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Article

Passion fruit (*Passiflora edulis*) leaf extract modulates the oxidative metabolism of rat peritoneal neutrophils in a model of inflammation

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This study was conducted to evaluate the effect of extracts of *Passiflora edulis* Sims leaves on the oxidative metabolism of rat peritoneal neutrophils using a model of acute inflammation. The extract was obtained by maceration in 70% ethanol, evaporation under reduced pressure and lyophilisation. Total phenolic content (TP) was determined by the Folin-Ciocalteu assay. The *P. edulis* extract, in different doses, was administered by gavage 1 h prior to inflammation induction by carrageenan (8 mg/kg, i.p.); five hours later, the neutrophils were obtained by intraperitoneal lavage. The tests performed in neutrophils were cytochrome C and chemiluminescence assay as well as myeloperoxidase (MPO), superoxide dismutase (SOD), glutathione peroxidase (GPx) and catalase (CAT) activities. The administration of the extract reduced the number of neutrophils recruited to the site of inflammation; however, the extract did not alter the activity of NADPH oxidase as well as SOD activity in these cells. The MPO and CAT activities in peritoneal neutrophils of rat treated with extract was lower than in the control group, and the GPx activity was increased. Based on the experimental model utilised, the anti-inflammatory potential of *P. edulis* leaf extract could be related to the presence of phenolic compounds in the extract.

Keywords: *Passiflora edulis* Sims. Inflammation. Neutrophil. Reactive oxygen species (ROS). Antioxidant enzymes.

INTRODUCTION

Neutrophils are recruited at the onset of the pathogen defence response as an essential component of the acute inflammatory process. The killing and microbicidal functions of neutrophils are directly related to activation of the NADPH oxidase system, which generates reactive oxygen species (ROS) in a process called oxidative burst. Molecular oxygen undergoes a one-electron reduction generating superoxide anions (O2•-), the precursor to a number of more reactive metabolites, such as hydroxyl radicals or hypochlorous acid (HOCl). These metabolites play essential roles in killing many types of bacteria and other invaders. HOCl is highly microbicidal and its formation requires myeloperoxidase (MPO), the principal enzyme located in azurophil granules of neutrophils (Bylund *et al*., 2010; Babior, 2002; Hampton *et al*., 1998; Klebanoff, 2005).

Despite the regulation of ROS production and use, this oxidative metabolism can set off an ongoing inflammatory process, leading to an oxidative stress status that can cause serious damage to the host organism. In such cases, endogenous antioxidants are often unable to prevent oxidative damage and require exogenous scavengers that

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are obtained from the diet, such as vitamins, minerals, carotenoids and polyphenols (Ciz *et al*., 2012; Pruchniak *et al*., 2013; Silva *et al*., 2014; Zhang, Tsao, 2016).

Passion fruit (*Passiflora edulis* Sims) is popular for its pulp and the medicinal use of its leaves. The plant has been largely used in American and European countries as a sedative or tranquiliser (Dhawan *et al*., 2004; Coleta *et al*., 2006; Petry *et al*., 2001). Recently, the leaves have been recognised for their anti-inflammatory potential (Montanher *et al*., 2007). The predominant compounds of *Passiflora* species are polyphenols, which are known for their antioxidant properties (Ferreres *et al*., 2007; Zeraik, Yariwake, 2010; Medina *et al*., 2017).

In the literature, little data exist on the effects of *P. edulis* on the oxidative metabolism of neutrophils in inflammation. Thus, considering that oxidative stress has been linked to the development of several diseases (Carocho, Ferreira, 2013), this study evaluated the effects of P. edulis leaf extract on the oxidative metabolic processes in rat peritoneal neutrophils by assessing ROS production and the activity of antioxidant enzymes of these cells.

MATERIALS AND METHODS

Plant material

The leaves of *Passiflora edulis* Sims (Passifloraceae) were collected in Alfenas, Minas Gerais, Brazil, in September of 2012 and identified by Prof Dr Douglas Carvalho of the Department of Biology at the Universidade Federal de Lavras (UFLA), Lavras, Minas Gerais, Brazil. A voucher specimen was deposited in the Herbarium at the same university (n. 22356).

Extract preparation

P. edulis leaves were air-dried at 40 ºC for 3 days. The dried and powdered leaves were extracted by maceration using 70% ethanol (Sinth, Brazil) (plant solvent, 1:10, w/v) for seven days. The extract was then filtered, evaporated under reduced pressure, and lyophilised, yielding a dry residue, according to Salles (2017).

Total phenolic content

The total phenolic content of the *P. edulis* extract was determined according to the Folin-Ciocalteu method (Singleton *et al*., 1999). Briefly, crude extracts were dissolved in water for a final concentration of 1mg/mL prior to testing. An aliquot of 100µL of the extract solution was mixed with 500 µL of Folin–Ciocalteu reagent (Sigma Aldrich, USA). After 8 min, 400 µL of sodium carbonate solution 4% was added. The mixture was allowed to stand for 2 h at room temperature before the absorbance was measured spectrophotometrically at 740 nm. The experiment was conducted in triplicate. Gallic acid (Inlab, Brazil) was used to create a standard curve. The total phenolic content of the sample was expressed as gallic acid equivalents (GAE) to 1.0 g of extract.

Animals

In total, 42 adult Wistar male rats, weighing approximately 350 ± 50 g, were obtained from the Universidade Federal de Alfenas (UNIFAL-MG). Animals were kept at a controlled temperature of 23 ± 2 ºC with alternating 12 h periods of light and dark and were allowed access to food and water ad libitum. All *in vivo* experiments followed the guidelines of the International Council for Laboratory Animal Science and were approved by the Ethical Committee for Animal Experimentation of UNIFAL-MG (protocol number 523).

Experimental groups

The *P. edulis* extract, in different doses (5, 50 and 300 mg/kg), was administered by oral gavage (p.o.) 1 h prior to inflammation induction, around 7:00 a.m. We employed an experimental model of inflammation in which rat neutrophils are primed *in vivo* by the intraperitoneal (i.p.) administration of carrageenan (C) (Sigma Aldrich, USA) solubilised in saline. Animals were placed into 6 groups of 7 animals each: (S) animals not treated with carrageenan or extract, but were treated with sterile saline (10 mL/kg, i.p.) to obtain peritoneal lavage; (D) animals treated with dexamethasone (5 mg/kg, p.o.) plus carrageenan (8 mg/kg, i.p.); (E5) animals treated with extract (5 mg/kg, p.o.) plus carrageenan (8 mg/kg, i.p.); (E50) animals treated with extract (50 mg/kg, p.o.) plus carrageenan (8 mg/kg, i.p.); and (E300) animals treated with extract (300 mg/kg, p.o.) plus carrageenan (8 mg/kg, i.p.). The number of animals for each experimental and control groups is indicated in Figure 1. All data are presented as the mean ± standard deviation (SD) from independent experiments. In each experiment, one animal from each group was analysed. The determinations of the parameters analysed were performed in duplicate or triplicate, as indicated in each item.

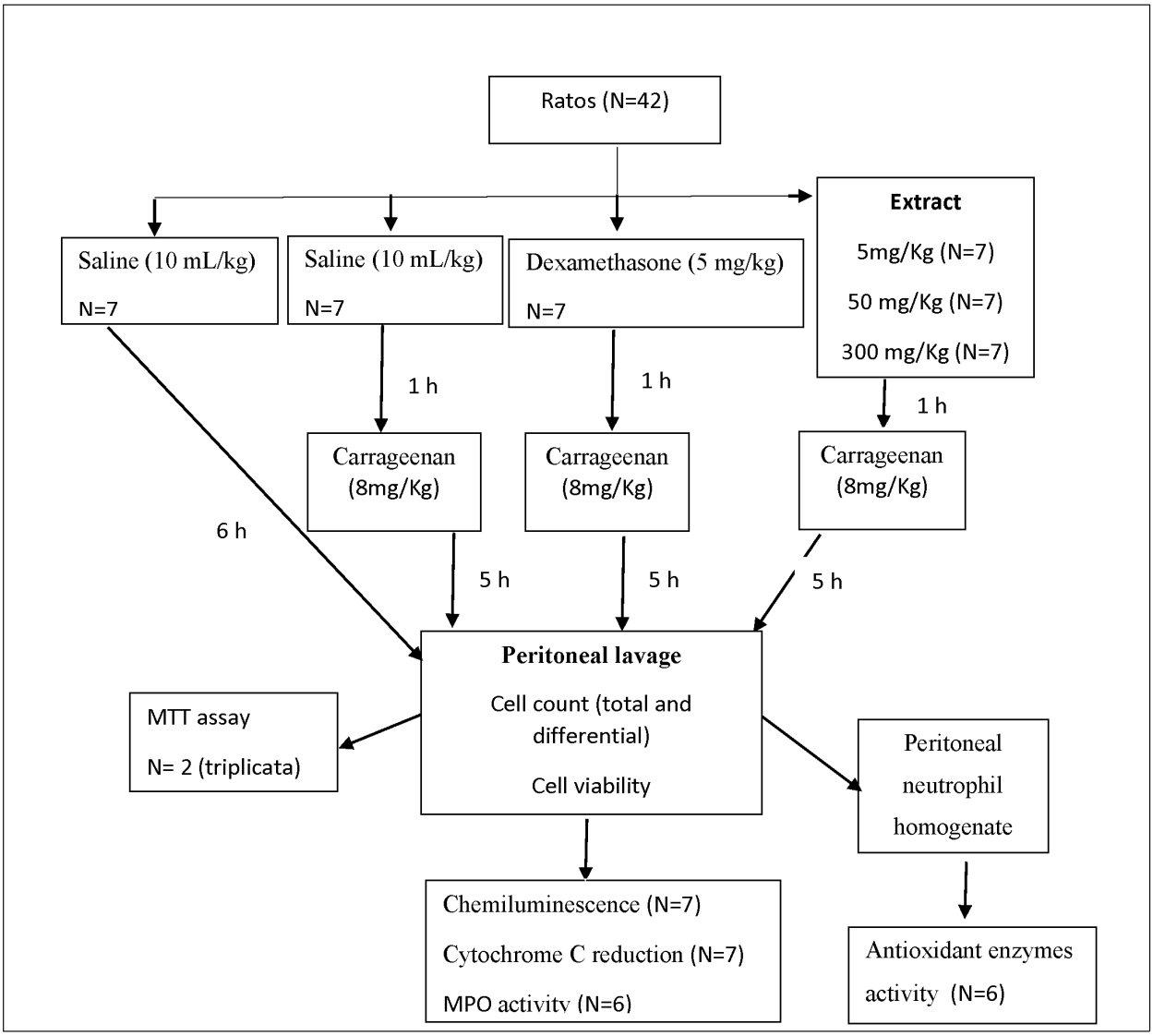

FIGURE 1 - Experimental delineation.

Neutrophil recruitment and cell preparation

The animals were sacrificed 5 hours after the carrageenan administration. Neutrophils were obtained by intraperitoneal lavage with 15 mL of phosphate-buffered saline (PBS, pH 7.4). After a gentle massage of the abdomen, the cell suspension was collected by aspiration with a syringe containing a 40 x 12 mm needle and was centrifuged at 4 ºC (1,500 rpm for 5 min). The cell pellet was re-suspended in glucose-PBS (PBS containing 0.5 mM MgCl2, 1 mM CaCl2 and 1 mg/mL of glucose). The counting of the peritoneum cells was performed in a Neubauer chamber, using 19 parts of Türk liquid (acetic acid 3% and methylene blue 1%) and 1 part of cell suspension (dilution 1/20). For the evaluation of cell morphology, the slides were stained by the MGG method modified by Rosenfeld (1947).The number of viable cells (>95% neutrophils) was determined in a Neubauer chamber (Knittel Glaser, Braunschweig, Germany) under an optical microscope by Trypan blue exclusion.

Cytotoxicity (MTT assay)

The cytotoxic effect of the extract on rat neutrophils was evaluated by the ability of living cells to reduce the yellow dye 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide (MTT – Sigma Aldrich, USA) to a purple formazan product (Mosmann, 1983).

Peritoneal neutrophils from the different treatment groups were re-suspended in RPMI-1640 medium and plated in 96-well plates (200 µL of 1 x 106 cells per millilitre in each well) with 20 µL of MTT solution (5 mg/mL RPMI-1640). The cells were incubated for 2 hours at 37°C and 5% CO2. The contents of each well were centrifuged (1200g, 10 min). The supernatant was then discarded and sediment was re-suspended adding 100 µL/well of isopropyl alcohol for dissolving the MTT formazan product. The optical density was read at 540 nm with a reference filter at 620 nm using a microplate reader. A second plate was prepared under the same conditions and incubated for 18 hours. All data are presented as the mean ± standard deviation (SD) from two independent experiments conducted in triplicate.

NADPH oxidase system activity (cytochrome C reduction)

The production of superoxide anions (O2•-) by NADPH oxidase in neutrophils was monitored by the reduction of cytochrome C. The test was conducted in a plastic bucket containing 2 x 106 neutrophils per millilitre, 100 µM cytochrome C (Sigma Aldrich, USA) and 20 µg/mL (958 U/mL) catalase (Sigma Aldrich, USA) in glucose-PBS. The buckets were kept at 37 ºC for 5 minutes. Superoxide production was initiated by the addition of PMA (Forbol 12-miristate 13-acetate, Sigma Aldrich, USA) (100 ng/mL). After the addition of PMA, the reaction was monitored by spectrophotometry for 3 min at a wavelength of 550 nm (Jones, Hancock, 1994).

Luminol-amplified chemiluminescence

The chemiluminescence of luminol is commonly used to evaluate the oxidative burst that occurs when intact phagocytes are activated by the addition of a stimulus such as PMA in an assay termed luminol-amplified chemiluminescence of activated phagocytes. For this assay, neutrophils were activated with PMA (53 ng per test) in a reaction mixture containing 1 mM of luminol (Sigma Aldrich, USA) and 1 x 106 neutrophils per millilitre. The chemiluminescent intensities of 0.3 mL samples were followed in a Geomax luminometer® 20/20. The chemiluminescence is expressed as relative light units per second (RLU/s). The reaction was performed in PBS, pH 7.4, and followed for 30 min. All measurements were made at 37 ºC (Souza Ferreira *et al*., 2012).

Myeloperoxidase activity (HOCl formation)

Peritoneal neutrophils (3 x 106 cells per millilitre) were incubated with 12 mM taurine (Sigma Aldrich, USA) in glucose-PBS at 37 ºC and were stimulated with PMA 100 ng/mL. After 30 min, the reactions were stopped by adding 20 µg/mL (958U/mL) catalase (Sigma Aldrich, USA) and placing the tubes in melting ice for 5 min. Cells were pelleted by centrifugation at 4 ºC (12 000 rpm for 10 min). The concentration of accumulated taurine chloramine present in the supernatants was quantified by measuring the oxidation of 5-thio-2-nitrobenzoic acid (TNB) to 5,5'-dithiobis-(2-nitrobenzoic acid) (DTNB) by the associated decrease in absorbance at 412 nm, as previously described (Kettle, Winterbourn, 1994).

Antioxidant enzymes activity

Peritoneal neutrophil homogenate

Peritoneal neutrophils were disrupted with sonication at 30 kHz output for two cycles of 30 seconds with 15 seconds rest intervals on melted ice. Unbroken cells were pelleted by centrifugation at 4 ºC (3000 rpm for 5 min) and the supernatant was used to determine the protein content and the activity of antioxidant enzymes.

Protein determination

The protein content of the peritoneal neutrophils homogenate was measured using the Bradford method (Bradford, 1976), using BSA (Sigma Aldrich, USA) as the standard. An aliquot corresponding to 35 µg of protein of this homogenate was used to determine the activity of antioxidant enzymes.

Superoxide dismutase (SOD) activity

SOD activity was assayed by the method of Oyanagui (Oyanagui, 1984). Aliquots of the peritoneal neutrophil homogenate were incubated with 3 mM hydroxylamine (Sinth, Brazil), 0.07 mM hypoxanthine (Sigma Aldrich, USA) and 3.4 x 10-3 U/mL xanthine oxidase (Sigma Aldrich, USA) at 37 ºC for 30 minutes in the dark. After incubation, a solution of sulfanilic acid (Sinth, Brazil), naphthalene diamine (Sigma Aldrich, USA) and glacial acetic acid (Acros organics, USA) was added and kept at room temperature for 20 minutes. The absorbance was

determined at 550 nm. SOD activity was expressed in units per milligram of protein (U/mg protein). One SOD unit (U) was defined as the enzyme activity that inhibits 50% of the reaction in this condition.

Catalase (CAT) activity

CAT activity was estimated as described by Aebi (1984). Aliquots of the peritoneal neutrophil homogenate were incubated in PBS (pH 7.4) and the reduction of 10 mM H2O2 (Sinth, Brazil) was monitored by measuring the absorbance at 240 nm for 1 minute. The activity was calculated by using a molar absorption coefficient and the enzyme activity was expressed in U/mg protein. One CAT U was defined as the enzyme activity that decomposes 1 µmol of H2O2 per minute.

Glutathione peroxidase (GPx) activity

GPx was measured using the method described by Sinet and co-workers (Sinet *et al*., 1975) employing t-butyl hydroperoxide (Sigma Aldrich, USA) as the substrate. Aliquots of the peritoneal neutrophil homogenate were incubated in PBS (pH 7.4) with 1 mM reduced glutathione (Sigma Aldrich, USA), 0.2 mM NADPH (Sigma Aldrich, USA) and 1.8 U/mL glutathione reductase (Sigma Aldrich, USA) for 3 minutes at 37 ºC. The absorbance was monitored at 340 nm. After 3 minutes of incubation, the reaction was initiated by the addition of 0.9 mM *t*-butyl hydroperoxide. The absorbance was monitored for five more minutes at 340 nm. GPx activity was expressed in U/mg protein. One GPx U was defined as the enzyme activity that consumes 1 µmol of NADPH/min.

Statistical analysis

The results were expressed as the mean ± standard deviation; they were also submitted to one-way analysis of variance and compared using the Scott-Knott test at 5% significance.

RESULTS

Yield and total phenolic content

Overall, 18.6 g of extract of *Passiflora edulis* Sims leaves was obtained for each 100 g of dry and pulverised vegetable matter.

We used gallic acid to create a standard curve (y = 101.68x + 0.0249, R2 = 0.9994) and the total phenolic content in *P. edulis* extract was determined to be equivalent to 46.7 ± 1.15 mg GAE g-1.

Neutrophil recruitment and viability

Animals were treated with *P. edulis* extract 1 h before carrageenan was used to induce inflammation. The administration of carrageenan to rats induced a significant amount of neutrophil migration compared with the S group treated intraperitoneally with saline. Animals treated with *P. edulis* extract showed significantly decreased neutrophil migration at all tested doses (Figure 2). As expected, animals that were treated with dexamethasone, which was used as a positive control at a dose of 5 mg/kg, showed a strong inhibition of neutrophil migration.

Figure 3 shows results from the cell viability assay measuring the effect of *P. edulis* extract on peritoneal neutrophils from each of the different treatment groups. Viable neutrophils were quantified by the MTT assay, an established method for probing cell death. There were no significant differences in the peritoneal neutrophil viability between the groups for 2 or 18 hours. These data indicate that the tested doses of *P. edulis* extract (5, 50 and 300 mg/kg) are all nontoxic over at least an 18-h period and can be used safely for further biological studies.

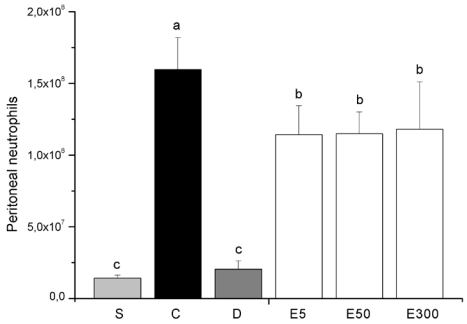

FIGURE 2 - Effect of the treatments on rats' peritoneal neutrophils migration. Results are presented as means ± SD of seven animals per group. S = saline group; C = carrageenan group; D = dexamethasone group; E5, E50 and E300 = extracts groups. Different letters indicate statistical significance ($p<0.05$).

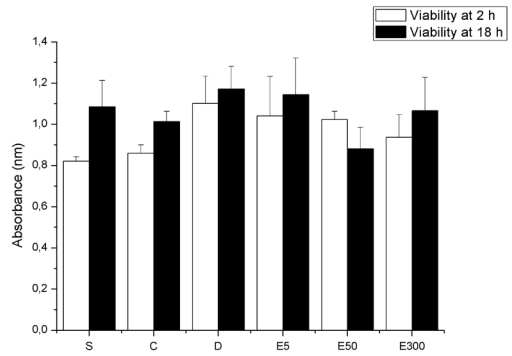

FIGURE 3 - Effect of the treatments on cell viability of rats' peritoneal neutrophils. Cells were incubated for 2 and 18 h, and the viability was measured with MTT assay. Results are presented as means ± SD from two experiments performed in triplicate. S = saline group; C = carrageenan group; D = dexamethasone group; E5, E50 and E300 = extracts groups.

NADPH oxidase activity and ROS production

Cytochrome C reduction and a chemiluminescence assay were used to assess the generation of O2•- and ROS by the NADPH oxidase system. Carrageenan increased both O2•- and ROS in peritoneal neutrophils compared with the S group (Figures 4A and 4B). The O2•- production by peritoneal neutrophils treated with *P. edulis* extract did not differ from that by the C group (Figure 4A). The chemiluminescence assay measured both ROS formation and O2•- generation (Figure 4B), showing that treatment with *P. edulis* extract decreases the amount of ROS produced by peritoneal neutrophils when compared with the C group. The most significant decrease was observed in the E300 group, which had approximately 69% lower levels of ROS than observed in the C group.

MPO activity

A TNB oxidation assay was used to indirectly measure HOCl concentration. The peritoneal neutrophils from the C group showed a increase in HOCl generation compared with the S group. The pre-treatment with *P. edulis* extract decreased HOCl generation compared with the C group (Figure 5) in a dose-dependent manner (reduction of approximately 22% in the E5 group, 43% in the E50 group and 56% in the E300 group).

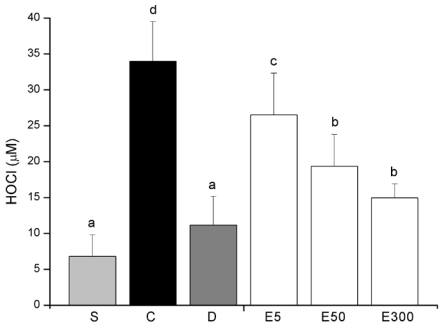

FIGURE 5 - Effect of the treatments on HOCl production from rats' peritoneal neutrophils. Results are presented as means ± SD of six animals per group. S = saline group; C = carrageenan group; D = dexamethasone group; E5, E50 and E300 = extracts groups. Different letters indicate statistical significance ($p<0.05$).

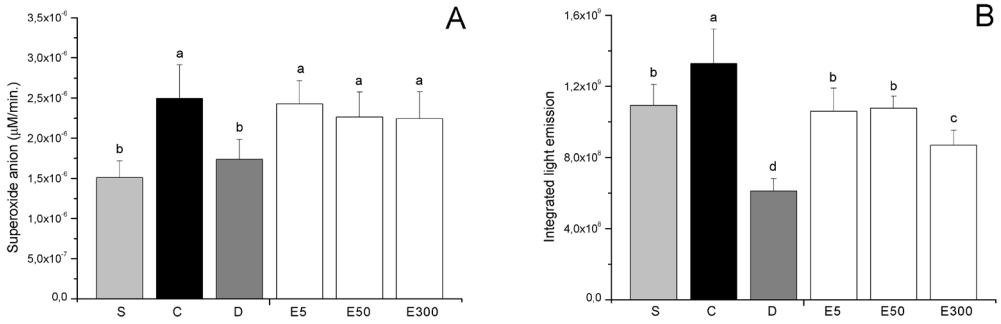

FIGURE 4 - A. Effect of the treatments on O_2•- production by rats peritoneal neutrophils. Results are presented as means ± SD of seven determinations of the reduction of cytochrome C 100 µM by rats peritoneal neutrophils (2 x 10^6 cells per millilitre) stimulated with PMA (100 ng/mL). B. Effects of the treatments on ROS production. Results are presented as means ± SD of the integrated area of light emission obtained for the oxidation of luminol 1 mM promoted by rats peritoneal neutrophils (1 x 10^6 cells per millilitre) stimulated with PMA (53 ng/mL) from seven experiments of each group. S = saline group; C = carrageenan group; D = dexamethasone group; E5, E50 and E300 = extracts groups. Different letters indicate statistical significance ($p<0.05$).

Activity of antioxidant enzymes

The increased level of oxidative stress is related to an overproduction of ROS or a deficiency in the antioxidant defence system. The treatment with *P. edulis* extract decreases the ROS production. With regard to effects on antioxidant enzymes, no significant differences in the SOD activity were observed in peritoneal neutrophils from groups treated with the *P. edulis* extract compared to the C group (Figure 6A). These results are consistent with those obtained regarding the O2•- production (Figure 4A). However, the CAT activity in peritoneal neutrophils from the E300 group was approximately 55% lower than that in the control group (Figure 6B), and the GPx activity was increased in a dose-dependent manner (Figure 6C), achieving results similar to those in the S and D groups.

DISCUSSION

This study evaluated the anti-inflammatory potential of *P. edulis* leaf extract by employing a model of acute peritonitis, which was induced by the administration of carrageenan. We observed that *P. edulis* extract reduced the number of cells recruited to the site of inflammation and modulated the oxidative metabolism of rat peritoneal neutrophils by decreasing the ROS production and modulating the activity of antioxidant enzymes.

P. edulis is rich in phenolic compounds, especially flavonoids C-glycosides derivatives of apigenin and luteolin, such as vitexin, isovitexin, orientin and isoorientin (Dhawan *et al*., 2004; Ferreres *et al*., 2007). Salles (2017) confirmed the presence of flavonoid isoorietin and suggested the presence of several flavonoids with an OH group at C-3', 4', 5 and 7 in the *P. edulis* leaf extract.

Many studies over the past years have shown that polyphenols found in dietary and medicinal plants often possess antioxidant activity that inhibits oxidative damage and may consequently prevent inflammation (Khanna *et al*. 2007; Manach *et al*., 2004; Rudnicki *et al*., 2007). Our results highlight the fact that the *P. edulis* leaf extract represents a source of phenolic compounds and high antioxidant potential.

Neutrophils are important for host defence; by releasing a series of mediators, neutrophils can induce the accumulation of inflammatory cells (Weiss, 1989). These phagocytic cells play a key role in the front-line defence, as they are recruited almost immediately to the site of injury or infection (Pruchniak *et al*., 2013). In our study, 5 hours after the administration of carrageenan (8 mg/kg, i.p.) in rats, peritoneal neutrophils were collected. The inflammatory effect induced by carrageenan has been linked to neutrophil infiltration and the production of neutrophil-derived ROS (Chang *et al*., 2012). The data obtained in this study showed that treatment with *P. edulis* leaf extract reduces the level of

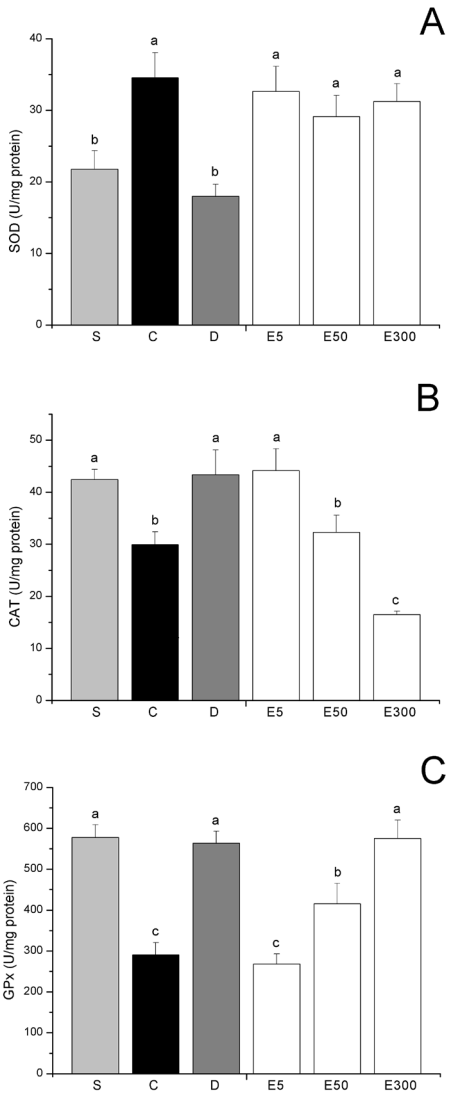

FIGURE 6 - Effect of the treatments on the activity of antioxidant enzymes in rats' peritoneal neutrophils. A. Superoxide anion (SOD). B. Catalase (CAT). C. Glutathione peroxidase (GPx). Results are presented as means ± SD of six animals per group. S = saline group; C = carrageenan group; D = dexamethasone group; E5, E50 and E300 = extracts groups. Different letters indicate statistical significance ($p<0.05$).

neutrophil migration to the site of inflammation (Figure 2). Moreover, the extract did not show a cytotoxic effect on the cells according to the MTT assay (Figure 3).

Upon activation, neutrophils generate ROS through the NADPH oxidase system in a process known as respiratory burst. The ROS generated by neutrophil activation are able to oxidise molecules such as luminol to excited and unstable intermediates, which can be measured by chemiluminescence (Gasbarrini *et al*., 1998). In this study, O2•- production was evaluated using a cytochrome C reduction assay (Figure 4A), and the ROS pool was monitored using chemiluminescence (Figure 4B). Injection of carrageenan into the peritoneal cavity of rats elicited an acute inflammatory response characterised by the accumulation of neutrophils in the peritoneal cavity and increased production of O2•, ROS and HOCl (Figures 4A, 4B and 5). The NADPH oxidase system activity, reflected by the O2•- production, was similar in the groups treated with *P. edulis* extract and the C group. However, the ROS pool was decreased in the extract-treated groups when compared to the C group, especially at the dose of 300 mg/kg. These data show that treatment with the extract did not change the NADPH oxidase activity, yet it interfered with the generation of by-products that result from the activation of this complex or other pathways involved in ROS generation.

The O2•- is a primary product of NADPH oxidase activation, it is required for the generation of more potent molecules, including one of the most potent microbicidal molecules, HOCl. HOCl is produced by the MPO enzyme in a reaction involving H2O2 and a chloride ion (Hampton *et al*., 1998; Dahlgren, Karlsson, 1999; Winterbourn *et al*., 2000; Biswas, 2016). MPO activity, which is abundant in neutrophil azurophilic granules, was shown to decrease in a dose-dependent manner upon treatment with *P. edulis* extract, as shown by the HOCl generation in treated groups compared with the C group (Figure 5).

MPO-deficient neutrophils are classified as "low responders" when ROS are measured by luminol-amplified chemiluminescence (Bylund *et al*., 2010). As HOCl participates in luminol oxidation, which can be detected by chemiluminescence, the decreased ROS produced by peritoneal neutrophils (Figure 4B) can be explained by the decreased HOCl observed in extract-treated groups.

Polyphenols have powerful antioxidant activities, mediated by their ability to scavenge a wide range of ROS. Therefore, the effects of various polyphenols on the respiratory burst of phagocytes can be at least partially explained by their antioxidant properties, which diminish adverse oxidative stress by scavenging ROS that have already been produced, including hypochlorous acid (Ciz *et al*., 2012; Zhang, Tsao, 2016). Thus, the results observed in this study could be attributed to the presence of phenolic compounds in the extract.

Zeraik and co-workers (2011) have demonstrated strong inhibitory effects of *P. edulis* rind extract on MPO, an effect that correlated with the extract's isoorientin content. The results indicated that this compound is able to interact directly at the active site or by otherwise altering the structure of MPO. Thus, the observed dose-dependent decrease in HOCl production in response to *P. edulis* extract is likely related to the extract's polyphenolic substances. These could act both on the enzyme and HOCl (Zeraik *et al*. 2011; Zhang, Tsao, 2016).

In most cases, the generation and elimination of ROS function in a dynamic balance. However, the disruption of this balance can lead to a state of oxidative stress, which can damage the body's own cells and tissues. The increased oxidative stress is related to a hyperproduction of ROS or a deficiency in the antioxidant defence system, which consists of enzymatic (SOD, CAT and GPx) and non-enzymatic scavenger components (Ciz *et al*., 2012).

Our results do not show any difference between the SOD activity in peritoneal neutrophils from the P. edulis extract-treated groups compared with that in the C group (Figure 6A). SOD acts as a first line of defence against O2•- by catalysing the dismutation of this species to H2O2. Therefore, the SOD activity results are consistent with the results obtained with the cytochrome C reduction assay (Figure 3A), which showed that the O2•- production in extract-treated groups did not differ from that in the C group.

Generated H2O2 is detoxified by conversion into molecular oxygen and water by CAT or GPx (Lei *et al*., 2014). Our results show that treatment with *P. edulis* extract decreases the CAT activity in peritoneal neutrophils (Figure 6B), while the extract increases the GPx activity of these cells (Figure 6C) in a dose-dependent manner.

A previous study showed that flavonoids can inhibit the CAT enzyme (Zhu *et al*., 2007; Krych, Gebicka, 2013). This inhibitory effect is, at least partially, due to the formation of hydrogen bonds between CAT and flavonoids, which causes conformational changes in the enzyme that inhibit the reaction of H2O2 with the

haem centre of the enzyme. The decreased CAT activity should result in an accumulation of H2O2 in neutrophils, promoting the formation of the highly reactive and damaging hydroxyl radical, which can be formed by the reaction of H2O2 with Fe2+ (via the Fenton reaction). A compensatory mechanism for the decreased CAT activity may include increased GPx activity, which is considered one of the most effective enzymes in reducing oxidative stress by the conversion of glutathione into oxidized glutathione (Lubos *et al*., 2011).

MPO enzyme activity also functions in the elimination of excess H2O2. Thus, the antioxidant enzymes results associated with reduced HOCl production leads to two hypotheses about the effects of *P. edulis* extract on the oxidative metabolism of peritoneal neutrophils. The first is that the inhibition of CAT by the extract's phenolic compounds increases the levels of H2O2, leading to an adaptive response that includes an increase in GPx activity. This regulatory response may sufficiently reduce MPO substrate levels, thereby decreasing HOCl production. The second hypothesis is that the extract inhibits CAT and MPO activities, resulting in increased H2O2 levels and a consequential modulation of GPx enzyme activity. Further studies are necessary to thoroughly assess these hypotheses.

CONCLUSIONS

We demonstrated that *P. edulis* leaf extract is a potential source of strong antioxidant molecules and that it anti-inflammatory and antioxidant potential. According to the results, the extract may modulate oxidative stress and diminish the intensity and endurance of the inflammatory process.

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