y el hígado. En los pulmones, la curcumina no tuvo ningún efecto sobre la n-acetil-glicosaminidasa. La curcumina no previno los cambios en el glutatión reducido, los hidroperóxidos de lípidos o la mieloperoxidasa en los riñones. La IR intestinal provocó estrés oxidativo e inflamación en el íleon, los pulmones y los riñones y, en menor grado, en el hígado. Debido a su distribución sistémica, la curcumina previno cambios principalmente en el íleon, pulmones e hígado y en menor grado en los riñones.

Palabras clave: Estrés oxidativo; Inflamación; Arteria Mesentérica Superior.

1. Introduction

Intestinal ischemia is characterized by a decrease in or suspended blood supply to an organ that is caused by trauma or pathological processes, such as mesenteric embolism, thrombosis, hernia, hemorrhagic shock, hypovolemic shock, infection, abdominal angina, and small bowel transplantation (Mallick et al., 2004). Although its incidence is relatively low (0.09-0.02% of admissions to emergency units), its mortality rate is high (50-80%) (Acosta & Björck, 2003; Stoney & Cunningham, 1993). Ischemia causes a metabolic imbalance in tissue that can lead to tissue damage. However, these lesions are aggravated at the moment blood flow is reestablished (i.e., reperfusion) (Stallion et al., 2002). The consequences of intestinal ischemia/reperfusion (IR) include the accentuated production of reactive oxygen species (ROS), mainly by the enzyme xanthine oxidase, which transforms metabolic products into free radicals, generates oxidative stress in tissue, and overloads endogenous antioxidant systems (McCord, 1985).

Enteric neuronal loss and morphological changes are among the complications of intestinal IR, which affect essential functions of the gastrointestinal tract, including motility (Hakgüder et al., 2002). Moreover, IR causes the infiltration and activation of immune system cells, such as neutrophils and macrophages, and the production of nitric oxide (NO) in the intestine, thus characterizing the inflammatory process in the organ (Paterno & Longo, 2008). The intestinal epithelial barrier is disturbed during ischemia, a condition that causes bacterial translocation. At this point, the immune system is activated, causing systemic inflammation that can result in multiple organ failure, such as the lungs, liver, and kidneys (Vinardi et al., 2003). Therefore, because of the potential severity of IR, it is clinically important to discover substances that can be used as therapies to prevent IR-induced damage.

Curcumin is a polyphenolic compound that is derived from *Curcuma longa* with antioxidant and anti-inflammatory properties. It is a natural substance with potential to mitigate damage after ischemic disease (Fan et al., 2014; Guzel et al., 2013; Lin, 2007). Previous studies evaluated the effects of 100 and 200 mg/kg curcumin on intestinal IR in the intestine and other organs, but the reperfusion times in these studies ranged from 60 to 120 min (Fan et al., 2014; Guzel et al., 2013). According to Lindeström and Ekblad (Lindeström & Ekblad, 2004), changes that are caused by intestinal IR may continue to occur over a longer reperfusion time.

Studying longer reperfusion period may help to understand how intestinal IR impairs distant organs. For this, several parameters must be analyzed. However, studies have analyzed a few parameters (Guzel et al., 2013; Onder et al., 2012) and this makes it difficult to understand the mechanism of the disease. Considering that several organs are being studied, we search for an antioxidant that, in low dose, has a positive effect on all of them.

Therefore, to achieve a better understanding of the degree of injury that affects each organ after 45 min of ischemia and 72 h of intestinal reperfusion, we analyzed several parameters of oxidative and inflammatory stress in the ileum, liver, lungs, and kidneys. We also explored the ability of 40 mg/kg curcumin to prevent intestinal IR-induced damage in these organs.

2. Methodology

The research was carried out in the Animal Histology laboratory of the Department of Morphological Sciences (DCM) of the State University of Maringá. All analyzes performed in this experiment are quantitative in nature (Pereira et al., 2018).

2.1 Animals

The experimental procedures were approved by the Commission of Ethics in the Use of Animals of the State University of Maringá (CEUA no. 8219300317). Thirty male Wistar rats (*Rattus norvegicus*), weighing 264 ± 3.708 g, were used. The male rats were obtained from the Central Bioterium of the State University of Maringá and housed in the Animal Room of the Department of Morphological Sciences, State University of Maringá, under a 12 h/12 h light/dark cycle and 22°C ± 2°C. Female rats were excluded from the experiment because hormonal influence may alter the results. The male rats were fed standard chow (Nuvilab) and water *ad libitum*. We divided the animals into five groups (n = 6/group; Table 1): Control (not operated) (C), Control *sham* (SC), Treated *sham* (ST), Ischemic control (vehicle) (IC) and Ischemic treated (IT).

Table 1. Experimental groups and treatments.

Groups			SMA[1]	Treatment
C	Control	Not operated	-	Vehicle
SC	Control *sham*	Underwent surgery	Not occluded	Vehicle
ST	Treated *sham*	Underwent surgery	Not occluded	Curcumin
IC	Ischemic control	Underwent surgery	Occluded	Vehicle
IT	Ischemic treated	Underwent surgery	Occluded	Curcumin

[1]SMA Superior Mesenteric Artery. Source: Authors.

The experimental groups were divided according to the occlusion or not of the superior mesenteric artery and according to the type of treatment received (vehicle or curcumin), as seen in table 1. Groups surgery operated

2.2 Ischemia and intestinal reperfusion induction

After a 15-h fast, the animals were anesthetized with ketamine (100 mg/kg body weight; Ceva Saúde Animal, Paulínia, SP, Brazil) and xylazine (20 mg/kg body weight; Ceva

Saúde Animal Ltda, Paulínia, SP, Brazil) intramuscularly. All of the groups except for the control group underwent abdominal laparotomy in the midline. The IC and IT groups were subjected to intestinal ischemia by occluding the superior mesenteric artery using a microvascular clamp and lateral irrigation between the ischemic and non-ischemic regions was blocked by loops lashings. Blood flow was obstructed for 45 min. The clamp and lashings were then removed, thus initiating the reperfusion period, which lasted 72 h. The SC and ST groups only underwent anesthesia, laparotomy, and the intestinal manipulation procedures and were not subjected to superior mesenteric artery occlusion. The abdomen was sutured with 3-0 nylon thread. Saline solution was heated to 37°C and used to hydrate the organ and maintain the animals' internal temperature during the surgical procedure.

2.3 Treatment with curcumin

Treatment was performed via gavage, 30 min before ischemia induction and daily during the reperfusion time in the morning. The last dose was administered 2 h before euthanasia. The ST and IT groups received curcumin (*Curcuma longa*, Sigma Aldrich, Darmstadt, Germany), dissolved in corn oil, at a dose of 40 mg/kg body weight. The C, SC, and IC groups received only corn oil, following the same design as the groups treated with curcumin

2.4 Euthanasia and sample collection

The animals received a lethal dose of thiopental sodium (120 mg/kg body weight; Cristália - Produtos Químicos Farmacêuticos, São Paulo, Brazil) intraperitoneally. We harvested the kidneys, lungs, liver, and distal portion of the ileum and distributed them for the oxidative stress and inflammatory analyses. We placed the tissue samples in liquid nitrogen and kept them in a freezer at -80ºC. We also collected a portion of the ileum for nitrite quantification.

2.5 Biochemical analyses

2.5.1 Sample preparation

The samples underwent the experimental protocols similarly to Borges et al (Borges et al., 2018). The samples were crushed and homogenized in 200 mM potassium phosphate

buffer, pH 6.5. A portion of the samples was separated to quantify the levels of reduced glutathione (GSH). Subsequently, the material was centrifuged at 9,000 × *g* for 20 min. The resulting supernatant was allocated to determinations of the levels of catalase (CAT), superoxide dismutase (SOD), glutathione *S*-transferase (GST), and lipid hydroperoxides (LOOH). The pellet was used to evaluate the enzymatic activity of n-acetyl-glycosaminidase (NAG) and myeloperoxidase (MPO). All of the reactions were performed in a 96-well plate and read in a spectrophotometer.

2.5.2 Oxidative stress

Reduced glutathione levels were evaluated after protein precipitation with trichloroacetic acid and reaction with 5,5'-dithiol-2-nitrobenzoic acid. Values that were read at 412 nm were interpolated on a standard GSH curve. The results are expressed as μg GSH/g tissue.

The enzymatic activity of CAT was based on the Aebi (Aebi, 1984) method, which evaluates the degradation of hydrogen peroxide by CAT. Absorbance was set at 240 nm, and the results are expressed as μmol/min/mg protein. We used the Marklund and Marklund (Marklund & Marklund, 1974) method for determination of the enzymatic activity of SOD. The protocol was based on the self-oxidation of pyrogallol that can be inhibited by SOD. Absorbance was set at 405 nm, and the results are expressed as U of SOD/mg protein.

The activity of GST was evaluated according to Warholm et al. (Warholm et al., 1985) using a GSH-containing assay. Absorbance was set at 340 nm, and an extinction coefficient of 9.6 mmolar 1/cm was used. To determine LOOH levels, the samples were evaluated by iron II oxidation in the presence of xylenol orange (Jiang et al., 1991). The results are expressed as mmol/mg of tissue after using the extinction coefficient of 4.3 mmolar 1/cm. Absorbance was set at 560 nm.

2.5.3 Inflammation

The enzymatic activity of MPO and NAG was assessed by placing the pellet in 0.08 M potassium phosphate buffer with hexadecyltrimethylammonium (pH 5.4). The samples were then centrifuged at 11,000 × *g* for 20 min. The MPO reaction occurred in the presence of tetramethylbenzidine and hydrogen peroxide. Absorbance was set at 620 nm. The activity of NAG was evaluated in the presence of 50 mM citrate buffer (pH 4.5) and a solution of 4-

nitrophenyl N-acetyl-β-D-glucosamine at 2.24 mM. After incubation, the reaction was interrupted with 200 mM glycine buffer (pH 10.4), and readings were performed at 405 nm. The results are expressed as optical density (OD)/min/mg of protein for both analyses.

2.5.4 Nitrite

We used the Griess reaction to indirectly assess NO by quantifying its byproduct, nitrite, a method that was adapted from Tiwari et al (Tiwari et al., 2011). The ileum was homogenized in 0.1 M sodium phosphate buffer (pH 7.4), and the material was centrifuged at 3,000 × *g* for 10 min. The reaction was performed with the addition of phosphoric acid, sulfanilamide, and n-1-naphthalylethylenediamide to the sample in a 96-well plate. We performed readings at 540 nm in a spectrophotometer. Nitrite values were evaluated relative to the standard curve of dilutions of sodium nitrite. The results are expressed as μM.

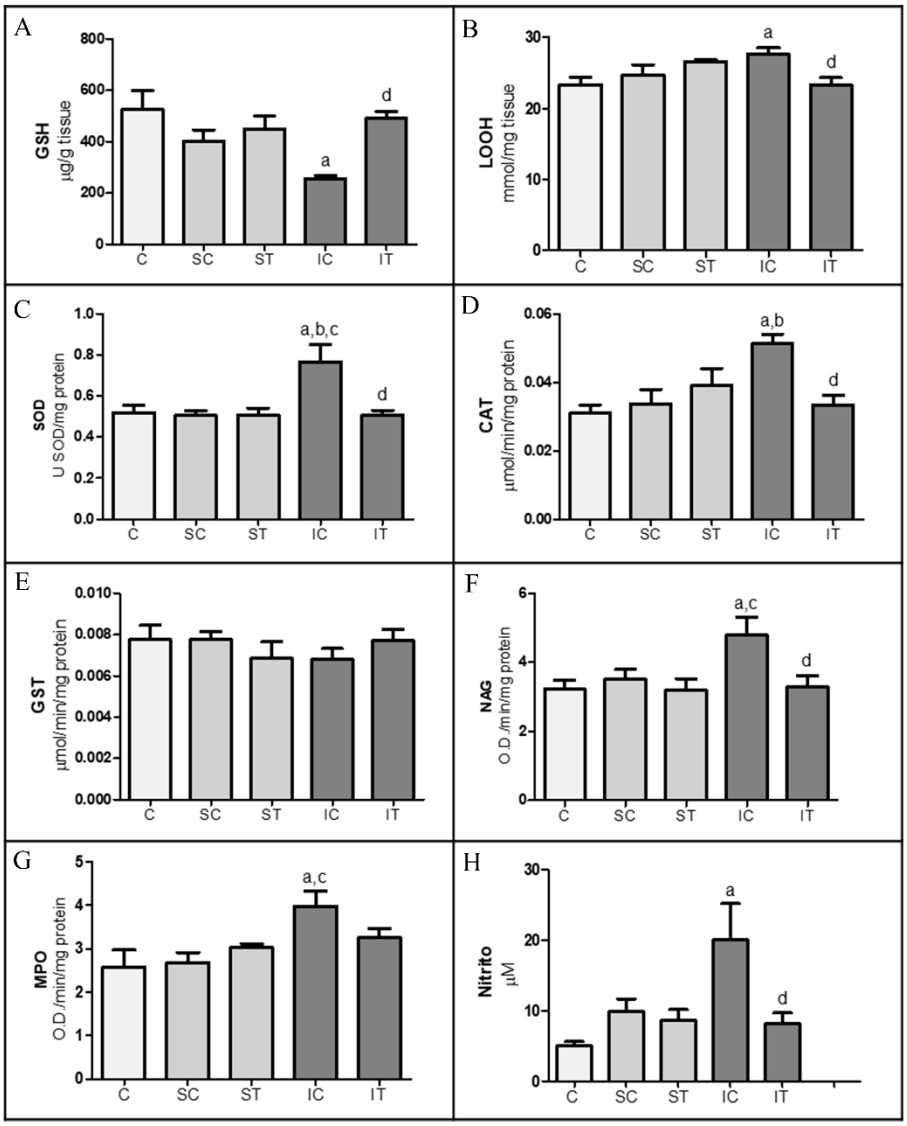
2.6 Statistical analysis

Data with a normal distribution were analyzed using one-way analysis of variance (ANOVA), followed by *Tukey's* post hoc test. Data that did not present a normal distribution were analyzed using the nonparametric *Kruskal-Wallis* test, followed by *Dunns* post hoc test. GraphPad Prism 5 software was used to perform the statistical analyses. The results are expressed as mean ± SE. Values of $p < 0.05$ were considered statistically significant.

3. Results

Intestinal IR caused changes in the oxidative state of the four organs that were studied. Animals in the IC group presented a decrease in GSH levels in the ileum ($p < 0.01$; Figure 1A), lungs ($p < 0.05$; Figure 3A), and kidneys ($p < 0.01$) compared with controls. An increase in LOOH levels was also observed in the ileum ($p < 0.05$; Figure 1B), liver ($p < 0.01$; Figure 3B), and kidneys ($p < 0.01$; Figure 4B).

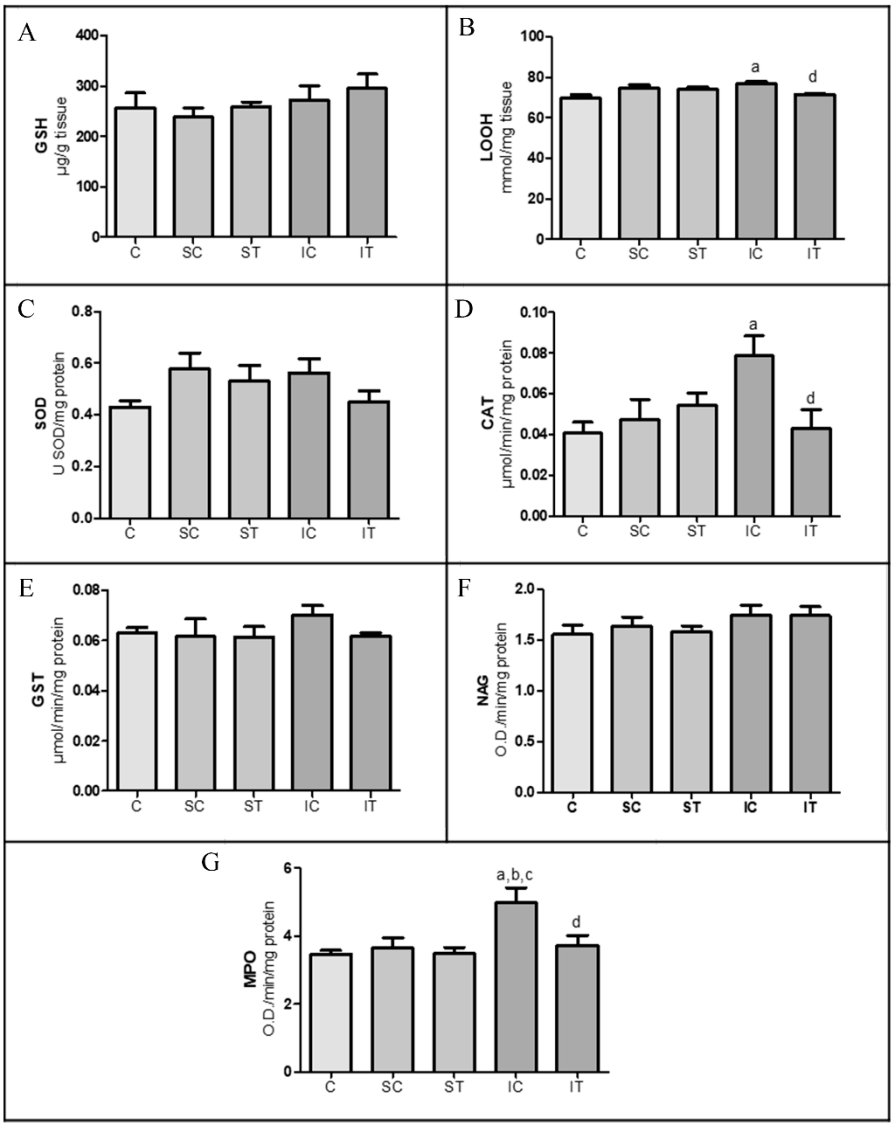
Figure 1 - Biochemical parameters of the ileum.



Levels of glutathione (GSH) (A) and lipid hydroperoxides (LOOH) (B). Enzymatic activity of superoxide dismutase (SOD) (C), catalase (CAT) (D), glutathione S-transferase (GST) (E), n-acetyl-glycosaminidase (NAG) (F) and myeloperoxidase (MPO) (G). Levels of nitrite (H). a = Significant difference from control group (C). b = Significant difference from sham control group (SC). c = Significant difference from treated sham group (ST). D = Significant difference from ischemic control group (IC). The results represent the mean ± standard error (n = 6). Source: Authors.

Comparison of inflammatory parameters MPO and NAG, oxidative stress parameters (SOD, CAT, GSH, LOOH, and GST) and nitrite in the ileum of rats after 45 minutes of intestinal ischemia and 72 hours of reperfusion, as seen in Figure 1.

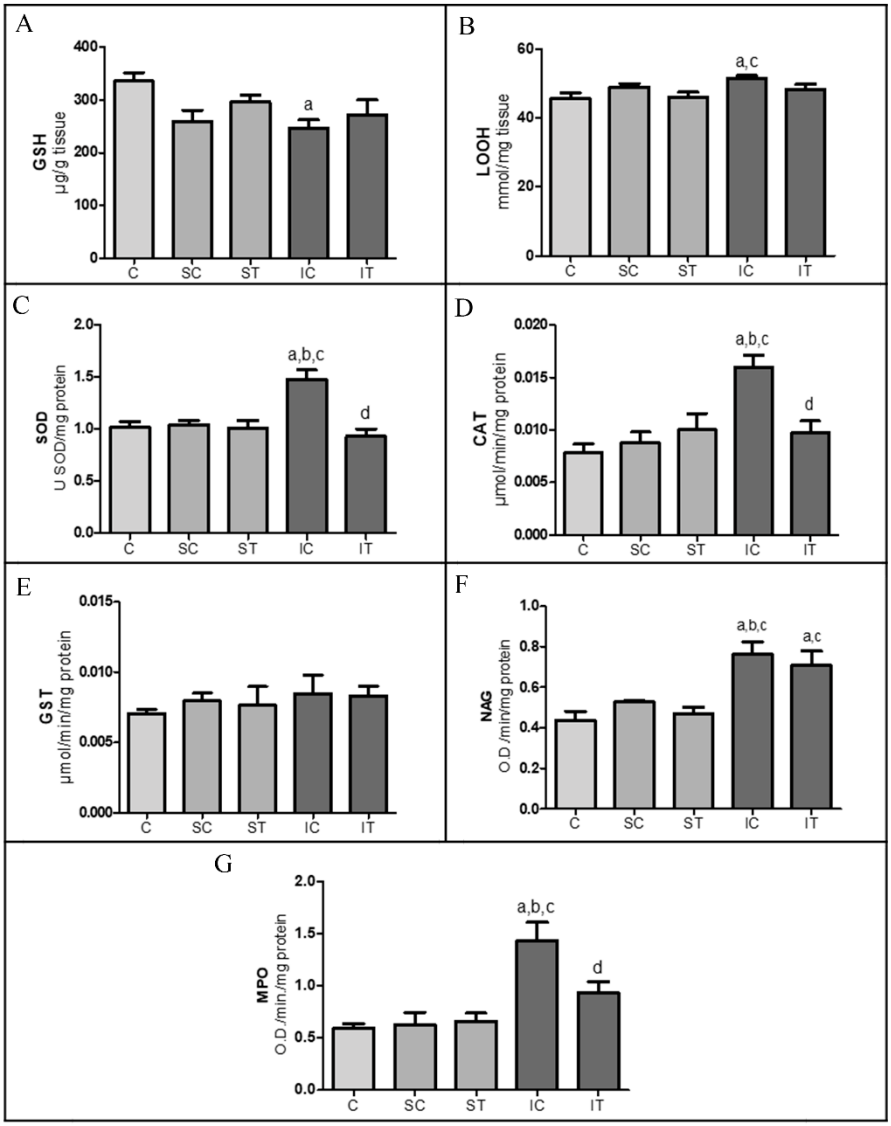
Figure 2 - Biochemical parameters of the liver.



Levels of reduced glutathione (GSH) (A) and lipid hydroperoxides (LOOH) (B). Enzymatic activity of superoxide dismutase (SOD) (C), catalase (CAT) (D), glutathione S-transferase (GST) (E), n-acetyl-glycosaminidase (NAG) (F) and myeloperoxidase (MPO) (G). a = Significant difference from control group (C). b = Significant difference from sham control group (SC). c = Significant difference from treated sham group (ST). d = Significant difference from ischemic Control group (IC). The results represent the mean ± standard error (n = 6). Source: Authors.

Comparison of inflammatory parameters MPO and NAG, oxidative stress parameters (SOD, CAT, GSH, LOOH, and GST) in the liver of rats after 45 minutes of intestinal ischemia and 72 hours of reperfusion, as seen in Figure 2.

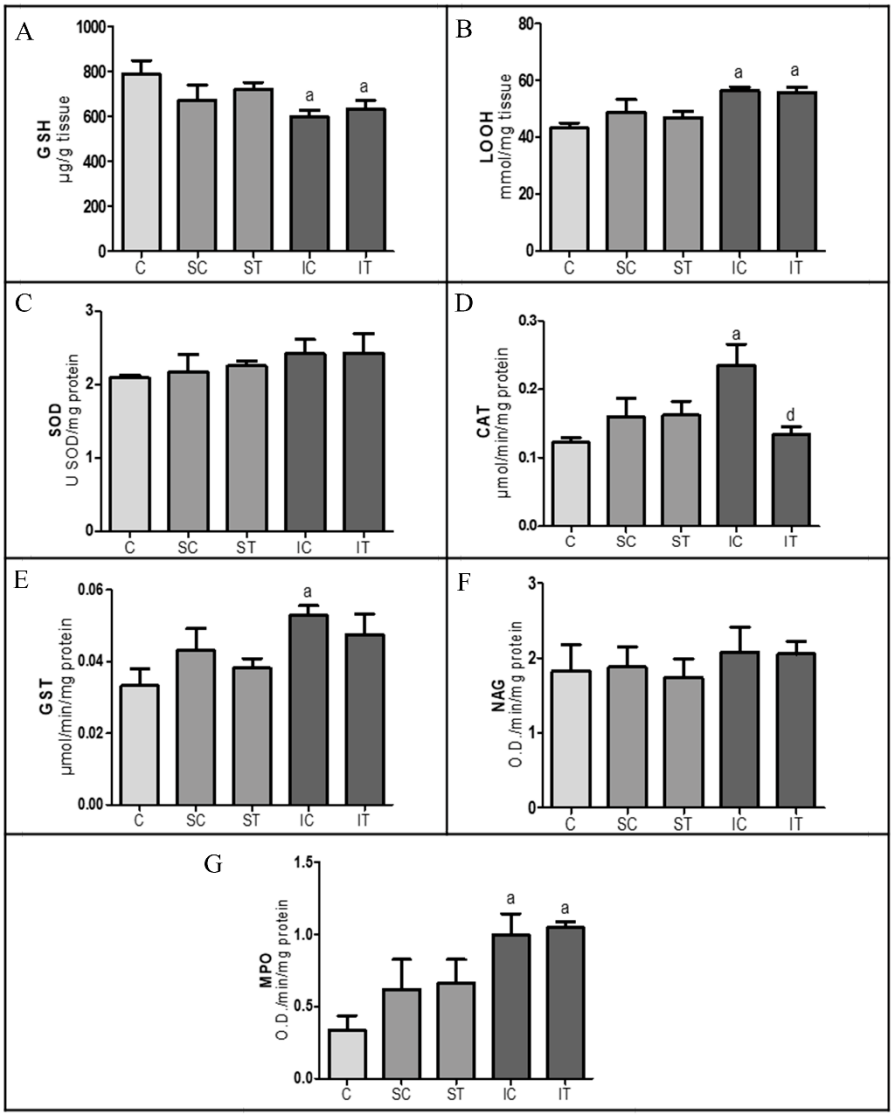
Figure 3 - Biochemical parameters of the lung.



Levels of reduced glutathione (GSH) (A), and lipid hydroperoxides (LOOH) (B). Enzymatic activity of superoxide dismutase (SOD) (C), catalase (CAT) (D), glutathione S-transferase (GST) (E), n-acetyl-glycosaminidase (NAG) (F) and myeloperoxidase (MPO) (G). a = Significant difference from control group (C). b = Significant difference from sham control group (SC). c = Significant difference from treated sham group (ST). d = ST from ischemic control group (IC). The results represent the mean ± standard error (n = 6). Source: Authors.

Comparison of inflammatory parameters MPO and NAG, oxidative stress parameters (SOD, CAT, GSH, LOOH, and GST) in the lung of rats after 45 minutes of intestinal ischemia and 72 hours of reperfusion, as seen in Figure 3.

Figure 4 - Biochemical parameters of the kidney.



Levels of reduced glutathione (GSH) (A) and lipid hydroperoxides (LOOH) (B). Enzymatic activity of superoxide dismutase (SOD) (C), catalase (CAT) (D), glutathione S-transferase (GST) (E), n-acetyl-glycosaminidase (NAG) (F) and myeloperoxidase (MPO) (G). a = Significant difference from control group (C). b = Significant difference from sham control group (SC). c = Significant difference from treated sham group (ST). d = Significant difference from ischemic control group (IC). The results represent the mean ± standard error (n = 6). Source: Authors.

Comparison of inflammatory parameters MPO and NAG, oxidative stress parameters (SOD, CAT, GSH, LOOH, and GST) in the kidney of rats after 45 minutes of intestinal ischemia and 72 hours of reperfusion, as seen in Figure 4.

A 47% increase in the enzymatic activity of SOD was observed in the ileum (Figure 1C), and a 45% increase in SOD activity was observed in the lungs (Figure 3C) as a compensatory response to oxidative stress that was caused by intestinal IR. Catalase activity increased by 65% in the ileum (Figure 1D), 93% in the liver (Figure 2D), 102% in the lungs (Figure 3D), and 91% in the kidneys Figure 4D) in the IC group compared with controls.

Changes in GST activity were observed only in the kidneys, in which an increase was observed in the IC group ($p < 0.01$; Figure 4E).

In addition to alterations of the oxidative state, intestinal IR caused an inflammatory response, reflected by a 49% increase in NAG activity in the ileum (Figure 1F) and a 74% increase in NAG activity in the lungs (Figure 3F) in the IC group compared with controls. Myeloperoxidase activity increased by 54% in the ileum (Figure 3G), 43% in the liver, 140% in the lungs, and 198% in the kidneys (Figure 4G) in the IC group compared with controls. Nitrite levels, an indirect measure of NO production, increased by 300% in the ileum in the IC group compared with controls (Figure 1H).

Treatment with curcumin in the IT group maintained GSH and LOOH at levels that were similar to the C group in the ileum (Figure 1A, B), liver (Figure 2A, B), and lungs (Figure 3A, B). Curcumin treatment did not prevent the decrease in GSH levels ($p < 0.05$) and did not prevent the increase in LOOH levels ($p < 0.05$) in the kidneys, in which these levels were still significantly different from controls (Figure 4A, B). Curcumin treatment also maintained the enzymatic activity of SOD, CAT, and GST at levels that were similar to the C group in the four organs that were studied. The enzymatic activity of NAG in the curcumin-treated group was similar to controls in the ileum (Figure 1F), liver (Figure 2F), and kidneys (Figure 4F), but a significant difference was found in the lungs ($p < 0.01$; Figure 3F). Myeloperoxidase activity was similar to controls in the ileum (Figure 1G), liver (Figure 2G), and lungs (Figure 3G), but a significant difference was found in the kidneys ($p < 0.05$; Figure 4G). Nitrite levels in the ileum were similar to controls (Figure 1H).

4. Discussion

The present study found that 45 min of intestinal ischemia followed by 72 h of reperfusion caused oxidative stress in the different organs that were studied. Treatment with 40 mg/kg curcumin mitigated these detrimental effects. We found different degrees of alterations of oxidative stress parameters. This damage can be explained by a sequence of biochemical reactions that occur after ischemia and throughout the intestinal reperfusion period. Ischemia that is followed by reperfusion triggers a series of biochemical processes that result in the production of the superoxide anion ($O2^{\bullet-}$) (Chung et al., 1997; Lee et al., 2014). This is a highly deleterious ROS for cells (Thomas et al., 1985) that can be converted to hydrogen peroxide (H_2O_2) through the Fenton reaction, whereby it is transformed into the hydroxyl radical (OH^-) (Lee et al., 2014), which can cause lipid peroxidation of the cell

membrane. We observed a significant increase in LOOH formation, a byproduct of lipid peroxidation, in the four organs that were studied. Increases in lipid peroxidation have been reported after intestinal IR in such organs as the intestine (Turan et al., 2017), liver (Saidi et al., 2017), and lungs (Barut et al., 2016). Despite these deleterious effects of IR in the ischemic group in the present study, the levels of LOOH in the ileum, lungs, and liver in the IT group that was treated with curcumin remained similar to the control group. This effect is likely attributable to the antioxidant activity of curcumin, which is related to its chemical structure. Curcumin can transfer electrons from its phenolic rings (Jankun et al., 2016), neutralizing ROS and preventing their reactions with cellular constituents. It can also reduce the production of $O2^{\bullet-}$ (Lin, 2007).

Among other oxidative stress alterations, we observed an increase in the activity of the antioxidant enzymes SOD, CAT, and GST after intestinal IR. This occurred as a compensatory mechanism against the increase in free radical production when blood flow was reestablished (i.e., reperfusion). This condition overloads endogenous antioxidant defense mechanisms, altering enzymatic activity and aggravating organ damage (Nordberg & Arnér, 2001; Parks & Granger, 1988). Several authors reported such changes in antioxidant enzymes after intestinal IR in the intestine (Akinrinmade et al., 2015; Demir et al., 2014), liver (Fan et al., 2014), lungs (Guzel et al., 2013), and kidneys (Kiliç et al., 2012) with shorter reperfusion times (i.e., 60 and 120 min). Curcumin treatment at doses of 100 and 200 mg/kg modulated the activity of antioxidant enzymes during oxidative stress after intestinal IR in the liver (Fan et al., 2014), lungs (Guzel et al., 2013), and intestine (Onder et al., 2012). Treatment with 40 mg/kg curcumin maintained the levels of SOD and CAT at values that were similar to the control group in all of the organs. These results indicate that even a lower dose of curcumin can have beneficial effects.

In addition to oxidant enzymes, GSH, a non-protein thiol, plays a key role in protecting against oxidative stress, in which it reacts with ROS or participates as a cofactor of antioxidant enzymes (Jones, 2002). In the present study, GSH decreased in the ileum, lungs, and kidneys in the control ischemic group. Akinrinmade et al. (Akinrinmade et al., 2015) also reported a decrease in GSH levels in the intestine 45 min after intestinal IR. Barut et al. (Barut et al., 2016) reported a reduction of GSH levels in the lungs after intestinal ischemia followed by 3 h of reperfusion. In the present study, curcumin treatment maintained GSH levels at values that were similar to controls, especially in the ileum. Previous studies have shown that curcumin increases GSH biosynthesis, mainly by modulating expression of the glutamate cysteine ligase (GCLC) gene (Lin, 2007).

Intestinal IR also triggers inflammatory processes in both the ileum and other affected organs. This can be measured by assessing the activity of two enzymes, MPO and NAG. The enzymatic activity of MPO reflects the accumulation of active neutrophils in tissue and is often used as a marker of inflammatory processes (Faith et al., 2008). Myeloperoxidase is expressed mainly in azurophil granules of neutrophils, catalyzing the reaction of hydrogen peroxide with chloride and forming free radicals and oxidizing substances with antimicrobial actions. However, these substances also contribute to an increase in oxidative stress and tissue injury (Aratani, 2018). The lysosomal enzyme NAG is produced mainly by macrophages, and its activity can be assessed to indirectly evaluate the activation of macrophages (Lamaita et al., 2012). In the present study, we observed an increase in MPO and NAG activity, which may be justified by the loss of integrity of the intestinal epithelial barrier. Previous studies showed that 45 min of mesenteric ischemia was related to an increase in intestinal permeability (Grootjans et al., 2010). This causes the translocation of bacterial products into the bloodstream. These substances interact with membrane receptors on immune cells, resulting in a signaling overload that activates transcription factors, such as nuclear factor-κB (NF-κB), leading to the production of proinflammatory cytokines, such as tumor necrosis factor α, interleukins, and chemokines (Iadecola & Anrather, 2011). These substances activate and recruit innate and adaptive immune cells, such as neutrophils and macrophages, to the damaged tissue, producing an inflammatory response that intensifies tissue damage (Chen & Nuñez, 2010). Although we did not evaluate these transcription factors and the immune response, we found that, in our experiment, curcumin treatment was able to maintain MPO activity in the ischemic group similar to the control group in all studied organs. Increases in NAG were observed only in the lungs and ileum, and NAG activity remained similar to the control group only in the ileum. This improvement that was afforded by curcumin treatment is likely attributable to its ability to reduce the infiltration of immune cells, including neutrophils and macrophages, into tissues under inflammatory conditions (Ukil et al., 2003) by inhibiting NF-κB transcription (Lin, 2007).

Another important inflammatory mediator is NO, which we measured only in the ileum. To perform this evaluation, we used an indirect method of measuring nitrite levels (Tiwari et al., 2011). We observed a considerable increase in nitrite levels in the ischemic group. Nitric oxide is produced by three different isoforms of the enzyme nitric oxide synthase (NOS): neuronal NOS (nNOS), endothelial NOS (eNOS), and inducible NOS (iNOS) (Cuzzocrea et al., 2002). Intestinal IR-induced lesions have been mainly associated with an increase in iNOS production through the activation of NF-κB, which is responsible

for inducing iNOS transcription through inflammatory mediators (Montalto et al., 2003). Nitric oxide that is produced by iNOS activation has cytotoxic effects that result from its direct action on cellular constituents or from its reaction with compounds that are released during the inflammatory process. Activated cells, such as macrophages, neutrophils, and endothelial cells, simultaneously release NO and ROS-producing enzymes. In the present study, curcumin prevented the increase in NO production in the ileum, likely because of its ability to inhibit NF-κB, preventing iNOS activation (Lin, 2007).

Hepatic injury that is caused by intestinal IR is frequently observed, mainly because the liver and intestine share a common anatomical pathway (i.e., the portal vein), through which substances that are absorbed by the intestine are transported to the liver for metabolism. As a result, occlusion of the mesenteric artery reduces blood flow in the liver, producing significant metabolic changes (Horie et al., 1996). However, the liver has a substantial metabolic reserve capacity that makes changes in the liver initially undetectable, thus explaining why the liver was less affected in the present study with a 72-h reperfusion time. We found no alterations of NAG activity in the liver with this time of reperfusion. However, we observed a significant increase in MPO, which is related to neutrophil infiltration. We observed more significant alterations of the lungs that were caused by intestinal IR. In the lungs, respiratory failure is common after ischemia. The lungs are also highly susceptible to the systemic inflammatory response because they are highly vascularized. Intestinal IR can promote lung injury, especially by increasing neutrophil infiltration and activating alveolar macrophages (Börjesson et al., 2000). The significant increases in MPO and NAG activity that we observed in the lungs support this theory. Moreover, these enzymes produce ROS, which contribute to the increase in oxidative stress in the lungs, which was also found in the present study. The kidneys were also sensitive to intestinal IR but to a lesser extent than the lungs. Renal dysfunction has also been observed after intestinal IR in several studies (Aldemir et al., 2003; Fayez et al., 2014). However, the mechanisms by which renal injury occurs are still poorly understood.

We found that each of the four organs that were evaluated in the present study responded differently to intestinal IR. Unsurprisingly, their responses to curcumin treatment were also different. Such responses involve cellular metabolism, collateral circulation, and local humoral factors. We observed more pronounced antioxidant and anti-inflammatory effects of curcumin in the ileum, liver, and lungs, whereas the effect of curcumin in the kidneys was more discreet. This may be attributable to the fact that the tissue distribution of curcumin varies in different organs (Wang et al., 2018). Curcumin is metabolized in the

intestine and liver, so its concentrations are higher in these organs (Shoba et al., 1998). Marczylo et al. (Marczylo et al., 2009) measured curcumin levels in various organs using ultra-performance liquid chromatography after the oral administration of 340 mg/kg. These authors reported levels of 16.1 ng/ml in plasma, 2.0 ng/ml in urine, 1.4 mg/g in intestinal mucosa, 3671.8 ng/g in the liver, 807.6 ng/g in the heart, and 206.8 ng/g in the kidneys. Based on the findings of Marczylo et al. (Marczylo et al., 2009), curcumin levels can be expected to be lower in the kidneys relative to the intestine and liver, thus explaining why curcumin did not exert pronounced effects in this organ in the present study. Additionally, curcumin has low free bioavailability when administered orally, which may be a limiting factor in the present study. One way to overcome this limitation would be to administer curcumin in the form of nanocapsules or nanoparticles, which have controlled release and can improve its absorption by the body and thus therapeutic effects (Xu et al., 2016).

5. Final Considerations

In summary, 45 min of intestinal ischemia followed by 72 h of reperfusion caused oxidative stress and inflammation in the ileum, liver, lungs, and kidneys, characterized by alterations of oxidative and inflammatory parameters. Curcumin at the dose of 40 mg/kg exerted antioxidant and antiinflammatory activity, preventing IR-induced alterations of several parameters in different organs. The ileum, lungs, and kidneys were affected to a greater degree than the liver by intestinal IR. The effect of curcumin treatment varied depending on the organ that was studied. More pronounced effects of curcumin were observed in the ileum, lungs, and liver, likely because of its tissue distribution.

Overall, these results indicate that curcumin is effective as a treatment for injuries caused by ischemia and intestinal reperfusion. Still, it is necessary that further studies are carried out in order to elucidate the ideal dose of curcumin needed for each organ, in order to prevent the damage caused by intestinal IR.

References

Acosta, S., & Björck, M. (2003). Acute thrombo-embolic occlusion of the superior mesenteric artery: a prospective study in a well defined population. *Eur J Vasc Endovasc Surg*, *26*(2), 179–183. https://www.ncbi.nlm.nih.gov/pubmed/12917835

Aebi, H. (1984). Catalase in vitro. *Methods Enzymol*, *105*, 121–126. https://www.ncbi.nlm.nih.gov/pubmed/6727660

Akinrinmade, J. F., Akinrinde, S. A., Odejobi, A., & Oyagbemi, A. A. (2015). Evidence of attenuation of intestinal ischemia-reperfusion injury following pre-treatment with methanolic extracts from Chromolena odorata in rats. *J Complement Integr Med*, *12*(1), 23–32. https://doi.org/10.1515/jcim-2014-0034

Aldemir, D., Tufan, H., Tecder-Unal, M., Türkoğlu, S., Oğüs, E., Kayhan, Z., & Haberal, M. (2003). Age-related alterations of oxidative stress and arginase activity as a response to intestinal ischemia-reperfusion in rat kidney and liver. *Transplant Proc*, *35*(7), 2811–2815. https://www.ncbi.nlm.nih.gov/pubmed/14612127

Aratani, Y. (2018). Myeloperoxidase: Its role for host defense, inflammation, and neutrophil function. *Arch Biochem Biophys*, *640*, 47–52. https://doi.org/10.1016/j.abb.2018.01.004

Barut, F., Ozacmak, V. H., Turan, I., Sayan-Ozacmak, H., & Aktunc, E. (2016). Reduction of Acute Lung Injury by Administration of Spironolactone After Intestinal Ischemia and Reperfusion in Rats. *Clin Invest Med*, *39*(1), E15-24. https://www.ncbi.nlm.nih.gov/pubmed/26833169

Borges, S. C., Ferreira, P. E. B., da Silva, L. M., de Paula Werner, M. F., Irache, J. M., Cavalcanti, O. A., & Buttow, N. C. (2018). Evaluation of the treatment with resveratrol-loaded nanoparticles in intestinal injury model caused by ischemia and reperfusion. *Toxicology*, *396–397*. https://doi.org/10.1016/j.tox.2018.02.002

Börjesson, A., Wang, X., Sun, Z., Wallén, R., Deng, X., Johansson, E., & Andersson, R. (2000). Effects of N-acetylcysteine on pulmonary macrophage activity after intestinal ischemia and reperfusion in rats / with invited commentaries. *Dig Surg*, *17*(4), 379. https://doi.org/10.1159/000018882

Chen, G. Y., & Nuñez, G. (2010). Sterile inflammation: sensing and reacting to damage. *Nat Rev Immunol*, *10*(12), 826–837. https://doi.org/10.1038/nri2873

Chung, H. Y., Baek, B. S., Song, S. H., Kim, M. S., Huh, J. I., Shim, K. H., Kim, K. W., & Lee, K. H. (1997). Xanthine dehydrogenase/xanthine oxidase and oxidative stress. *Age (Omaha)*, *20*(3), 127–140. https://doi.org/10.1007/s11357-997-0012-2

Cuzzocrea, S., Chatterjee, P. K., Mazzon, E., Dugo, L., De Sarro, A., de Loo, F. A., Caputi, A. P., & Thiemermann, C. (2002). Role of induced nitric oxide in the initiation of the inflammatory response after postischemic injury. *Shock*, *18*(2), 169–176. https://www.ncbi.nlm.nih.gov/pubmed/12166782

Demir, M., Amanvermez, R., Kamalı Polat, A., Karabıçak, I., Cınar, H., Kesicioğlu, T., & Polat, C. (2014). The effect of silymarin on mesenteric ischemia-reperfusion injury. *Med Princ Pract*, *23*(2), 140–144. https://doi.org/10.1159/000356860

Faith, M., Sukumaran, A., Pulimood, A. B., & Jacob, M. (2008). How reliable an indicator of inflammation is myeloperoxidase activity? *Clin Chim Acta*, *396*(1–2), 23–25. https://doi.org/10.1016/j.cca.2008.06.016

Fan, Z., Jing, H., Yao, J., Li, Y., Hu, X., Shao, H., Shen, G., Pan, J., Luo, F., & Tian, X. (2014). The protective effects of curcumin on experimental acute liver lesion induced by intestinal ischemia-reperfusion through inhibiting the pathway of NF-κB in a rat model. *Oxid Med Cell Longev*, *2014*, 191624. https://doi.org/10.1155/2014/191624

Fayez, A. M., Awad, A. S., El-Naa, M. M., Kenawy, S. A., & El-Sayed, M. E. (2014). Beneficial effects of thymoquinone and omega-3 on intestinal ischemia/reperfusion-induced renal dysfunction in rats. *Bulletin of Faculty of Pharmacy, Cairo University*, *52*(2), 171–177. https://doi.org/10.1016/J.BFOPCU.2014.05.003

Grootjans, J., Lenaerts, K., Derikx, J. P., Matthijsen, R. A., de Bruïne, A. P., van Bijnen, A. A., van Dam, R. M., Dejong, C. H., & Buurman, W. A. (2010). Human intestinal ischemia-reperfusion-induced inflammation characterized: experiences from a new translational model. *Am J Pathol*, *176*(5), 2283–2291. https://doi.org/10.2353/ajpath.2010.091069

Guzel, A., Kanter, M., Yucel, A. F., & Erboga, M. (2013). Protective effect of curcumin on acute lung injury induced by intestinal ischaemia/reperfusion. *Toxicol Ind Health*, *29*(7), 633–

642. https://doi.org/10.1177/0748233711430984

Hakgüder, G., Akgür, F. M., Ateş, O., Olguner, M., Aktuğ, T., & Ozer, E. (2002). Short-term intestinal ischemia-reperfusion alters intestinal motility that can be preserved by xanthine oxidase inhibition. *Dig Dis Sci*, *47*(6), 1279–1283. https://www.ncbi.nlm.nih.gov/pubmed/12064802

Horie, Y., Wolf, R., Miyasaka, M., Anderson, D. C., & Granger, D. N. (1996). Leukocyte adhesion and hepatic microvascular responses to intestinal ischemia/reperfusion in rats. *Gastroenterology*, *111*(3), 666–673. https://www.ncbi.nlm.nih.gov/pubmed/8780571

Iadecola, C., & Anrather, J. (2011). The immunology of stroke: from mechanisms to translation. *Nat Med*, *17*(7), 796–808. https://doi.org/10.1038/nm.2399

Jankun, J., Wyganowska-Świątkowska, M., Dettlaff, K., Jelińska, A., Surdacka, A., Wątróbska-Świetlikowska, D., & Skrzypczak-Jankun, E. (2016). Determining whether curcumin degradation/condensation is actually bioactivation (Review). *Int J Mol Med*, *37*(5), 1151–1158. https://doi.org/10.3892/ijmm.2016.2524

Jiang, Z. Y., Woollard, A. C., & Wolff, S. P. (1991). Lipid hydroperoxide measurement by oxidation of Fe2+ in the presence of xylenol orange. Comparison with the TBA assay and an iodometric method. *Lipids*, *26*(10), 853–856. https://www.ncbi.nlm.nih.gov/pubmed/1795606
Jones, D. P. (2002). Redox potential of GSH/GSSG couple: assay and biological significance. *Methods Enzymol*, *348*, 93–112. https://www.ncbi.nlm.nih.gov/pubmed/11885298

Kiliç, K., Hanci, V., Selek, S., Sözmen, M., Kiliç, N., Citil, M., Yurtlu, D. A., & Yurtlu, B. S. (2012). The effects of dexmedetomidine on mesenteric arterial occlusion-associated gut ischemia and reperfusion-induced gut and kidney injury in rabbits. *J Surg Res*, *178*(1), 223–232. https://doi.org/10.1016/j.jss.2012.03.073

Lamaita, R. M., Pontes, A., Belo, A. V, Caetano, J. P., Andrade, S. P., Cândido, E. B., Carneiro, M. M., & Silva-Filho, A. L. (2012). Evaluation of N-acetilglucosaminidase and myeloperoxidase activity in patients with endometriosis-related infertility undergoing intracytoplasmic sperm injection. *J Obstet Gynaecol Res*, *38*(5), 810–816.

https://doi.org/10.1111/j.1447-0756.2011.01805.x

Lee, M. C., Velayutham, M., Komatsu, T., Hille, R., & Zweier, J. L. (2014). Measurement and characterization of superoxide generation from xanthine dehydrogenase: a redox-regulated pathway of radical generation in ischemic tissues. *Biochemistry*, *53*(41), 6615–6623. https://doi.org/10.1021/bi500582r

Lin, J. K. (2007). Molecular targets of curcumin. *Adv Exp Med Biol*, *595*, 227–243. https://doi.org/10.1007/978-0-387-46401-5_10

Lindeström, L. M., & Ekblad, E. (2004). Structural and neuronal changes in rat ileum after ischemia with reperfusion. *Dig Dis Sci*, *49*(7–8), 1212–1222. http://www.ncbi.nlm.nih.gov/pubmed/15387349

Mallick, I. H., Yang, W., Winslet, M. C., & Seifalian, A. M. (2004). Ischemia-reperfusion injury of the intestine and protective strategies against injury. *Dig Dis Sci*, *49*(9), 1359–1377. http://www.ncbi.nlm.nih.gov/pubmed/15481305

Marczylo, T. H., Steward, W. P., & Gescher, A. J. (2009). Rapid analysis of curcumin and curcumin metabolites in rat biomatrices using a novel ultraperformance liquid chromatography (UPLC) method. *J Agric Food Chem*, *57*(3), 797–803. https://doi.org/10.1021/jf803038f

Marklund, S., & Marklund, G. (1974). Involvement of the superoxide anion radical in the autoxidation of pyrogallol and a convenient assay for superoxide dismutase. *Eur J Biochem*, *47*(3), 469–474. https://www.ncbi.nlm.nih.gov/pubmed/4215654

McCord, J. M. (1985). Oxygen-derived free radicals in postischemic tissue injury. *N Engl J Med*, *312*(3), 159–163. https://doi.org/10.1056/NEJM198501173120305

Montalto, M. C., Hart, M. L., Jordan, J. E., Wada, K., & Stahl, G. L. (2003). Role for complement in mediating intestinal nitric oxide synthase-2 and superoxide dismutase expression. *Am J Physiol Gastrointest Liver Physiol*, *285*(1), G197-206. https://doi.org/10.1152/ajpgi.00029.2003

Nordberg, J., & Arnér, E. S. (2001). Reactive oxygen species, antioxidants, and the mammalian thioredoxin system. *Free Radic Biol Med*, *31*(11), 1287–1312. https://www.ncbi.nlm.nih.gov/pubmed/11728801

Onder, A., Kapan, M., Gümüş, M., Yüksel, H., Böyük, A., Alp, H., Başarili, M. K., & Firat, U. (2012). The protective effects of curcumin on intestine and remote organs against mesenteric ischemia/reperfusion injury. *Turk J Gastroenterol*, *23*(2), 141–147. https://www.ncbi.nlm.nih.gov/pubmed/22706742

Parks, D. A., & Granger, D. N. (1988). Ischemia-reperfusion injury: a radical view. *Hepatology*, *8*(3), 680–682. https://www.ncbi.nlm.nih.gov/pubmed/3286463

Paterno, F., & Longo, W. E. (2008). The etiology and pathogenesis of vascular disorders of the intestine. *Radiol Clin North Am*, *46*(5), 877–885. https://doi.org/10.1016/j.rcl.2008.06.005

Pereira, A. S., Shitsuka, D. M., Parreira, F. J., & Shitsuka, R. (2018). Método Qualitativo, Quantitativo ou Quali-Quanti. In *Metodologia da Pesquisa Científica*. https://repositorio.ufsm.br/bitstream/handle/1/15824/Lic_Computacao_Metodologia-Pesquisa-Cientifica.pdf?sequence=1.

Saidi, S. A., Ncir, M., Chaaben, R., Jamoussi, K., van Pelt, J., & Elfeki, A. (2017). Liver injury following small intestinal ischemia reperfusion in rats is attenuated by Pistacia lentiscus oil: antioxidant and anti-inflammatory effects. *Arch Physiol Biochem*, *123*(4), 199–205. https://doi.org/10.1080/13813455.2017.1302961

Shoba, G., Joy, D., Joseph, T., Majeed, M., Rajendran, R., & Srinivas, P. S. (1998). Influence of piperine on the pharmacokinetics of curcumin in animals and human volunteers. *Planta Med*, *64*(4), 353–356. https://doi.org/10.1055/s-2006-957450

Stallion, A., Kou, T. D., Miller, K. A., Dahms, B. B., Dudgeon, D. L., & Levine, A. D. (2002). IL-10 is not protective in intestinal ischemia reperfusion injury. *J Surg Res*, *105*(2), 145–152. https://www.ncbi.nlm.nih.gov/pubmed/12121701

Stoney, R. J., & Cunningham, C. G. (1993). Acute mesenteric ischemia. *Surgery*, *114*(3), 489–490. http://www.ncbi.nlm.nih.gov/pubmed/8367801

Thomas, C. E., Morehouse, L. A., & Aust, S. D. (1985). Ferritin and superoxide-dependent lipid peroxidation. *J Biol Chem*, *260*(6), 3275–3280. https://www.ncbi.nlm.nih.gov/pubmed/2982854

Tiwari, V., Kuhad, A., & Chopra, K. (2011). Emblica officinalis corrects functional, biochemical and molecular deficits in experimental diabetic neuropathy by targeting the oxido-nitrosative stress mediated inflammatory cascade. *Phytother Res*, *25*(10), 1527–1536. https://doi.org/10.1002/ptr.3440

Turan, I., Ozacmak, H. S., Ozacmak, V. H., Barut, F., & Araslı, M. (2017). Agmatine attenuates intestinal ischemia and reperfusion injury by reducing oxidative stress and inflammatory reaction in rats. *Life Sci*, *189*, 23–28. https://doi.org/10.1016/j.lfs.2017.08.032

Ukil, A., Maity, S., Karmakar, S., Datta, N., Vedasiromoni, J. R., & Das, P. K. (2003). Curcumin, the major component of food flavour turmeric, reduces mucosal injury in trinitrobenzene sulphonic acid-induced colitis. *Br J Pharmacol*, *139*(2), 209–218. https://doi.org/10.1038/sj.bjp.0705241

Vinardi, S., Pierro, A., Parkinson, E. J., Vejchapipat, P., Stefanutti, G., Spitz, L., & Eaton, S. (2003). Hypothermia throughout intestinal ischaemia-reperfusion injury attenuates lung neutrophil infiltration. *J Pediatr Surg*, *38*(1), 88–91. https://doi.org/10.1053/jpsu.2003.50017

Wang, J., Yu, X., Zhang, L., Wang, L., Peng, Z., & Chen, Y. (2018). The pharmacokinetics and tissue distribution of curcumin and its metabolites in mice. *Biomed Chromatogr*, e4267. https://doi.org/10.1002/bmc.4267

Warholm, M., Guthenberg, C., von Bahr, C., & Mannervik, B. (1985). Glutathione transferases from human liver. *Methods Enzymol*, *113*, 499–504. https://www.ncbi.nlm.nih.gov/pubmed/3003505

Xu, Y., Hu, N., Jiang, W., Yuan, H. F., & Zheng, D. H. (2016). Curcumin-carrying nanoparticles prevent ischemia-reperfusion injury in human renal cells. *Oncotarget*, *7*(52),

87390–87401. https://doi.org/10.18632/oncotarget.13626

Percentage of participation of each author in manuscript

Elouisa Bringhentti – 30%

Stephanie Carvalho Borges - 30%

Camila Quaglio Neves – 10%

Nilza Cristina Buttow – 30%