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The Amentoflavone-Rich Extract of *Ouratea fieldingiana* Leaves Presents Antioxidant and Anti-inflammatory Activity in Mice

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Aims: To evaluate the antioxidant and anti-inflammatory effects of the *Ouratea fieldingiana* leaf extract (OFE) and its main constituent amentoflavone (AMT).
Study Design: The phenolic compounds of *Ouratea fieldingiana* leaves (OFE) were quantified, the chemical structure characterized and the anti-inflammatory effect of OFE and amentoflavone was 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 Physio pharmacology Laboratory, between July 2020 and June 2021.
Methodology: The OFE was obtained from 70% ethanol extract of *O. fieldingiana*. OFE was subjected to classic chromatographic column furnishing AMT. The phenolic compounds were characterized by HPLC and quantified by Follin-Ciocalteu methodology. The antiradical potential was evaluated by DPPH and ABTS methods. Anti-inflammatory activity was determined in female Swiss mice received per oral OFE (0.1-10 mg/kg) or sterile saline 60 min before stimulation with carrageenan (300 mg) for quantification of the parameters: edema, abdominal hypernociception, neutrophil migration and oxidative stress markers (reduced glutathione, malondialdehyde, myeloperoxidase and catalase activities).
Results: AMT was identified as the main compound in the OFE (292.64 ± 3.87 mg/g extract). OFE has an average inhibitory concentration of 9.81 ± 0.17 µg/mL 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 abdominal hypernociception (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 activity *in vitro* and *in vivo,* and anti-inflammatory activity *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 [1], and other inflammatory disorders [2].*Ouratea fieldingiana* (Gardner) Engl. is a tree belonging to the Ochnaceae family and Theales order [3]. This family is distributed in tropical and subtropical areas, comprising arboreal, shrub and rarely herbaceous representatives [4], and encompasses around 40 genera and 600 species, mainly in South America [5]. In Brazil, approximately 9 genera with 105 species are found [6]. In the Northeast, species of this genus are known as batiputá [7]. *O. fieldingiana* is frequently found in the Caatinga biome, showing bloodcolored flower buds that turn yellow when blooming, in addition to a mild smell. This tree is common on the coast and stands out for its canoe-shaped leaves, with teeth close to the apex, light green, shiny and oblique veins, as well as for the clusters of flowers with five yellow petals during flowering [8]. The seeds produce an oil, by decoction, that has been prescribed for stomachache, erysipelas, uterine wounds and rheumatism [9] and anticholinesterase activities [10,11]. The polar fraction of this oil contains amentoflavone as the main constituent, but also other flavonoids, such as rutin, kaempferol-3-*O*-rutinoside, isoquercitrin, quercetin and apigenin, all of them reported as anti-inflammatory [12-15]. Amentoflavone also possesses the properties antidiabetic [16], antitumoral [17], anti-SARS-CoV-2 [18] and antioxidant, demonstrated *in vitro* [19,20]. *Ouratea* genus generally shows similarities in its chemical composition, containing a variety of active compounds such as phytosterols, phytoestrogens, free or fatty-acid

esterified triterpenes, lectins, lignans, and flavonoids [21].

Antioxidants, such as plant polyphenols, have been linked to control inflammation due to their ability to neutralize free radicals and reduce oxidative stress in cells. This can lead to reduced production of pro-inflammatory molecules such as nitric oxide (NO) and the cytokine TNF-α. Therefore, antioxidants may contribute to the modulation of the inflammatory response and be useful for the treatment of inflammatory conditions [22]. Recent investigations have shown that inflammation is an important factor for the progression of several acute and chronic disorders, such as diabetes, cancer, cardiovascular and arthritis, being directly related to oxidative damage [23,24]. The most prescribed anti-inflammatory drugs, although effective, are limited due to its adverse effects, causing gastric, renal, cardiovascular disorders and impairing host defenses against infections [25]. 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 [26].

Thus, this study aimed to validate the popular use of O. *fieldingiana*, and to evaluate 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 *in vivo* on the mice models of paw edema and peritonitis induced by carrageenan.

2. EXPERIMENTAL DETAILS

2.1 Collection 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).

2.2 Obtaining of *Ouratea fieldingiana* extract (OFE) and Amentoflavone

Dried leaves of *O. fieldingiana* were macerated with 70 % v/v (1:1) ethanol for 7 days, evaporated until remotion of alcohol, giving a remaining aqueous extract mixed with a fatty green insoluble material. This fatty material was removed by filtration, in a Buchner funnel and the aqueous solution was lyophilized to obtain the *O. fieldingiana* extract (OFE) [10]. The isolation and structural characterization of the main constituent amentoflavone (AMT) was performed according to previously published procedures [20].

2.3 Quantification of Total Phenols and Flavonoidsof OFE

The determination of the total phenolic content was carried out by spectroscopy in the visible region using the Folin-Ciocalteu reagent, following the methodology of Sousa et al. [27]. For the quantification of flavonoids, 2.5% aluminum chloride ($AlCl_3$) was used according to Funari and Ferro [28].

2.4 High Performance Liquid Chromatography (HPLC) with the Diode Array Detector (DAD) of OFE

To identify the phenolic compounds, the OFE methanolic solution at 10 mg/mL was injected into the HPLC equipment. Standards were purchased from Sigma Chemical Co. (St. Louis, MO, USA). The solvents used for extraction were of analytical (Vetec®) and of HPLC grade (J.T. BAKER®). Chromatographic analyses 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. The 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 wavelength of 350 nm. The peaks related to the constituents present in the HPLC chromatogram were confirmed by the retention time of the reference standards and by DAD spectra (200 to 400 nm).

2.5 *In vitro* antioxidant Activity Assay of OFE

The antioxidant activity was evaluated using the DPPH (2,2-diphenyl-1-picrylhydrazyl) method, following the methodology adapted from Becker

et al. [29] and by the ABTS method (2,2'-azino-bis(3-ethylbenzothiazoline-6sulfonic acid)) described by Re et al. [30]. Both assays were performed in 96-well flat-bottom microplates in Elisa BioTek reader, model ELX 800. The results were 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 was the solution with all reagents, except the sample. The antioxidant Quercetin and Gallic acid were used for comparison.

2.6 Animals Treatment

Mice 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 λ-carrageenan (Sigma-Aldrich® code 22049) for evaluation of the parameters (edema, abdominal hypernociception, neutrophil migration and oxidative stress markers) in the models of paw edema and peritonitis.

2.7 Paw Edema Model

Edema was induced in left/right hind paw of mice by subcutaneous administration of λ-carrageenan (300 µg). Paw volumes were measured by hydroplethysmometry, immediately before (zero time) and after induction (1-5 h). Edema was expressed as Δ paw volume (µL) from basal value or area under the curve AUC (arbitrary units) [31].

2.8 Peritonitis Model

Peritonitis was induced by intraperitoneal injection of λ-carrageenan (300 µg) for evaluation of abdominal hypernociception, leucocyte migration and oxidative stress markers.

The hypernociception was evaluated by the application of a non-flexible filament coupled to a digital algesimeter, until occurrence of nociceptive response (abdominal licking and withdrawal). Values (in g) were obtained immediately before (zero time) and after (1-5 h) the peritonitis induction [32].

Five hours after peritonitis induction mice were euthanized under anesthesia (xylazine 10 mg/kg + ketamine 100 mg/kg i.p.), the peritoneal fluid was harvested (saline + 5 IU heparin) for quantification of leukocyte migration by optical microscopy (total leukocyte, neutrophil, mononuclear) [33], and oxidative stress markers: catalase [34], at A240 nm, expressed as U/proteins; reduced glutathione (GSH) at A412 nm [35], expressed as µmol/mL of peritoneal fluid; malondialdehyde (MDA), at A535 nm [36], expressed as U/mL; and myeloperoxidase (MPO) activity at A450 nm, expressed as MPO U/mL [37].

2.9 Statistical Analysis

Data were expressed as mean ± standard error of mean. Statistical differences of 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 that OFE presents high content of phenolic compounds (338.62 ± 0.12 mg GAE/g extract) and antiradical activity against DPPH and ABTS, features widely described for polyphenolic compounds [38]. The extraction method influences the type and quantity of phenolic compounds. For example, the extraction of amentoflavone (AMT) from *O. fieldingiana* leaves by HPLC-DAD using ethanol showed the content of 86.88 ± 1.08 mg/g in the extract [20, 21], while, 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 292.64 ± 3.87 mg/g of extract (Table 2), and with an improvement in the IC_{50} value (9.81 ± 0.17 µg/mL) of extract necessary to inhibit the radical (Table 1).

The characterization of the compounds present in the aqueous extract of *O. fieldingiana* by high-performance liquid chromatography, presents as the major constituent the biflavonoid amentoflavone showing higher amount compared to the other constituents (Table 2), suggesting that probably the activities of the plant can be mainly associated to this compound. Amentoflavone presents anti-inflammatory activity in Gentamicin-induced kidney damage in rats [39]; kaempferol-3-*O*-rutinoside displays antinociceptive activity in the Zebrafish model [40]; and rutin has anti-inflammatory activity *in vitro* and *in vivo* studies [41]. Thus, the various bioactive flavonoids

present in the OFE, all together can be responsible for the great anti-inflammatory potential of this plant.

The λ-carrageenan induced paw edema initiated at the first 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) (Fig. 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)] (Fig. 1B) and in the second phase [2-4 h: 19% (0.1 mg/kg); 22% (1 mg/kg) and 45% (10 mg/kg)] (Fig. 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) (Fig. 1B and 1C).

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

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) (Fig. 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) (Fig. 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) (Fig. 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) (Fig. 2A) and MPO activity (5.11 ± 0.60 *vs*. carrageenan: 19.63 ± 1.38 *vs.* saline: 1. 23 ± 0.58 U/mL) by 73% (Fig. 2B).

Carrageenan reduced the mechanical threshold required for the animal's withdrawal response, which is an indication of abdominal hypernociception (Fig. 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%, at the 2nd (9.70 ± 0.87 *vs.* carrageenan: 6.56 ± 0.48 *vs.* saline: 10.89 ± 2.22 g) by 32%, at the 3rd hour (10.00 ± 0.69 *vs*. carrageenan: 6.36 ± 0.99 *vs*. saline: 11.15 ± 2.04 g) by 36%, and at

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

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

Table 2. Characterization 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

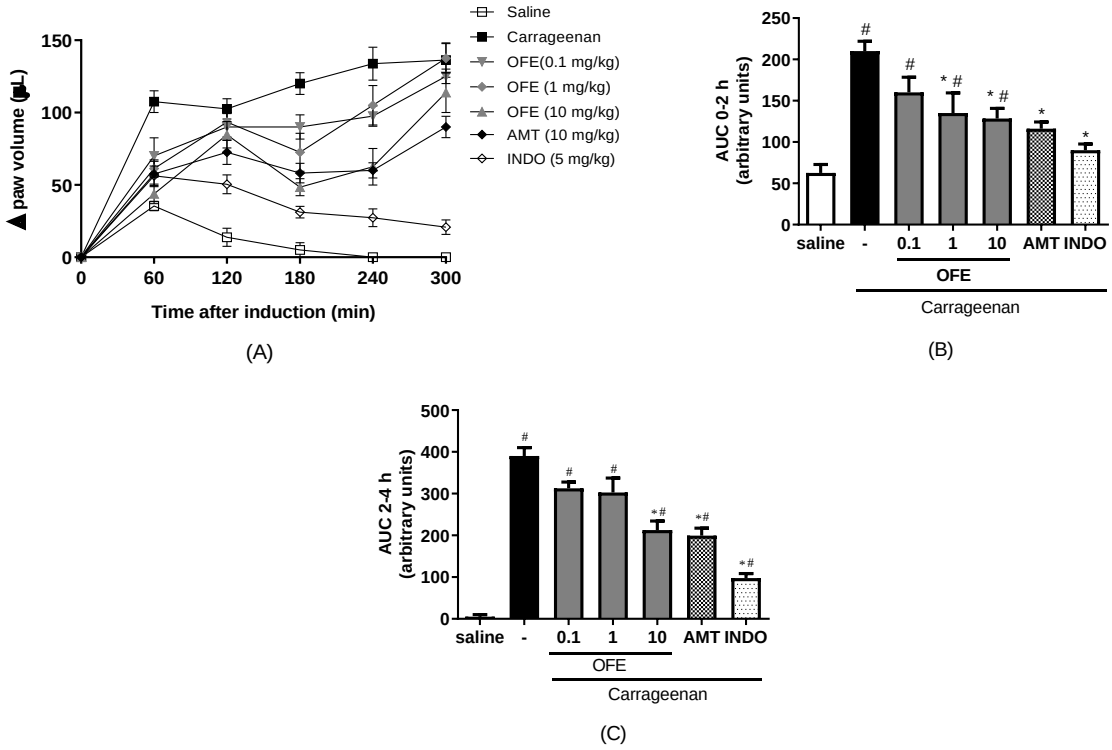

Fig. 1. The amentoflavone-rich extract of *Ouratea fieldingiana* leaves and amentoflavone reduce the mice paw edema induced by carrageenan

*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.). The 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*

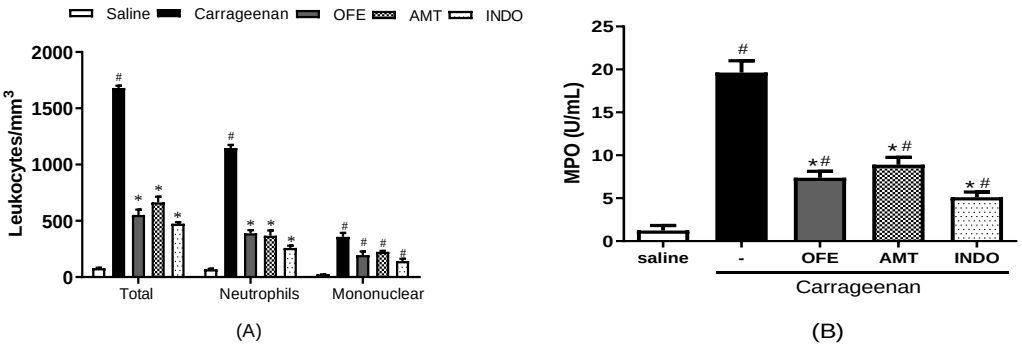

Fig. 2. The amentoflavone-rich extract of *Ouratea fieldingiana* leaves and amentoflavone reduce the leukocyte migration induced by carrageenan

*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 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*

the 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%, at the 2nd (9.30 ± 2.4 *vs*. carrageenan: 6.56 ± 1.76 *vs.* saline: 10.89 ± 2.22 g) by 30%, at the 3rd (9.84 ± 1.5 *vs*. carrageenan:

6.36 ± 2.5 *vs*. saline: 11.15 ± 2.04 g) by 35% and at the 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%, at the 2nd (9.68 ± 2.21 *vs.* carrageenan: 6.56 ± 0.48 g) by 32%, at the 3rd (9.02 ± 1.88 *vs*. carrageenan: 6.36 ± 0.99 *vs.* saline: 11.15 ± 2.1 g) by 30% and at the 4th hour (8.97 ± 0.5 *vs*. carrageenan: 5.32 ± 1.5 *vs.* saline: 10.92 ± 1.81 g) by 40%.

Acute inflammation is characterized by increase in vascular permeability, leading to edema, and leukocyte infiltration to the inflammatory site [42]. 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 [43]. Our data demonstrates the anti-edematogenic 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 [44], 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 [43], 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 agrees with other studies that demonstrated similarly to other flavonoids activity on neutrophils [45], microglial cells, macrophages, COX-2, iNOS [46-48] 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 [49]. Regarding the effect of AMT, previous studies suggest that it has a potent inhibitory effect on leukocyte degranulation, activity of phospholipase A2 and cyclooxygenase, in addition to the analgesic effect in inflammatory nociception [50, 51].

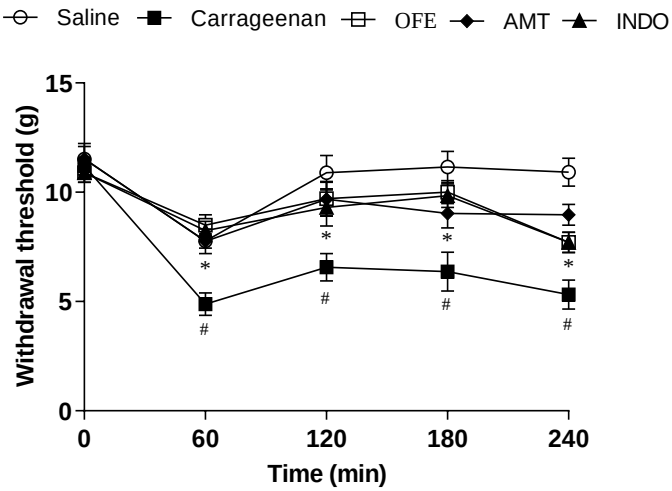

Fig. 3. The amentoflavone-rich extract of *Ouratea fieldingiana* leaves and amentoflavone reduce visceral hypernociception induced by carrageenan

*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*

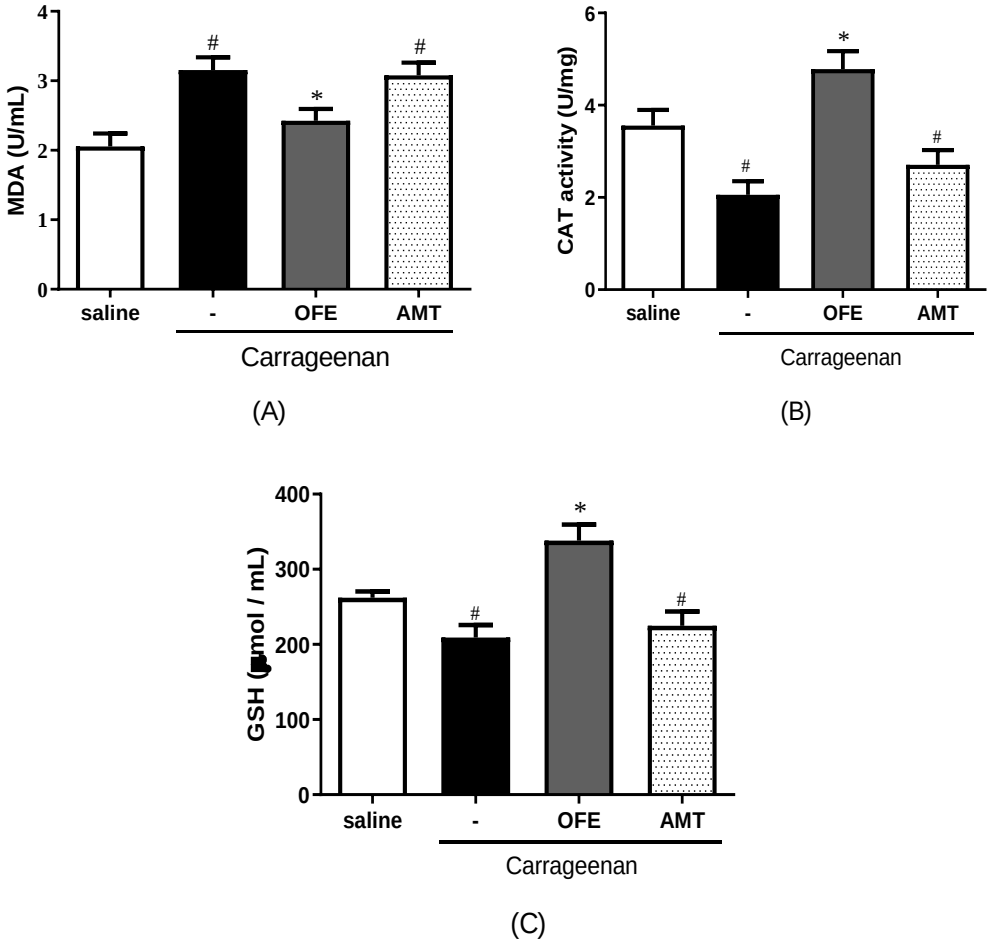

Fig. 4. The amentoflavone-rich extract of *Ouratea fieldingiana* leaves (OFE) reduces oxidative stress markers

*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) (Fig. 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% (Fig. 4B and 4C). AMT did not alter MDA, GSH or catalase activity (Fig. 4). 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/cavity 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*

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 [44]. Such mediators contribute to sensitization of primary sensory nociceptive neurons and hypernociception [52]. 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 [24]. 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 [43,53]. Based on the increased levels of MDA induced by carrageenan, which is an indirect marker of oxidative damage [54], 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 have made this suggestion [55]. Thus, our findings suggest the OFE potential as a novel anti-inflammatory product derived from plants.

4. CONCLUSION

Ouratea fieldingiana leaf extract (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 phase of 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.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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