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FULL ARTICLE

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Aqueous extract of *Paullinia cupana* attenuates renal and hematological effects associated with ketoprofen

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Abstract

This study aimed to evaluate the effect of aqueous extract of *Paullinia cupana* (AEG) against ketoprofen side effects, through biochemical, hematological, and histological parameters. AEG showed antioxidant activity in the DPPH$^{\bullet}$ scavenging (IC_{50} = 17.00 ± 1.00 μg/ml) and HPLC analysis revealed that this extract is constituted by antioxidants (caffeine, catechins, theobromine, and polyphenols). In vivo experiments in female Wistar rats demonstrated that alterations in urea, creatinine, and uric acid levels promoted ($p < .05$) by ketoprofen were reversed when AEG was co-administered. Ketoprofen significantly decreased the catalase levels of animal tissues ($p < .05$), which were restored when AEG was co-administered with the mentioned drug. Histological analysis showed that AEG protected tissues from damages caused by ketoprofen. Moreover, AEG reestablished the number of white blood cells, which had decreased when ketoprofen was administered. In conclusion, this study suggested that the association between ketoprofen and AEG may be an alternative to reduce health damages caused by this drug.

Practical applications

Paullinia cupana, popularly known as guaraná, is commonly consumed as a beverage in Brazil and exhibits pharmacological and beneficial effects to humans. Ketoprofen is an efficacious drug employed in the treatment of inflammatory processes. However, this drug can cause several side effects in humans. Thus, the usage of natural products and plant extracts that can reduce such undesirable effects consists in a valuable strategy to be applied in therapeutic interventions.

KEYWORDS

antioxidant, catalase, DPPH, guarana, ketoprofen, kidney damage

1 | INTRODUCTION

The treatment of various inflammatory diseases involves the use of nonsteroidal anti-inflammatory drugs (NSAIDs), such as ketoprofen (Gomaa, 2018; Wongrakpanich et al., 2018). Such drugs present pharmacological activities related to the nonselective inhibition of cyclooxygenase enzymes (COX 1 and COX 2), thus leading to the inhibition of prostaglandin (PGs) (Levoin et al., 2004).

Ketoprofen (2-(3-benzoylphenyl)propanoic acid) is an effective drug, and is commonly used for the treatment of rheumatoid arthritis, musculoskeletal injuries, osteoarthritis and acute gouty arthritis, bursitis, and tendinitis (Gigante & Tagarro, 2012; Rençber et al., 2009). However, its use can cause some severe side effects, such as the reduction of the biosynthesis of the protecting gastric mucosa (PGs), which can result in ulceration, bleeding, increased intestinal mucosa permeability, and gastric hypermotility (Radi & Khan, 2006). Besides that, ketoprofen can cause renal dysfunction and decreased creatinine clearance, as well as damage to the liver (Lee et al., 2005; Tomic et al., 2008).

The mechanism involved in the side effects resulting from the use of ketoprofen has not been completely elucidated yet. Studies have suggested that these effects may be related to the inhibition of PG synthesis (which is responsible for important physiological functions), to the changes caused to the hydrophobicity of the gastrointestinal mucosa, or even to free radicals and reactive oxygen and nitrogen species generation (ROS and RNS) (Batlouni, 2010; de Lastra et al., 2002; Markiewicz & Pasenkiewicz-gierula, 2011). Studies have also indicated that the use of ketoprofen can lead to reduced levels of NADPH and reduced glutathione (GSH) (Asensio et al., 2007), and decreased levels of superoxide dismutase (SOD) enzyme (de Lastra et al., 2002), which generates $O_2^{\bullet -}$ in the plasmatic membrane by NADPH oxidase activation (Domínguez-Luis et al., 2013). Although free radicals and ROS perform some physiological functions, these species are also highly reactive and potentially harmful (Duracková, 2010). Thus, the organism possesses antioxidant systems in order to reduce or prevent damage caused by the excess of ROS, such as catalase (CAT) and SOD, as well as local products (e.g., glutathione and ubiquinone), or even substances from the human diet, such as vitamins C and E, carotenoids, and polyphenols (Birben et al., 2012).

Guaraná (*Paullinia cupana* var. Sobillis, Sapindaceae), is a plant species native from the Amazon region in Brazil, and is commercially used in cosmetics and soft drink industries (Marques et al., 2019). A number of studies have proved the biological effects of guaraná such as aphrodisiac, energetic (Smith & Atroch, 2007), antioxidant (Basile et al., 2005; Carvalho et al., 2016), antimicrobial, and antiplatelet (Jippo et al., 2009; Yamaguti-sasaki et al., 2007). Guaraná has also demonstrated to possess antiallergic, genoprotector, and gastroprotective properties (Campos et al., 2005; Jippo et al., 2009), besides being able to prevent alterations in lipid metabolism and metabolic diseases (Krewer et al., 2011; Portella et al., 2013). Recent studies have showed that guaraná can also help to prevent and treat of some aging-associated disorders due to its antioxidant activity (Arantes et al., 2018; Peixoto et al., 2017). Moreover, some works revealed the beneficial effects of guaraná on weight loss (Marques et al., 2019). The chemical composition of this species also reveals the presence of a high amount of bioactive compounds, such as methylxanthines, condensed tannins, proanthocyanidins (flavan-3-ol), catechins, epicatechins, saponins, and polyphenols (Carvalho et al., 2016; Machado et al., 2018; Mattei et al., 1998; Portella et al., 2013; Santana & Macedo, 2019).

Considering that guaraná presents interesting antioxidant properties, the aim of this study was to investigate the in vitro and in vivo protective effects of aqueous extract of guarana (AEG), in model systems (oxidative, renal, liver, and hematologic systems in animals) that may be affected by the typical side effects of ketoprofen.

2 | MATERIALS AND METHODS

2.1 | Chemicals

All chemicals and solvents used in this work were of analytical grade. 2,2-diphenyl-1-picrylhydrazyl (DPPH), reduced glutathione (GSH), hydrogen peroxide (H_2O_2), ethylenediaminetetraacetic acid (EDTA), Tris–HCl, Folin–Ciocalteu' reagent, sodium carbonate (Na_2CO_3), gallic acid, resveratrol, potassium chloride (KCl), 5-5′-dithio-2-nitrobenzoic acid (DTNB), trichloroacetic acid (TCA), sodium phosphate, and formaldehyde were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Ketoprofen was purchased from a local supplier. All aqueous solutions were prepared in deionized water.

2.2 | Plant extract preparation

This study has an authorized agreement providing access and remittance to the Brazilian genetic heritage and is part of a project approved by the Genetic Heritage Management (CGEN) (ABSP nº 010547/2013-4). Guaraná powder was acquired by the Brazilian Agricultural Research Corporation (2013/2014 crop) located in the Western Amazon (Maués, Amazonas state, Brazil). The sample was stored in dry conditions at −20°C until the preparation of the extract. Aqueous crude extract was prepared by soaking guaraná powder (100 g) in boiling water (97.3°C) for 10 min. Gentle shakes were applied at intervals of 5 and 10 min. The remaining solution was filtered through Whatman No. 8 paper, frozen at −8°C in amber flasks, and lyophilized.

2.3 | Determination of bioactive compounds in AEG

The total content of condensed tannins in AEG was performed according to the method described by Morrison et al. (1995). The absorbance was measured at 500 nm and the results were expressed as mg gallic acid (GA)/100 g of AEG. The determination of hydrolysable tannins was performed as described by Brune et al. (1991) and the results were expressed in mg catechin (CAE)/100 g of AEG.

The total alkaloid content was assessed by acidification, and subsequently addition of Dragendorff's reagent as described by Sreevidya and Mehrotra (2003). The absorbance was measured at 435 nm, and the results were expressed as mg berberine (BBR)/100 g of AEG. Total phenolic content was determined using Folin–Ciocalteu' reagent (FCR), as described by Chandra and de Mejia (2004). Samples of AEG were mixed with FCR and 20% (w/v) Na_2CO_3 solutions. The absorbance was measured at 730 nm after 10 min of incubation at room temperature. The results were expressed as gallic acid (GA) equivalents/100 g of AEG dry weight.

2.4 | Quantification of caffeine, theobromine, and catechins

High-performance liquid chromatography (HPLC) was performed using a Shimadzu LC Prominence-20A chromatograph with a LC-20AT quaternary pump, a SIL-20A automatic injector, a DGU-20A5 in-line degasser, and a SPD-20AV diode array detector (DAD). An ODS-3 column (4.6 mm × 150 mm) packed with 5 μm diameter particles (Phenomenex Prodig ODS-2 100) was employed for component separation. The mobile phase was composed by 0.1% phosphoric acid and 100% acetonitrile. AEG and mobile phase were filtered using 0.45 μm membrane filter (Millipore) and degassed using an ultrasonic bath prior to HPLC analysis. The flow rate and the wavelength were set to 1 ml/min and 272 nm, respectively. All chromatographic operations were carried out at room temperature and performed in triplicate (Andrews et al., 2007; Barreto Alves & Bragagnolo, 2002).

2.5 | DPPH• radical scavenging activity

Antioxidant activity of AEG was evaluated by DPPH• scavenging assay as described by Soares et al. (2008). Samples were previously dissolved in 99% (v/v) ethanol and incubated with 60 μM DPPH• ethanolic solution for 10 min at room temperature in the absence of light. Afterwards, the absorbance was measured at 531 nm. The radical scavenging activity was measured as a decrease in the absorbance of DPPH• and calculated using the following equation:

$$\%\ \text{inhibition} = 100 - \left[(\text{Abs sample} - \text{Abs blank}) / \text{Abs control} \times 100\right].$$

Antioxidant activity results were expressed as IC_{50} values and resveratrol was used as a positive control.

2.6 | Animal model

The animal experimental protocol was approved by the Ethics Committee for Research on the Use of Animals at the State University of Ponta Grossa (Number 039/2014). Sixty-four female Wistar rats (*Rattus norvegicus* var. Albinus) (150–250 g, 11–13 weeks) were maintained throughout the experimental period in special cages for rodents, in a temperature-controlled room (21 ± 2°C), with light-dark cycle of 12/12 hr and free access to food and water ad libitum. The animals were distributed in six groups according to the administered substance: 0.9% saline (4 ml kg^{-1} day^{-1}, $n = 11$) ("C" group—control); 20 mg kg^{-1} day^{-1} of ketoprofen, $n = 11$ ("Ket" group—ketoprofen); AEG 0.1 mg g^{-1} day^{-1}, $n = 10$ ("AEG 0.1" group—AEG 0.1); AEG 1 mg g^{-1} day^{-1}, $n = 10$ ("AEG 1" group—aqueous extract of guaraná 1); Ketoprofen 20 mg kg^{-1} day^{-1} + AEG 0.1 mg g^{-1} day^{-1}, $n = 11$ ("Ket + AEG 0.1" group); Ketoprofen 20 mg kg^{-1} day^{-1} + AEG 1 mg g^{-1} day^{-1}, $n = 11$ ("Ket + AEG 1" group). The animals were treated with the mentioned samples for 7 days by oral route. The doses of ketoprofen and AEG were based to the studies reported by Zhao et al. (2007) and Fukumasu et al. (2006), respectively.

On the 8th day of treatment, all the animals were anesthetized with a mixture of anesthetics (ketamine hydrochloride 75 mg/kg and xilasine 15 mg/kg intraperitoneally), and the blood was collected from the vena cava. Then, the rats were sacrificed by deepening the anesthesia, and the kidneys and liver were removed aiming the determination of oxidative stress biomarkers.

2.7 | Preparation of blood, liver, and kidneys samples

Blood was collected from the vena cava in tubes containing EDTA (7.5 mM), or in the absence of any anticoagulant in order to obtain the plasma and serum, respectively, after centrifugation for 15 min at 2,000 rpm. For the determination of non-protein thiol groups, proteins of liver and kidney were precipitated in 50% (w/v) TCA aqueous solution. Then, the tissues were homogenized in 0.02 M EDTA and the supernatant was used for the determination of the NP-SH groups. For the determination of CAT levels, tissues were homogenized in sodium phosphate buffer (50 mM; pH 7.4 containing 150 mM KCl and 200 mM EDTA) and centrifuged for 30 min at 4°C.

2.8 | Evaluation of hematological parameters

Whole blood counts (EDTA)—total number of leukocytes, erythrocytes, hemoglobin, hematocrit, mean corpuscular volume (MCV), and platelets—were performed using the electronic counter Hemacounter 60® (Hemogram).

2.9 | Evaluation of biochemical parameters

Aspartate aminotransferase (AST), alanine aminotransferase (ALT), uric acid, urea, and creatinine levels were determined in the serum of the animals using commercial kits (Diasys, Germany, Holzheim) in automated routine (Respons 910, Diasys), following the manufacturer's guidelines.

2.10 | Determination of non-protein thiol groups in the liver and kidneys

The determination of non-protein thiol groups in the liver and kidneys was carried out as described by Sedlak and Lindsay (1968). The reaction was performed in Tris–HCl buffer solution (0.4 M, pH 8.9) in the presence of 0.16 mM DTNB, and the absorbance value was measured at 412 nm. For the determination, an analytical curve using reduced GSH was constructed, and the results were expressed as μM NP-SH/g tissue.

2.11 | Determination of catalase activity in liver and kidney tissues

Catalase activity in the liver and kidneys was measured as described by Aebi (1984). The reaction was carried out with the supernatant in sodium phosphate buffer (50 mM, pH 7.4), and 10.5 mM H_2O_2 was used as the enzyme substrate. The absorbance value was monitored for 2 min at 240 nm and the results were expressed as μM H_2O_2/g tissue/min.

2.12 | Histological investigations

The liver and kidneys of animals were fixed in ethanol, formaldehyde, and acetic acid solution for 18 hr–24 hr. Then, following histological processing (dehydration and diaphanization), the samples were finally embedded in paraffin. Histological sections of 4 μm were obtained in rotary microtome, and stained with hematoxylin-eosin (H&E). The slides were analyzed using a light microscope (Zeiss Primo Star) with objective lens (5x, 10x, 20x, and 40x). The analysis of the hepatic tissue included: general structure of the liver, sinusoid space, presence of neutrophils in sinusoid space, morphological changes in hepatocytes, presence of congestion, type (acute, chronic, and mixed) and intensity (mild, moderate, and intense) of inflammatory infiltrate in door space, intra-lobular inflammatory agglomerates, areas of necrosis, and mitotic index (400 × magnification). The analysis of the renal tissue included the following aspects: integrity of Bowman's capsule, urinary space, proximal and distal ducts, presence of bleeding and presence of congestion, and the pith. Type (acute, chronic, and mixed) and intensity (mild, moderate, and intense) of inflammatory infiltrate and presence of fibrosis.

2.13 | Statistical analysis

The results were expressed as mean ± standard deviation (*SD*). All in vitro data were performed in triplicate and analyzed statistically using GraphPad Prism (version 5.01, San Diego, California, USA) software. Statistical differences between the different groups were assessed by one-way analysis of variance ANOVA) followed by Tukey's test. The level of significance for the analysis was set to $p < .05$.

3 | RESULTS

3.1 | Determination of bioactive compounds from AEG

Preliminary phytochemical analysis revealed that AEG is constituted by different classes of bioactive compounds. The total phenolic content of AEG was 452.7 ± 19.1 mg Eq GA/100 g dry weight and the alkaloid content was 77.5 ± 23.9 mg Eq BBR. The content of hydrolyzed and condensed tannins was 169.2 ± 5.3 mg Eq CAE and 1,198.0 ± 121.4 mg Eq GA, respectively. HPLC analysis also revealed that AEG was constituted by caffeine 223.5 ± 13.8 μg/0.002 g, theobromine 3.67 ± 1.1 μg/0.002 g, and catechins 39.9 ± 2.3 μg/0.002 g.

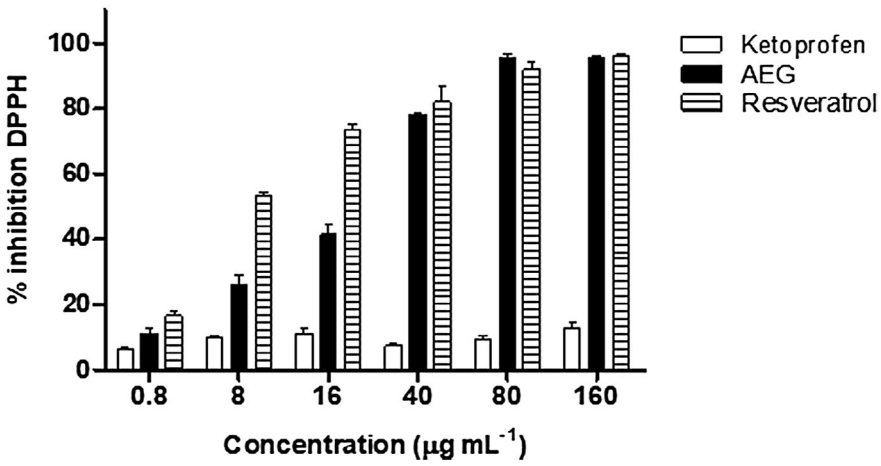

FIGURE 1 Scavenger Activity DPPH• expressed in percentage (%) of inhibition—ketoprofen, AEG, and resveratrol (0.8; 8; 16; 40; 80; and 160 μg/ml)

TABLE 1 Effect of treatment with ketoprofen and/or AEG on the hematological parameters of the rats

Group	Leukocytes $\times 10^3/\mu l$	Erythrocytes $\times 10^6/\mu l$	Hemoglobin g/Dl	Hematocrit (%)	Mean corpuscular volume (fl)
C	5.04 ± 0.39a	6.28 ± 0.20a	13.32 ± 0.26a	38.90 ± 1.22a	62.00 ± 0.31a
Ket	8.40 ± 0.31b	3.39 ± 0.21b	8.55 ± 0.61b	23.94 ± 1.29b	66.55 ± 1.37b
AEG 0.1	4.12 ± 0.44a	5.85 ± 0.11a	13.13 ± 0.25a	36.64 ± 0.70a	62.69 ± 0.54a
AEG 1	5.40 ± 0.32a	6.00 ± 0.15a	13.34 ± 0.35a	37.48 ± 1.00a	62.77 ± 0.60a
Ket + AEG 0.1	6.30 ± 0.61a	3.40 ± 0.12b	8.08 ± 0.28b	23.68 ± 0.88b	69.74 ± 1.34b
Ket + AEG1	6.75 ± 0.63ab	3.48 ± 0.18b	7.56 ± 0.29b	22.86 ± 0.97b	66.08 ± 0.93b

*Values are expressed as mean ± standard deviations (*SD*) of experiments performed in triplicate. Averages followed by different letters differ statistically in the same column ($p < .05$).

3.2 | DPPH• radical scavenging activity

The antioxidant activity of AEG and ketoprofen is demonstrated in Figure 1. It can be seen that the maximal inhibition of DPPH• radicals caused by ketoprofen (160 μg/ml) was nearly 11%. Moreover, AEG (80 μg/ml) reached a maximal inhibition of approximately 95%, with $IC_{50} = 17.00 \pm 1.00$ μg/ml. At the highest AEG concentrations (80 and 160 μg/ml), it was observed that the percentage of DPPH• inhibition was similar to that obtained for resveratrol ($IC_{50} = 7.70 \pm 0.70$ μg/ml) (Figure 1), which was able to cause approximately 92% DPPH• inhibition at the same concentrations.

3.3 | Evaluation of hematological parameters

Blood count and morphotypes were microscopically analyzed, showing no significant differences between the groups with regard to the platelets (data not shown). The group that received ketoprofen showed a greater difference in white and red blood cell counts, hemoglobin, hematocrit, and mean corpuscular volume (MCV), when compared to the control group, showing anemia (Table 1). Moreover, the groups that were only treated with AEG showed results that were similar to the control group for all hematological parameters analyzed. Interestingly, when AEG was administered with ketoprofen, a reduced total leukocyte count was noticed when compared to the group that only received ketoprofen, thus restoring the levels observed for the control group. However, the AEG was not able to prevent changes in the red blood cells count caused by ketoprofen when administered together with this drug.

3.4 | Evaluation of biochemical parameters in liver and kidney tissues

As it can be seen in Figure 2, the administration of ketoprofen and AEG (individually or combined) was not able to alter the serum levels of AST (Figure 2a) and ALT (Figure 2b) hepatic enzymes using the doses employed in this study, when compared to the control group.

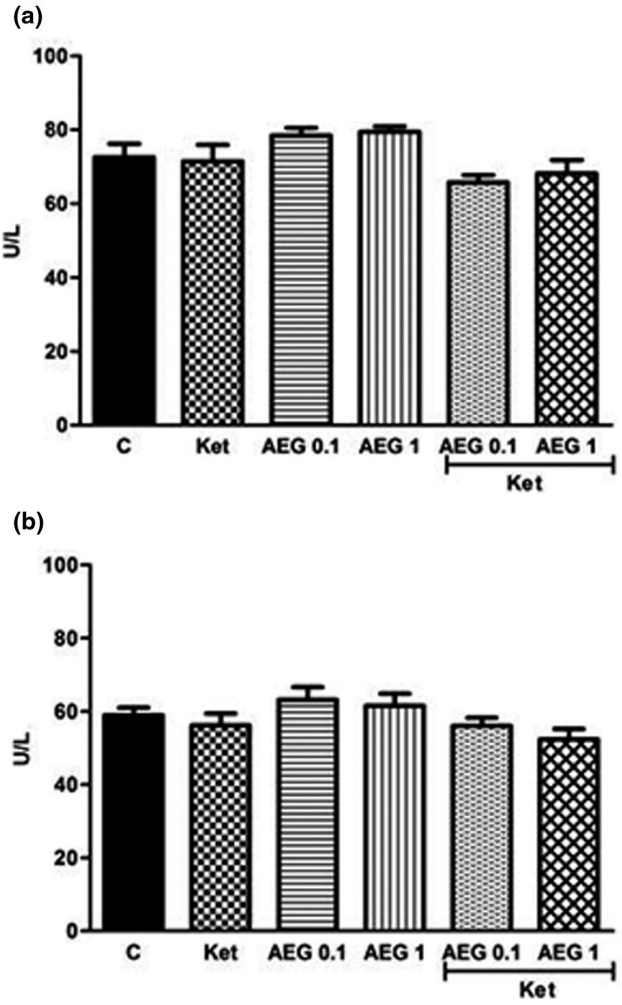

FIGURE 2 Effect of treatment with AEG in AST (a) and ALT (b) serum in animals treated with ketoprofen. *Indicates a statistical difference ($p < .05$) with the control group. #Indicates a statistical difference ($p < .05$) with the ketoprofen group

The effect of treatment with ketoprofen and/or AEG on serum biomarkers of kidney functions (urea, creatinine, and uric acid) is demonstrated in Figure 3. The results showed that the treatment with ketoprofen led to an increase around 20% in serum urea levels, when compared to the control group ($p < .05$). Moreover, a significant reduction in urea levels was observed for the groups treated

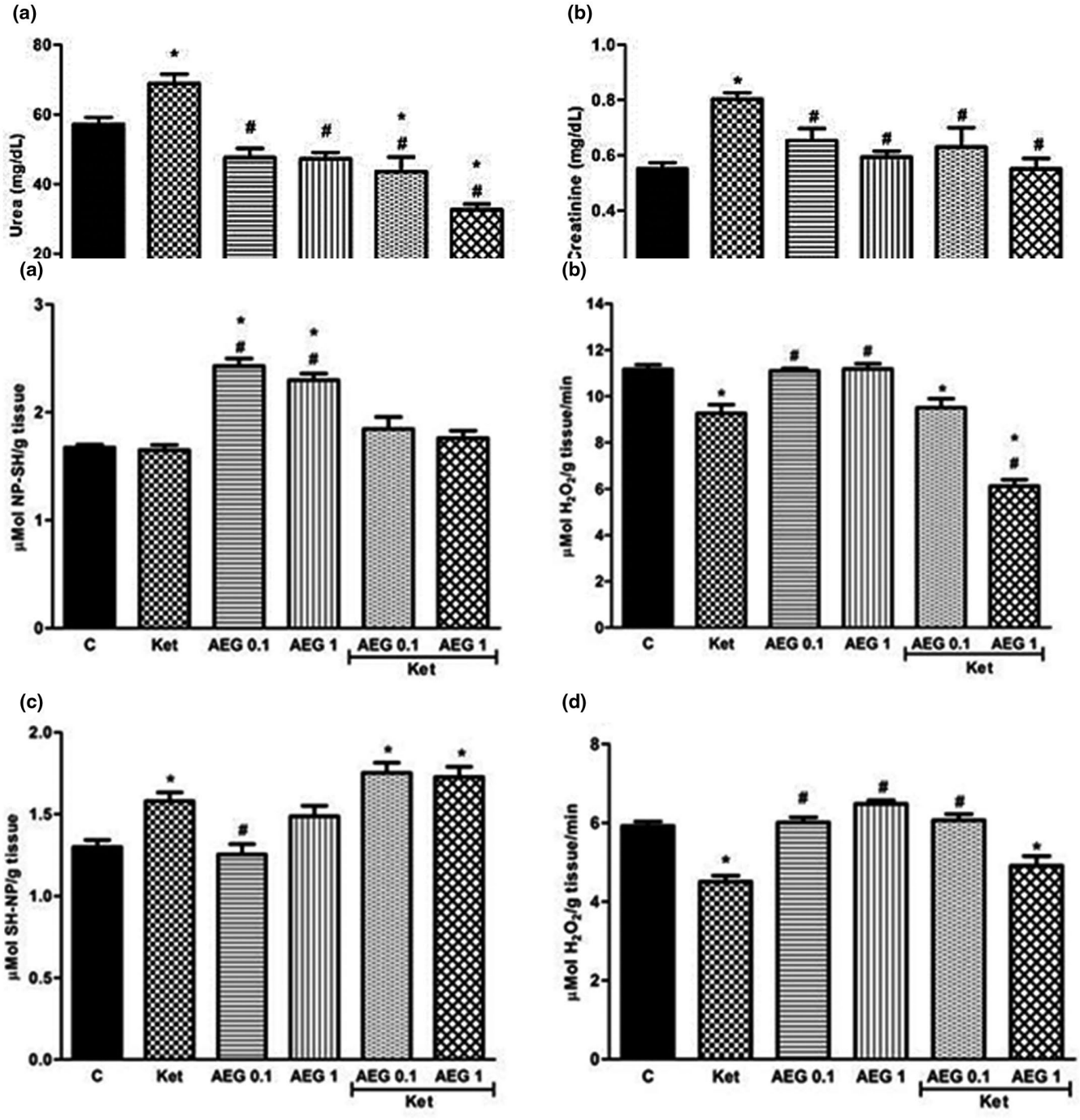

FIGURE 4 Effect of AEG on NP-SH levels (a) and catalase activity (b) of the liver of animals treated with ketoprofen. *Indicates statistical difference ($p < .05$) compared with the control group. #Indicates statistical difference ($p < .05$) compared with the ketoprofen group

FIGURE 3 Effect of AEG on the serum urea (a); creatinine (b), and uric acid (c) levels in animals treated with ketoprofen. *Indicates statistical difference ($p < .05$) compared with the control group. #Indicates statistical difference ($p < .05$) compared with the ketoprofen group

with ketoprofen and AEG. For instance, when the highest dose of AEG was co-administered with ketoprofen, the serum urea levels decreased 43% when compared to control group ($p < .05$) (Figure 3a). The treatment with ketoprofen caused an increase of approximately 45% in the creatinine levels in relation to the control group ($p < .05$). However, when animals were simultaneously treated with ketoprofen and AEG, it was observed that the serum creatinine levels were similar to those of the control group (Figure 3b). Uric acid levels were increased about 121% in the treatment with ketoprofen, which may be an evidence of interference in the excretory renal function

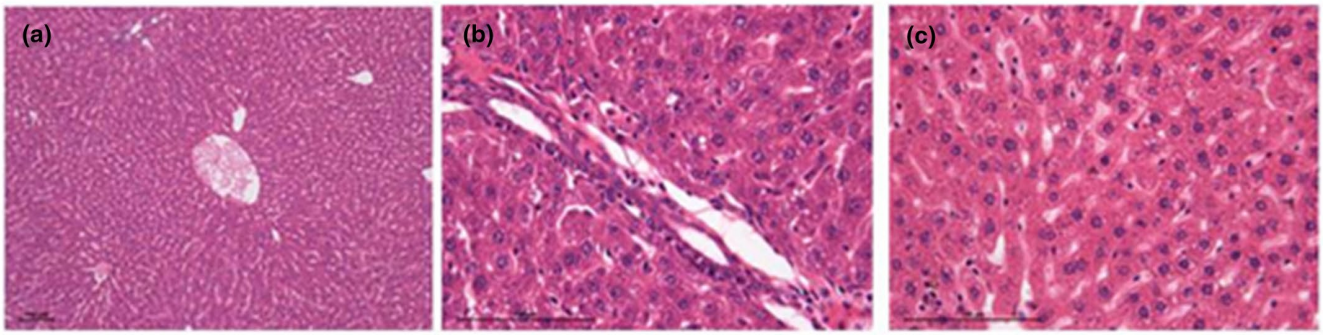

FIGURE 5 Presence of congestion (a); inflammatory infiltrate in the portal space and (b); sinusoid spaces (c), observed in liver tissue of the groups

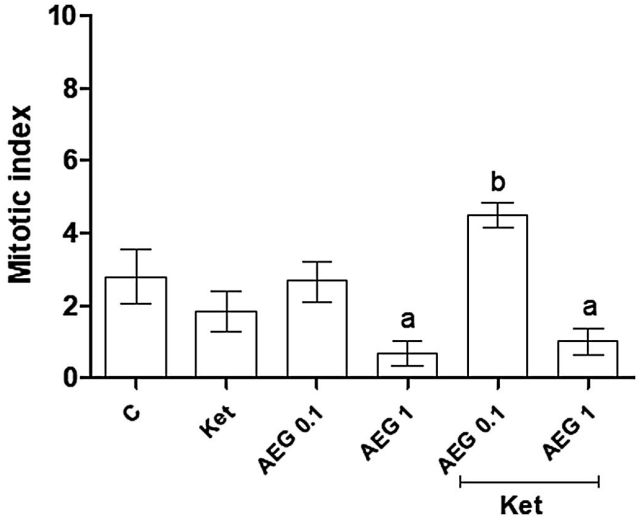

FIGURE 6 Effect of AEG on the mitotic index in hepatocytes. Averages followed by different letters differ statistically by Tukey's test at $p < .05$

($p < .05$). Interestingly, the groups that received AEG and ketoprofen had blood uric acid levels similar to those obtained for the control group (Figure 3c).

3.5 | Determination of non-protein thiol groups in liver and kidneys tissues

Ketoprofen did not cause changes in the hepatic NP-SH levels, whereas AEG (when used in isolation, and regardless of the dose) caused an increase of approximately 45% in such levels ($p < .05$) (Figure 4a). This suggest a significant enhancement in nonenzymatic antioxidant defenses in the liver after treatment with the natural extract.

In the evaluation of the renal antioxidant parameters, the results showed an increase of about 28% in the NP-SH levels in the group which was treated with ketoprofen ($p < .05$). However, the groups which received this drug combined with AEG presented, an increase around 35% in SH-NP levels in relation to the control group ($p < .05$) (Figure 4c).

3.6 | Determination of catalase activity in liver and kidneys tissues

Ketoprofen led to a reduction in CAT activity of about 17% when compared to the control group (Figure 4b) ($p < .05$), which suggested a possible interference in the enzymatic antioxidant defenses in the liver. When AEG was used alone, it was not possible to observe changes in CAT levels comparing to the control group. However, when AEG was administered in combination with ketoprofen, it was unable to prevent a reduction in the hepatic CAT levels (Figure 4b).

The renal CAT activity was also influenced by the treatment with ketoprofen (Figure 4d). The Ket group presented catalase levels that were approximately 24% lower compared to the control group ($p < .05$).Moreover, when the lower dose of AEG was combined with ketoprofen, the catalase activity was similar to the control group, which suggests a possible antioxidant protection provided by the natural extract (Figure 4d).

3.7 | Histological investigations

In the histological examinations of liver sections, it was found that the features of the different groups were maintained in terms of qualitative analysis, with normal sinusoid spaces, absence of neutrophils in sinusoid space, and normal-sized hepatocytes (Figure 5). It also noticed the presence of blood congestion (which corresponds to the congestion caused by venous or arterial process), mild chronic inflammatory infiltrate in the portal space region, sporadic presence of intralobular inflammatory clusters, and necrosis in all analyzed groups. Mitotic index results in hepatocytes are depicted in Figure 6, where a reduction in the cell renewal rate for AEG 1 ($p < .05$) and Ket + AEG 1 groups ($p < .05$) can be observed. Furthermore, no significant differences were observed between the groups regarding the presence of necrosis.

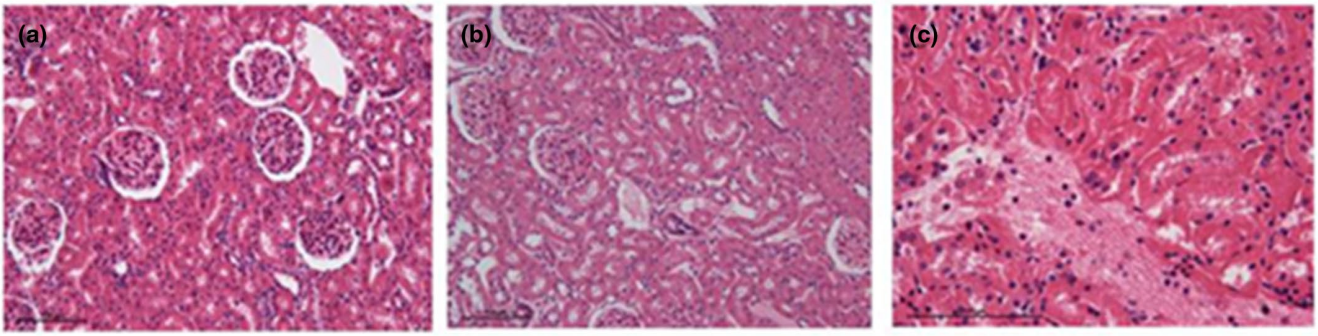

FIGURE 7 Normal urinary space (a); Hemorrhage and (b); Edema in the proximal ducts (C), observed in renal tissue of the groups

Figure 7 shows the histological analysis of renal tissue. The obtained results exhibited only discrete changes in the renal tissue in terms of qualitative analysis (Figure 7). Besides that, it was possible to observe that these organs exhibited normal glomeruli, intact Bowman's capsule, normal urinary space, normal distal ducts, and mild infiltration, which is characteristic of chronic inflammation. It was verified the presence of hemorrhage in the cortical region in all groups analyzed (possibly caused by the manipulation of the tissues at the moment of extraction). However, only the Ket + AEG 0.1 group showed edema in the proximal ducts.

4 | DISCUSSION

Herein, the protective properties of AEG regarding the side effects caused by ketoprofen were evaluated. For this purpose, biochemical, hematological, and histological parameters were assessed in tissue samples from rats treated with combinations of ketoprofen and AEG.

It is known that the management of the organism oxidative condition during an inflammatory process can be an alternative to promote the reduction of side effects caused by NSAIDs (Belló et al., 2015). For this reason, natural sources with potential antioxidant activity may be a promising alternative to be combined with NSAIDs. In this study, it was verified that the AEG was composed by phenolic compounds, alkaloids, hydrolyzed and condensed tannins, caffeine, theobromine, and catechins, which are probably involved in the antioxidant activity observed in the DPPH$^{\bullet}$ assay (Quideau et al., 2011). Thus, it is possible to suggest that the chemical compounds present in the extract have hydrogen-donating ability. Moreover, these results are in a good agreement with the study reported by Yamaguti-Sasaki et al. (2007), which demonstrated that AEG is rich in bioactive compounds and exhibited antioxidant activity. In contrast, it was verified that ketoprofen has a lower antioxidant effect, which corroborated the result by Manente et al. (2011).

To clarify the possible target tissues of side effects caused by ketoprofen and to evaluate if AEG would be able to attenuate such effects, we analyzed hepatic, renal, and hematological parameters in samples obtained from rats that were treated with the drug and/or AEG. The increase in the serum levels of liver enzymes (ALT and AST) are significant indicators of liver injury (Jiang et al., 2015). However, according to our results, the AEG and ketoprofen (Figure 2), whether combined or not, did not cause significant changes in the levels of these two enzymes. Hence, these results suggest that there were no hepatotoxicity related to damage to the membrane and mitochondria of the hepatocytes (Bertolami, 2005).

Histological analysis in liver tissue demonstrated that there was alterations in the cell renewal. Hepatocytes are rarely found in mitosis, although cell proliferation can occur by chemical and physical stimuli and in the presence of exogenous substances, which can have a deleterious effect and lead to cell death (Melo et al., 2003). The maintenance of liver functions is conferred by the cellular integrity, as well as the hepatic parenchyma (Melo et al., 2003). Our results showed that the highest dose of AEG was able to reduce the mitotic index (Figure 6) and did not lead to significant changes in the necrosis levels. This result indicates the protective effect of AEG, which there was less need for recovery and the tissue necrosis did not occur at a greater rate in the presence of the natural extract.

The evaluation of antioxidant parameters showed integrity for the nonenzymatic system but indicated a possible interference caused by ketoprofen on the enzymatic antioxidant defenses. This evidence may represent a stage in the biochemical changes caused by the tested doses. Moreover, the liver protective potential of AEG became evident for the evaluated antioxidant systems.

Although only a few histological changes were observed in the kidneys (Figure 7), the ketoprofen treatment promoted significant alterations in renal functions with an increase in serum levels of uric acid, urea, and creatinine levels (Figure 3). It was possible to observe that AEG did not affect the structure of kidney tissue such as Bowman's capsule. According to the literature, there are some factors that influence the toxicity of chemical compounds in renal functions, for example, shape and activity of chemicals, effective dose, distribution, and excretion of compounds, among others (Motamed Jahromi & Niami Jahromi, 2020). Somchit et al. (2014) suggested that these alterations are related to the inhibitory action of NSAIDs on PGs (PGE_2 and PGI_2), which can affect the renal blood supply and consequently kidney functions are affected.

Interestingly, the present study shows that the administration of AEG along with ketoprofen treatment was able to reverse these changes. Considering that removal of urea and creatinine mainly occurs by glomerular filtration (Sodré et al., 2007), it is possible to suggest that the AEG could positively interfere in the excretion of these molecules. Previous studies have reported that this diuretic effect is due to the presence of bioactive substances, such as methylxanthines, which have already been identified in AEG (Bolignano et al., 2007; Santana & Macedo, 2019; Yamaguti-sasaki et al., 2007). Besides that, it is known that diuresis effect occurs on adenosine A1 receptors predominate in the afferent arteriole (Rieg et al., 2005), especially in the outer cortex, inhibiting its sustained constriction (Vallon et al., 2006).

The uric acid excretion was also affected by the treatment with ketoprofen. Its elimination depends on four processes: glomerular filtration, almost complete absorption, secretion, and post-secretory reabsorption (Lipkowitz, 2012). After filtration, uric acid enters into the proximal tubule and the remaining stages of excretion dependent on transporters located along the tubule (Lipkowitz, 2012). The urate transporter (URAT1) is in the apical membrane of the proximal tubule cells and plays an essential role in the reabsorption of urate (Xu et al., 2017). The organic ion transporters (OAT1 and OAT3) are present in the basolateral membranes of these cells and, through the ion exchange, are able to participate in the secretion of urate (Lipkowitz, 2012). Khamdang et al. (2002) observed that several NSAIDs, including ketoprofen, are transported by OAT1 and OAT3, which suggests that these carriers are involved in the excretion of these drugs. In addition, these authors also suggested that such transporters are involved in the side effects caused by NSAIDs in the kidney (Khamdang et al., 2002). Besides that, there is evidence that NSAIDs may competitively inhibit the OAT1 carriers (Apiwattanakul et al., 1999), which can explain the increased uric acid levels in the serum of the animals from Ket group.

It was demonstrated that the groups which received AEG exhibited similar results to the control group regarding the uric acid levels (Figure 3), and this behavior might be associated with the presence of polyphenols in AEG. In fact, some studies have demonstrated that polyphenols, such as flavonoids, can have the opposite effect to that caused by ketoprofen in uric acid excretion (Hu et al., 2012; Wang et al., 2010). In a study conducted by Hu et al. (2012), it was demonstrated that quercetin increased the uric acid excretion in hyperuricemic animals without causing other changes. These authors suggested that the results are related to the expression of organic ion transporters in the kidney, since quercetin caused an increase in the expression of OAT1 and the transporters lowered the URAT1 expression, thus facilitating the excretion of uric acid.

While the NP-SH levels in the renal tissues were not reduced by any of the treatments employed, the CAT activity was decreased by the treatment with ketoprofen (Figure 4). CAT is an enzymatic endogenous antioxidant, and some compounds can inhibit this enzyme activity (Glorieux et al., 2015; Kodydková et al., 2014; Krych et al., 2014). For instance, ketoprofen has a benzene ring in its chemical structure, and may inhibit CAT activity by the interaction with the amino acids present in its catalytic site. Moreover, it was demonstrated that the lowest dose used for AEG was able to prevent damage to CAT caused by ketoprofen (Figure 4). This result may be related to the fact that the decomposition of H_2O_2 catalyzed by CAT prevails when the substrate concentration is higher than 10^{-4} M, and can be preserved in the presence of antioxidants that can react with H_2O_2 (Kodydková et al., 2014). Following the re-establishment of antioxidant defense, the oxidative renal environment returns to homeostasis, which can lead to the re-establishment of the renal function.

As it can be seen from Table 1, the analyzed hematological parameters were affected by the treatment with ketoprofen. It was possible to observe an increase in the number of leukocytes, which shows the response of the organism to different stimuli such as stress, adrenaline, and the presence of NSAIDs in the bloodstream (Yamamura et al., 1996). However, it was observed that the combination between ketoprofen and AEG reestablished the number of white blood cells (Table 1). Moreover, ketoprofen treatment caused a significant reduction in the number of erythrocytes, hemoglobin concentration and hematocrit. In the analysis of cell morphology a significant presence of polychromatophilia was observed, which was characterized by polychromatic macrocytes, denoting erythroid hyper-regeneration and possibly influenced the increase of MCV values (Medeiros & Dittrich, 2014). These results characterize the onset of anemia in group that received ketoprofen (Goldstein et al., 2011) evidencing that the use of NSAIDs aggravates the condition of anemia through a reduction in hemoglobin over time.

Several studies have shown that anemia could be associated with drug treatment, and should be classified as hemolytic anemia induced by drugs. However, the onset of anemia caused by various NSAIDs could also be related to the damage caused by these drugs in the gastrointestinal tract (Marlicz et al., 2014; Scarpignato & Hunt, 2010). In gastric mucosa, NSAIDs can cause damage through its direct action on the mucosa, which results in physical and chemical changes and effects mediated by the inhibition of PGs synthesis, such as the lowering of mucus production, bicarbonates and cell proliferation. These effects, combined with changes in microcirculation and the release of free radicals, result in damage to the structure and function of the gastrointestinal mucosa, with consequent hemorrhagic lesions (Scarpignato & Hunt, 2010).

Our work showed that the AEG did not cause any anemia-related changes through the use of ketoprofen. Afsana et al. (2004) demonstrated that the use of tannic acid, impaired iron absorption, and iron-induced anemia in the animals. In view of the large number of polyphenols found in the AEG, it could be supposed that this mechanism has been a contributory factor in the observed results in the present study.

5 | CONCLUSION

Our results confirmed that AEG contains promising antioxidants that can protect the body from side renal and hepatic effects caused by

ketoprofen. It was demonstrated that this drug was able to interfere with endogenous antioxidant defense, which was preserved by the previous treatment with AEG. The results outlined here are an indicative of how a potential joint application of medicinal and natural product in anti-inflammatory therapy can mitigate its side effects. Finally, it was underlined the importance of conducting studies that involve pharmacodynamics and pharmacokinetics of the substances used in this research, so that the safety and efficacy of both, drug and natural extract, can be ensured.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHOR CONTRIBUTIONS

Caroline Belló: Conceptualization; Data curation; Formal analysis; Investigation; Methodology. **Ana Paula Prestes:** Data curation; Formal analysis; Methodology. **Josiane Schemberger:** Formal analysis; Investigation; Methodology. **Ana Carolina Hacke:** Data curation; Investigation; Writing-original draft. **Romaiana Pereira:** Writing-review & editing. **Francine Manente:** Formal analysis; Investigation; Methodology. **Iracilda Carlos:** Methodology; Validation. **Cleverton Roberto Andrade:** Formal analysis. **Daniel Fernandes:** Data curation; Formal analysis. **Ivana BM da Cruz:** Formal analysis; Resources. **Tais Unfer:** Data curation; Formal analysis. **Jose Carlos Vellosa:** Conceptualization; Project administration; Resources; Writing-review & editing.

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