Original Research Article

Amentoflavone-rich extract of *Ouratea fieldingiana* leaves,with high content of phenolic compounds and antioxidant activity,reduces mice acute inflammation

ABSTRACT

Aims: To evaluate the antioxidant and anti-inflammatory effects of *Ouratea fieldingiana* leaf extract(OFE) and its main constituent amentoflavone (AMT).
Study design: The phenolic compounds of *Ouratea fieldingiana* leaves (OFE) were quantified and chemical structure characterized and the anti-inflammatory effects of OFE and its main constituent amentoflavone (AMT) were evaluated in mice paw edema and peritonitis.
Place and Duration of Study: The work involved the partnership of the Natural Products Chemistry Laboratory and Inflammation Physiopharmacology Laboratory, between July 2020 and June 2021.
Methodology: The OFE was obtained from *O. fieldingiana* 70%ethanolextract. OFE subjected to a classic chromatographic column furnished AMT.The phenolic compounds were characterized by HPLC and quantified by Follin-Ciocalteu methodology. The antiradicalpotential was evaluated by DPPH and ABTS methods. Anti-inflammatory activity was determinedusing Female Swiss mice received per oral OFE (0.1-10 mg/kg) or sterile saline 60 min before stimulation with carrageenan (300 mg) for evaluation of the parameters: edema, abdominal hypernociception, neutrophil migration and oxidative stress markers. In the peritonitis model, reduced glutathione, malondialdehyde, myeloperoxidase and catalase activities were measured.
Results:AMT was identified in the OFE as the main compound, with 292.64 ± 3.87 mg/g of extract. OFE has an average inhibitory concentration of 9.81 ± 0.17 μg/mL of extract to inhibit the DPPH radical. OFE and AMT inhibited paw edema [OFE: 32% (0-2 h), 28% (2-4 h); AMT: 55% (0-2h); 51% (2-4 h)], neutrophil migration (OFE: 65%; AMT: 67%); myeloperoxidase activity (OFE: 37%; AMT: 45%) and visceral nociception (OFE: 31%; AMT: 35%). OFE, but not AMT, increased catalase activity (57%) and reduced glutathione (62%), but decreased malondialdehyde (69%).
Conclusion: The amentoflavone-rich extract from *O. fieldingiana*leaves presents antioxidant activities *in vitro* and *in vivo* and anti-inflammatory activities *in vivo*, corroborating the popular use of the plant.

Keywords: Ouratea; Amentoflavone; Oxidative stress; Antioxidant activity;anti-inflammatory activity;Hypernociception.

1. INTRODUCTION

In the Brazilian traditional medicine, the aqueous infusions prepared from the stem barks of *Ouratea hexasperma* are used to treat cutaneous wounds and dermatophytosis[5 1], and other inflammatory disorders[7 2].*Ouratea* genus plants generally show similarities in their chemical composition, containing a variety of active compounds such as phytosterols, phytoestrogens, free or fatty-acid esterified triterpenes, lectins, lignans, and flavonoids[8 3].In respect to the specie *O. fieldingiana*, commonly known as Batiputá, the seeds produce through decoction an oil, that has been prescri stomach ache, erysipelas, uterine wounds and rheumatism[9 4] and anticholinesterase activities[6 5,10 6].The polar fraction of this oil and contains amentoflavone as the main constituent, but also other flavonoids such as rutin, kaempferol-3-*O*-rutinoside, isoquercitrin, quercetin and apigenin, all of them with reported anti-inflammatory activity[11-14

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7-10].Amentoflavone possess also the properties antidiabetic[15 11], antitumoral[16 12], anti-SARS-CoV-2[17 13] and antioxidant, demonstrated *in vitro*[18-19 14,15].

Recent investigations have shown that inflammation is an important factor for the progression of several acute or chronic disorders, such as diabetes, cancer, cardiovascular and arthritis, being directly related to oxidative damage[1,2 16,17]. The most prescribed anti-inflammatory drugs, although effective,are limited due to its adverse effects, causing gastric, renal, cardiovascular disorders and reduced host defenses against infections[3 18]. Thus, the search for novel anti-inflammatory and antioxidant molecules from natural sources that can suppress inflammation causing minimal adverse effects is a great challenge.

According to the World Health Organization, 80% of the world's population use traditional medicine for primary care using herbal extracts. The therapeutic effects of plant extracts and their active constituents have been validated by pharmacological studies[4-6 1,5,19].Thus, this study aimed to validate popular use of *O. fieldingiana* by evaluating the phenolic composition, the anti-inflammatory and antioxidant effects of *O. fieldingiana* leaf aqueous extract and its major constituent amentoflavone *in vitro* against free radicals and on the mice models of paw edema and peritonitis induced by carrageenan.

2. EXPERIMENTAL DETAILS

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2.1Collection of the plant material

O. fieldingiana leaves were collected in the municipality of Trairi, Ceará, Brazil, in March 2019 (3°13'01.9''S 39°23'20,1''W). An exsiccate of the plant was deposited at the Herbarium Prisco Bezerra -Federal University of Ceara (62392) in April 2019. In accordance with the Brazilian Federal Law No. 13123/2015, the assessment activity was registered at the National System for the Management of the Genetic Heritage and the Associated Traditional Knowledge (Code A67BFFF).

2.2Obtaining of aqueous extract from *Ouratea fieldingiana* and amentoflavone

Dried leaves of *O. fieldingiana* were macerated with 70 % v/v (1:1) ethanol for 7 days, was evaporated until removing all alcohol, leaving a remaining aqueous extract mixed with a fatty green insoluble material.This fattymaterial was removed from filtration, in a Buchner funnel and aqueous solution was lyophilized to obtain *O. fieldingiana*extract (OFE) used in all experiments [5].The isolation and structural characterization of the main constituent amentoflavone (AMT) was performed according to previously published procedures[15].

2.3 Quantification of total phenols and flavonoidsof OFE

The determination of the total phenolic content was carried out using spectroscopy in the visible region using the Folin-Ciocalteu reagent, following the methodology of Sousa et al. [20]. For the quantification of flavonoids. The reagent aluminum chloride ($AlCl_3$) at 2.5% was used following the methodology of Funari and Ferro [21].

2.4 High performance liquid chromatography (HPLC) with the diode array detector (DAD) of OFE

To identify the phenolic compounds, a methanolic solution of the OFE with a concentration of 10 mg/mL was injected into the equipment. Standards were purchased from Sigma Chemical Co. (St. Louis, MO, USA). The solvents used for extraction were of analytical grade (Vetec®), in the analyzes the solvents used were of HPLC grade (J.T. BAKER®). Chromatographic analyzes were performed on a Shim-pack reversed phase column (CLC) ODS GOLD (4.6x250mm, 5μm). Mobile phases C and D were acetonitrile and Milli-Q water acidified to pH 2.8 with phosphoric acid, correspondingly, solvent gradient was used as follows: 0-15 min, an isocratic elution with C:D (20:80 v/v); 17-25 min, linear variation up to C:D (40:60 v/v); 25-40 min, an isocratic elution with C:D (20:80 v/v). The flow rate was 1.0 mL.min-1, with an injection volume of 20 μL and a wavelength of 350 nm. The peaks relating to the constituents present in the HPLC chromatogram were confirmed by comparing their retention time with that of the reference standard and by DAD spectra (200 to 400 nm).

2.6. Antioxidant activity *in vitro* assay of OFE

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The antioxidant activity will be evaluated using the DPPH (2,2-diphenyl-1-picrylhydrazyl) method, following the methodology described by Becker et al. [22], with modifications, and by the ABTS method (2,2'-azino-bis(3-ethylbenzothiazoline-6sulfonic acid)) described by Re et al. [23]. Both tests will be performed in a 96-well flat-bottom microplate in an Elisa BioTek reader, model ELX 800. The results are expressed as percentage of inhibition, calculated by PI% = [(ACAS)/AC].100, where AC is the absorbance of the control solution DPPH or ABTS at the initial time and AS is the absorbance of the sample solution containing DPPH or ABTS at the final time. The negative control will be the solution with all reagents except the sample. The antioxidant Quercetin and Gallic acid will be used for comparison.

2.7Animals

Swiss mice (25 - 35 g) of both genders were maintained in the animal room of the Superior Institute of Biomedical Sciences - State University of Ceara (UECE), receiving filtered water and food ad libitum under adequate conditions (25 °C; 12 h light/dark cycle). Experimental protocols were conducted in accordance to the guidelines of the Brazilian College of Animal Experimentation (COBEA), and approved by the Animal Care and Use Committee of UECE (CEUA Nº 0559924-4). Animals were brought to laboratory 1 h before experiments for adaptation.

2.8Animals peritonitis treatment

Female Wistar mice (25-30 g) from our facilities were maintained under standard conditions (25 °C; cycle of 12 h light/12 h dark, and food and water *ad libitum*). The total of 36 animals were used, in groups of 8 animals. The procedures were conducted according to the guidelines of the NIH guidelines and were approved by the Animal Care and Use Committee/State University of Ceará, Brazil (CEUA/UECE, No. 4153018/2018). Animals received per oral treatment with OFE (0.1-10 mg/kg), AMT (10 mg/kg), indomethacin (5 mg/kg) or sterile saline (0.9%) 60 min prior the administration of inflammatory stimuli for evaluation of the following parameters: edema, abdominal hypernociception, neutrophil migration and oxidative stress markers in the models of paw edema and peritonitis.

2.9Paw edema induction

Edema was induced in left/right hind paw of mice by subcutaneous administration of carrageenan (300 µg). Paw volumes were measured by hydroplethysmometry, immediately before induction (0 hour) and every 60 min until five hours. Edema was expressed as Δ paw volume (µL) from basal value or area under the curve AUC (arbitrary units)[24].

2.10Peritonitis induction

Peritonitis was induced by intraperitoneal injection of carrageenan (300 µg) and the abdominal hypernociception evaluated by the application of a non-flexible filament coupled to a digital algesimeter, until occurrence of a withdrawal response. Values (in g) were obtained immediately before the peritonitis induction (0 hour) and every 60 min until hour 5[25].Five hours after peritonitis induction mice were euthanized by the section of great vessels, under anesthesia (xylazine 10 mg/kg + ketamine 100 mg/kg i.p.). Animals had their peritoneal cavity washed with 3 mL of heparinized saline solution (5 UI heparin). Total leukocyte influx was measured under optical microscopy using a Neubauer chamber (cells/mL). The fluid was centrifuged (670×g), and the pellet resuspended in 50 µL saline for preparation of smears slides and differential leukocyte count[26]. Supernatant was used to measure the protein content (mg/mL) by the Bradford method[27] and oxidative stress markers: a) catalase (U/mg protein) [28]; b) reduced glutathione (GSH, µmol/mL)[29]; c) malondialdehyde (MDA, U/mL)[30]; d) myeloperoxidase (MPO, U/mL)[31].

2.11Statistical Analysis

Data were expressed as mean ± standard error of mean. Statistical differences between parametric data were determined by one-way analysis of variance, followed by the Bonferroni's test. $P < 0.05$ was considered significant. GraphPad Prism v. 5.00 was used for data analyses and graphs plottage.

3. RESULTS AND DISCUSSION

Table 1 shows the quantification of phenolic compounds which revealed a high content of phenolic compounds (338.62 ± 0.12 mg GAE/g extract), and a high activity against DPPH and ABTS radicals as expected since flavonoids are polyphenolic compounds which displays antioxidant activity [32]. The extraction method influences the type and quantity of phenolic compounds, for example, using ethanol,*O. fieldingiana* leaves, through HPLC-DAD analysis showed as major compound amentoflavone (AMT) with 86.88 ± 1.08 mg/g of the extract. In the present work, using another extraction method, the amentoflavone was also identified in the extract as the main compound, but in a much higher concentration of

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292.64 ± 3.87 mg/g of extract, and with an improvement in the IC_{50} value, with 9.81 ± 0.17 μg/mL of extract being necessary to inhibit the radical.

Table 1. Quantification of phenols, flavonoids and antiradical activity against DPPH and ABTS of *O. fieldingiana*extract (OFE), amentoflavone, main phenolic constituent and antioxidant standards.

Sample	Total phenols (mg GAE/g)	Flavonoids (mg QE/g)	IC_{50}DPPH (μg/mL)	IC_{50}ABTS (μg/mL)
OFE	338.62 ± 0.12	107.57 ± 0.68	9.81 ± 0.17	15.69 ± 0.36
Amentoflavone	-	-	5.42 ± 0.12	10.63 ± 0.18
Quercetin (Standard)	-	-	2.74 ± 0.08	3.98 ± 0.13
Gallic acid (Standard)	-	-	1.94 ± 0.27	13.01 ± 0.03

OFE - *O. fieldingiana* extract; GAE - Gallic acid equivalent; QE - Quercetin equivalent; IC_{50}- Average inhibitory concentration

The characterization of the compounds present in the aqueous extract of *O. fieldingiana* by high-performance liquid chromatography, presents the majorconstituent the biflavonoid Amentoflavone with a very significant amount compared to other constituents (Table 2), that suggest that probably the activities of the plant can be linked mainly to this compound. Amentoflavone present anti-inflammatory activity inGentamicin-induced kidney damage in rats [33],nevertheless, other leaf constituents as kaempferol-3-*O*-rutinosidedisplays high antinociceptive activity in Zebrafish model [34] and rutin has anti-inflammatory activities *in vitro* and *in vivo*studies [35]. Then OFE is rich in various bioactive flavonoids that together can be responsible for its great anti-inflammatory potential of this plant.

Table 2. Caracterization of phenolic compounds in the*Ouratea fieldingiana* leaf extract (OFE) by high performance liquid chromatography (HPLC)

Component	mg/g of extract	R_t (min)
Rutin	29.73 ± 0.39	6.47
Isoquercitrin	19.19 ± 0.25	7.91
Kaempferol-3-*O*-rutinoside	23.12 ± 1.39	9.38
Quercetin	3.49 ± 0.10	22.36
Apigenin	0.60 ± 0.01	24.75
Amentoflavone	292.64 ± 3.87	27.17

R_t- Retention times

Carrageenan induced paw edema since the first one hour, with a plateau being reached at 240 min (133.75 vs. saline: 0.00 μL, p<0.0001). OFE elicited dose-dependent anti-edematogenic effect, showing reductions at 60, 180 and 240 min. The maximal effect of OFE (10 mg/kg) was reached at 180 min (48.5 ± 4.9 vs. carrageenan: 120 ± 21.4 vs. saline: 5 ± 1 μL) and 240 min (62.5 ± 12.6 vs. carrageenan:133 ± 11.4 vs. saline: 0.00 μL) (Figure 1A). The area under curve was reduced at all doses in the first phase [0-2h: 23% (0.1 mg/kg); 35% (1 mg/kg) and 38% (10 mg/kg)] (Figure 1B) and in the second phase [2-4 h: 19% (0.1 mg/kg); 22% (1 mg/kg) and 45% (10 mg/kg)] (Figure 1C). Amentoflavone (10 mg/kg) reduced the paw edema at both phases: by 55% the first phase (0-2 h) and by 51% the second phase (2-4 h) (Figure 1B and 1C).

Indometachin (5 mg/kg) reduced the paw edema by 58% the first phase (0-2 h) and by 75% the second phase (2-4) (Figure 1B and 1C).

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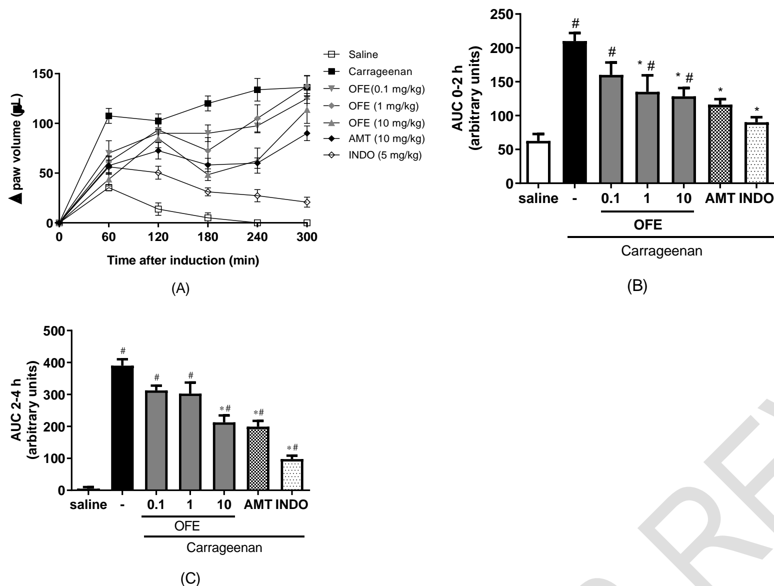

Fig. 1. The amentoflavone-rich extract of *Ouratea fieldingiana* leaves and amentoflavone reduce the mice paw edema induced by carrageenan. OFE (0.1, 1, 10 mg/kg; p.o.), AMT (10 mg/kg; p.o.) or INDO (5 mg/kg; p.o.) was given 60 min before carrageenan (300 µg; s.c.).Control group received 0.1% saline (s.c). (A) time-course, (B) AUC: 0-2 h, (C) AUC: 2-4 h. Mean ± E.P.M. One-way ANOVA, Bonferroni test. #P<0.05 vs. Saline. *P<0.05 vs. Carrageenan. OFE: Amentoflavone-rich extract; AMT: Amentoflavone; INDO: Indomethacin

Five hours after peritonitis induction, OFE (10 mg/kg) decreased the number of total leukocytes by 67% (553 ± 152 vs. carrageenan:1681 ± 68 vs. saline: 78.37 ± 11 cells/mm^3), mainly neutrophils by 66% (388 ± 57 vs. carrageenan:1147 ± 520 vs. saline: 69.25 ±12 cells/mm^3) (Figure 2A) and reduced the MPO activity by 37% (7.39 ± 0.74 vs. carrageenan: 19.63 ± 1.38 vs. saline: 1.23 ± 0.58 U/mL) (Figure 2B). Amentoflavone (10 mg/kg) also reduced total leukocytes and neutrophils, respectively, by 60% (664 ± 153 vs. carrageenan: 1672 ± 68 vs. saline: 78.37 ± 11 cells/mm^3) and by 67% (368 ± 150 vs. carrageenan: 1147 ± 80 vs. saline: 69.25 ±12 cells/mm^3), and MPO activity by 45% (8.90 ± 0.84 vs. carrageenan: 19.63 ± 1.38 vs. saline: 1. 23 ± 0.58 U/mL) (Figure 2B). Indomethacin decreased total leukocytes by 71% (474 ± 35 vs. carrageenan: 1681 ± 68 vs. saline: 78.37 ± 11 cells/mm^3), mainly neutrophils by 77 % (259 ± 40 vs. carrageenan:1147 ± 520 vs. saline: 69.25 ±12 cells/mm^3) (Figure 2A) and MPO activity (5.11 ± 0.60 vs. carrageenan: 19.63 ± 1.38 vs. saline: 1. 23 ± 0.58 U/mL) by 73% (Figure 2B).

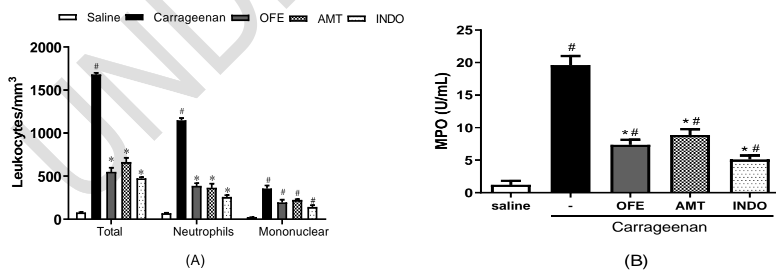

Fig. 2. The amentoflavone-rich extract of *Ouratea fieldingiana* leaves and amentoflavone reduce the leukocyte migration induced by carrageenan. OFE (10 mg/kg; p.o.), AMT (10 mg/kg; p.o.) or INDO (5 mg/kg; p.o.) was given 60 min before peritonitis induction. Control group received saline (0.1%; i.p). After 5 hours, peritoneal fluid was

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collected to assess total and differential leukocytes (A) and MPO activity (B). Mean ± E.P.M, One-way ANOVA, Bonferroni test. # P<0.05 vs. Saline. * P<0.05 vs. Carrageenan. OFE: Amentoflavone-rich extract; AMT: Amentoflavone; INDO: Indomethacin

Carrageenan reduced the mechanical threshold required for the animals withdrawal response, which is an indication of abdominal hypernociception (Figure 3). Such decrease was reduced by OFE (10 mg/kg) at the 1st (8.49 ± 0.8 vs. carrageenan: 4.88 ± 0.75 vs. saline: 7.76 g), by 42%, 2nd (9.70 ± 0.87 vs. carrageenan: 6.56 ± 0.48 vs. saline: 10.89 ± 2.22 g) by 32%, 3rd hour (10.00 ± 0.69 vs. carrageenan: 6.36 ± 0.99 vs. saline: 11.15 ± 2.04 g) by 36% and 4th hour (7.71 ± 0.5 vs. carrageenan: 5.32 ± 1.5 vs. saline: 10.92 ±1.81 g) by 30%. Similar effect was observed in the animals treated with amentoflavone (10 mg/kg), at the 1st (8.49 ± 1.35 vs. carrageenan: 4.88 ± 1.44 vs. saline: 7.0 ± 0.94 g) by 47%, 2nd (9.30 ± 2.4 vs. carrageenan: 6.56 ± 1.76 vs. saline: 10.89 ± 2.22 g) by 30 %, 3rd (9.84 ± 1.5 vs. carrageenan: 6.36 ± 2.5 vs. saline: 11.15 ± 2.04 g) by 35% and 4th hour (7.71 ± 1.32 vs. carrageenan: 5.32 ± 1.5 vs. saline: 10.92 ± 1.81 g) by 30%. The indomethacin attenuated hypernociception at the 1st (7.76 ± 1.6 vs. carrageenan: 4.88 ± 0.75 vs. saline: 7.76 g) by 37 %, 2nd (9.68 ± 2.21 vs. carrageenan: 6.56 ± 0.48 g) by 32%, 3rd (9.02 ± 1.88 vs. carrageenan: 6.36 ± 0.99 vs. 11.15 ± 2.1 g) by 30 % and 4th hour (8.97 ± 0.5 vs. carrageenan: 5.32 ± 1.5 vs. 10.92 ± 1.81 g) by 40%.

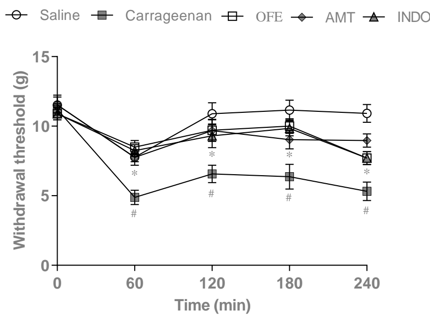

Fig. 3. The amentoflavone-rich extract of *Ouratea fieldingiana* leaves and amentoflavone reduce visceral hypernociception induced by carrageenan. OFE (10 mg/kg; p.o.), AMT (10 mg/kg; p.o.) or INDO (5 mg/kg; p.o.) was given 60 min before hypernociception induction by carrageenan (300 µg/cavity; i.p). Control group received saline (0.1%; i.p). Mean ± E.P.M, two-way ANOVA, Bonferroni test. #P<0.05 vs. Saline. *P<0.05 vs. Carrageenan. OFE: Amentoflavone-rich extract, AMT: Amentoflavone, INDO: Indomethacin

OFE reduced the oxidative stress marker MDA (2.42 ± 0.16 vs. carrageenan: 3.15 ± 0.18 vs. saline: 2.05 ± 0.18 U/mL) (Figure 4A) and enhanced the antioxidant markers GSH (338.2 ± 21.17 vs. carrageenan: 209.1 ± 16.76 vs. saline: 262.3 ± 8.06 µmol/mL) by 62% and catalase activity (4.78 ± 0.39 vs. carrageenan: 2.05 ± 0.29 vs. saline: 3.56 ± 0.33 U/mg) by 57% (Figure 4B and 4C). AMT did not alter MDA, GSH or catalase activity (Figure 4).

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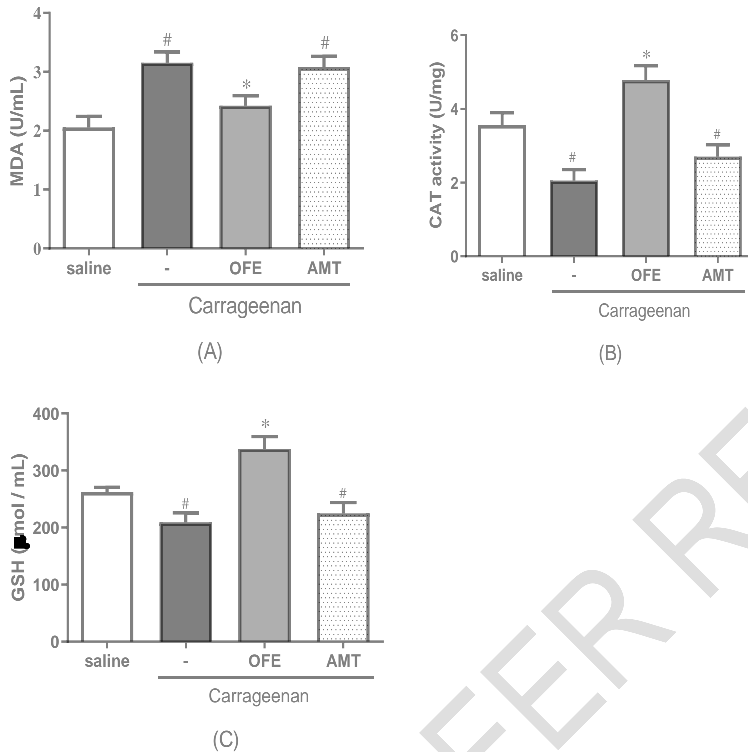

Fig. 4. The amentoflavone-rich extract of *Ouratea fieldingiana* leaves reduces oxidative stress markers. OFE (10 mg/kg; p.o.), AMT (10 mg/kg; p.o.) or INDO (5 mg/kg; p.o.) was given 60 min before carrageenan (300 µg/peritoneum; i.p.). Control group received saline (0.9%; i.p). After 5 hours, peritoneal fluid was collected to assess: (A) MDA (B) Catalase (C) GHS. Mean ± E.P.M, One-away ANOVA, Bonferroni test. # P<0.05 vs. Saline. * P<0.05 vs. carrageenan. OFE: *Ouratea fieldingiana* extract, AMT: Amentoflavone, CAT: Catalase

Acute inflammation is characterized by increase in vascular permeability, leading to edema, and leukocyte infiltration to the inflammatory site[36]. Carrageenan is a sulfated linear polysaccharide obtained from red seaweed that induces a biphasic paw edema after being injected into the hind paw of rodents and has been used in preclinical studies to test new anti-inflammatory drugs and elucidate mechanisms of inflammation[37]. Our data demonstrates the antiedematogenic effect of OFE and AMT at 10 mg/kg in the edema both phases. Since the late phase of carrageenan-induced inflammation is characterized by intense neutrophil infiltration[38], we evaluated the inhibitory effect of OFE and AMT on cellular events evoked by carrageenan in the mice peritoneum.

In the peritonitis model, five hours after carrageenan, OFE and AMT significantly decreased the leukocyte migration, mainly that of neutrophils. These data suggest a potential inhibitory effect on cellular events of carrageenan-induced acute inflammation. This hypothesis could be reinforced by the reduction in MPO activity, a marker of neutrophils and monocytes[37], and also on the neutrophil migration observed in the peritoneal lavage.

Regarding the anti-inflammatory effect of AMT, a flavonoid that is the main constituent of OFE, it is in agreement with other studies that demonstrated similarly to other flavonoids activity on neutrophils[39], microglial cells, macrophages, COX-2, iNOS[40-42] and suppression of inflammation via NF-kB pathway. Other flavonoids have demonstrated inhibitory effects in animal models of inflammation, particularly on the edema induced by carrageenan in mice, but also on phospholipase A2 synthesis, prostaglandin-E2, elastase release by human neutrophils and reactive oxygen species[43].

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Regarding the effect of AMT, previous studies suggest the it has a potent inhibitory effect on leukocyte degranulation, activity of phospholipase A2 and cyclooxygenase, in addition to the analgesic effect in inflammatory nociception[44, 45].

The carrageenan-induced inflammation is triggered by release of histamine and serotonin by resident cells and induction of a cytokine cascade involving TNF-α, IL-6, IL-1β and cytokine-induced neutrophil chemoattractant-1 (CINC-1), leading to prostaglandin synthesis and release of sympathetic amines [38]. Such mediators contribute to sensitization of primary sensory nociceptive neurons and hypernociception[46]. Since the maximal antinociceptive effect of OFE and amentoflavone were observed at 120 and 180 min, it is reasonable to consider that a limited prostaglandin production by resident or migrated cells contributes to the effect observed. Moreover, the leaf extract of *O. fieldingiana* contains kaempferol-3-*O*-rutinoside, which has relevant antinociceptive activities on orofacial nociception[34]. This compound is also present in OFE and may contribute to the antinociceptive effects observed and could be associated to possible synergism among OFE constituents, especially to the major component AMT.

Elevated levels of MPO in the peripheral circulation or extracellular fluids are associated to inflammation and increased local oxidative stress[17]. OFE and AMT appeared to reduce the oxidant markers, probably by the balance between the increased production of oxidant substances, such as catalase and GSH and the reduced of oxidizing agents, such as superoxide, peroxide and free radicals synthesized during the acute inflammation stimulated by carrageenan.

Previous studies indicate that carrageenan-stimulated macrophages produce the inflammatory cytokine IL1b by a complex intracellular pathway involving activation of TLR4/CD14/TRIF/Syk and increase in MPO production and ROS synthesis. There are evidences showing that ROS boost the expression of IL-1β, that leads formation of oxidizing agents[37,47]. Based on the increased levels of MDA induced by carrageenan, which is an indirect marker of oxidative damage[48], we could speculate that the inhibitory effect of OFE on this oxidizing agent, together with the lack of the *in vivo* effect of the major component of the extract, occurs via synergic effect among its components, such as phenols, tannins, flavones and flavanones. In fact, previous studies has made this suggestion [49]. Thus, our findings suggest the OFE potential as a novel anti-inflammatory product derived from plants.

4. CONCLUSION

Ouratea fieldingiana leafextract (OFE) is rich in phenolic compounds with the biflavonoid amentoflavone (AMT) as the main constituent and presents high anti-radical activity in *in vitro* studies, which may be related to *in vivo* studies that showed anti-radical properties. OFE and AMT significantly decreased the migration of leukocytes, especially neutrophils, acting both in the early phase and in the late phaseof inflammation. However, the *in vivo* antioxidant effect of OFE was more pronounced than that of amentoflavone, indicating a possible synergism between the phenolic constituents. These data suggest a potential inhibitory effect on the cellular events of acute inflammation induced by carrageenan, corroborating the popular use of the plant.

ETHICAL APPROVAL

Experimental protocols were conducted in accordance to the guidelines of the Brazilian College of Animal Experimentation (COBEA), and approved by the Animal Care and Use Committee of UECE (CEUA Nº 0559924-4).

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