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Original article

Chemical fingerprint, acute oral toxicity and anti-inflammatory activity of the hydroalcoholic extract of leaves from *Tocoyena formosa* (Cham. & Schlecht.) K. Schum

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ABSTRACT

Inflammation is a protective response of the organism against damaging agents, this process is considered beneficial, however in some situations, this response can be damage when exacerbated effect are present. This claim objective to evaluate the qualitative and quantitative chemical profile, acute toxic and anti-inflammatory effects of the hydroalcoholic extract of leaves from *Tocoyena formosa* (Cham. & Schlecht.) K. Schum. (HELTF). Quantitative and qualitative phytochemical analysis was performed by HPLC-DAD and colorimetric assay. The topical anti-inflammatory activity was determined in Croton oil-induced ear edema assay and systemic activity was performed in vascular permeability, paw edema induced by carrageenan and dextran. Phytochemical analysis of leaves from HELTF showed presence of tannin, flavonoid, saponins an other that confirmed by HPLC analysis. The extract did not cause significant with LD_{50} greater than 5000 mg/kg and did not promote significate reduction in topical inflammatory process. However, HELTF demonstrate significant reduction of paw edema induced by carrageenan and dextran. The HELTF (200 mg/kg) reduced the protein/cell migration in the intradermal carrageenan-induced inflammation. Our results demonstrated that the first time the chemical profile and describe the effective action in systemic anti-inflammatory, antiedematogenic activity and low acute toxicity. This activity presents, supporting its traditional use. However, new studies are necessary for the detection and clarification of the possible mechanism of action.

1. Introduction

Inflammatory process is characterized as a protective response of the organism against pathogenic agents to promote restoration/cure. The intensity of this process is controlled by pro- and anti-inflammatory elements. Inflammation is a process considered beneficial and necessary since its action, in conjunction with hormones and signaling molecules, is responsible for the regeneration and repair or damaged structures (Zaldivar et al., 2006).

The inflammatory response involves several cascades, pathways and several components, this hinders the initial control of the inflammatory process, with that the response can become exacerbated and lead to various damages to the body (Zaldivar et al., 2006). This exacerbated response leads to intense clinical or symptomatological conditions, even generating inflammatory pathologies. Global estimates suggest that about 3/4 of the world's population does not have access to synthetic therapies to treat inflammatory conditions, and such a population sector depends on medicinal plants.

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Research from plants with known popular use as anti-inflammatories can be seen as an excellent strategy in the search for new anti-inflammatory drugs, because in addition to being natural sources of raw material for new and important pharmacological molecules with active potential, they become an alternative/complement for the treatment of inflammatory diseases since the synthetic drugs for the treatment of these morbidities are of high cost and cause various adverse effects (Butler et al., 2014; Maroon et al., 2010; Newman et al., 2003). Many of these plants with excellent pharmacological activities exist in Brazil, although most of them are used by the population without any scientific basis (Bitu et al., 2015; Ribeiro et al., 2014; Souza et al., 2014).

Tocoyena formosa (Cham. & Schlecht.) K. Schum., for example, is a very popularly used plant in the treatment of bruises, sprains, pain and rheumatic problems, and other inflammatory processes in the city of Crato, Cariri region, South of Ceará, Brazil, and has an emphasized traditional importance. However, individuals making use of this species have no concrete information on the actual actions of this plant (Souza et al., 2014).

This species predominant of South American is found in Paraguay, Bolivia and Brazil, especially in xeromorphic formations like cerrado, sandbanks and heath. In Brazil, this species is traditionally known as "Jenipapo do campo" and has a huge range of distribution in the Northeast (Coelho et al., 2006), it is an ornamental species, which grows spontaneously in dry regions and in Cerrado, it is a Savannah and possesses a physiognomically floristic diverse vegetation being very impressive because of its yellow and green flowers (Bolzani et al., 1997; Hamerski et al., 2005).

Given the importance of evaluating the pharmacological action of this plant with information restricted to ethnopharmacological data in the region, *Tocoyena formosa* (Cham. & Schlecht) K. Schum may exhibit anti-inflammatory activity, justifying the development of studies for investigating its therapeutic potential to control and treat inflammatory diseases. From this, our work has as its objective to evaluate and investigate the acute toxicity and anti-inflammatory activity of the Hydroalcoholic Extract of Leaves from *Tocoyena formosa* (Cham. & Schlecht.) K. Schum (HELTF) in mice.

2. Materials and methods

2.1. Collection of botanic material, preparation of hydroalcoholic extract of leaves from Tocoyena formosa (Cham. & Schlecht.) K. Schum

Leaves from the *Tocoyena formosa* (Cham. & Schlecht.) K. Schum were collected from Chapada of Araripe, localized in the Northeast of Brazil, South of Ceará, in the Cariri region, in the County of Crato-CE, in Sitio Barreiro Grande (07°21′44.0″S and 39°28′41.0″W with an altitude of 901 m above sea level), and with 65.9 km proximity to the County of Serrita-PE with a total area of 76654.3 km^2. A representative sample of the species (voucher specimen) containing fruit was sent for identification to the Herbarium Dárdano de Andrade Lima Regional University of Cariri - URCA under N°. 2.770.

The botanic material (leaves) were exposed to the sun for drying, weighed and crushed, followed by subjection to solvent extraction in ethanol and distilled water (1:1 v/v), for 72 h. The hydroethanolic extract formulated was filtered for and subjected to extraction of chlorophyll by addition of activated carbon in the extract (10 g/100 ml) in a 30 min period, after, it was once again filtered for retention of the activated carbon. It was then concentrated by distilling of the solvent on a rotary evaporator, followed by placement on Bain Marie to provide complete evaporation of ethanol, after 24hs the sample was frozen and placed in a lyophilization process.

2.2. Phytochemical prospecting

Phytochemical prospecting of the extract and fractions were performed for determination of secondary metabolites presents in HELTF, according to the methodology by Matos (2009), with the following main classes evaluated: phenols, tannins, anthocyanins, anthocyanidins, flavonoids, leucoanthocyanidins, catechins, flavones, alkaloids, steroids, triterpenoids and saponins. This methodology addresses the visual observations of colorations and formation of precipitates after exposure to specific chemical reagents.

The lyophilized EHFTF (0.3 g) was initially diluted in 30 ml of 70% ethanol and distributed into six portions, where the first three tests were performed, the other three had different methods:

- Test for Phenols and Tannins: In one portion, 3 drops of ferric chloride were added and stirred, the color and precipitate formation was observed relative to the white test with water and ferric chloride.
- Test for Anthocyanins, Anthocyanidins and Flavonoids: 3 parts were used, in the first one was added ammonium hydroxide, alkalizing to pH = 8.5, in the second was added hydrochloric acid, acidifying to pH = 3 and in the third was added hydroxide of sodium 1%, alkalizing the medium to pH = 11.
- Test for Leucoanthocyanidins, catechins and flavones: 2 parts were used, in the first one was added 1% sodium hydroxide, alkalizing the medium to pH = 11, in the second one was added hydrochloric acid, acidifying to pH = 3, and both were heated for 2–3 min.
- Test for Alkaloids: 0.3 g of EHFTF was diluted in 30 ml of 5% acetic acid and subjected to heating until the boil of the preparation. Alkalisation was carried out with ammonium hydroxide (15 ml), followed by the addition of chloroform (15 ml), homogenization and standing on a separatory funnel. The chloroform phase was collected in the beaker and the solvent evaporated (chloroform) by heating and 1% hydrochloric acid was added and homogenized, then this solution was applied to a slide and a drop of draggent reagent was administered observing the formation of precipitate which is indicative of the presence of alkaloids.
- Test for Steroids and Triterpenoids: 0.3 g of EHFTF was dissolved in 6 ml of chloroform, then filtered into a funnel with a cotton ball covered with anhydrous sodium sulfate, the filtrate was separated into a test tube and 1 ml of acetic anhydride and 3 drops of sulfuric acid, stirred, observing the rapid change of colors.
- Test for Saponins: 0.3 g of EHFTF was diluted in 10 ml of chloroform and 10 ml of distilled water, then filtered in a cotton ball funnel, stirred for 3 min observing foaming.

Chromatographic analyses were carried out under gradient conditions using C_{18} column (4.6 mm × 250 mm) in reverse phase, packed with 5 μm diameter particles; the mobile phase was water containing 2% acetic acid (A) and methanol (B), and the composition gradient was: 8% of B until 5 min and changed to obtain 20%, 30%, 50%, 60%, 70%, 20% and 10% B at 20, 30, 40, 50, 60, 70 and 80 min, respectively, following the method described by Silva et al. (2014) with slight modifications. *Tocoyena formosa (Cham. & Schlecht.) K. Schum* hydroalcoholic extract and mobile phase were filtered through 0.45 μm membrane filter (Millipore) and then degassed by ultrasonic bath prior to use, the hydroalcoholic extract was analyzed at a concentration of 20 mg/mL. The flow rate was 0.6 mL/min, injection volume 50 μl and the wavelength were 270 for gallic acid and ellagic acid, 280 nm for catechin, 327 nm for chlorogenic acid and caffeic acid, and 365 nm for quercetin, luteolin and rutin.

All the samples and mobile phase were filtered through 0.45 μm membrane filter (Millipore) and then degassed by ultrasonic bath prior to use. Stock solutions of standards references were prepared in the HPLC mobile phase at a concentration range of 0.020–0.350 mg/ml for catechin, quercetin, luteolin and rutin; and 0.025–0.300 mg/ml for ellagic, gallic, caffeic and chlorogenic acids. Chromatography peaks were confirmed by comparing its retention time with those of reference standards and by DAD spectra (200–500 nm). Calibration curve for gallic acid: Y = 12609x + 1358.5 (r = 0.9998); catechin: Y = 13626x + 1197.6 (r = 0.9995); chlorogenic acid: Y = 13074x + 1258.3 (r = 0.9999); caffeic acid: Y = 12745x + 1236.9 (r = 0.9995); ellagic acid: Y = 11873x + 1305.6 (r = 0.9998); rutin: Y = 13670x + 1271.9 (r = 0.9999), luteolin: Y = 12745x + 1308.5 (r = 0.9991) and quercetin: Y = 13165x + 1175.8 (r = 0.9996). All chromatography operations were carried out at ambient temperature and in triplicate.

The limit of detection (LOD) and limit of quantification (LOQ) were calculated based on the standard deviation of the responses and the slope using three independent analytical curves. LOD and LOQ were calculated as 3.3 and 10 σ/S, respectively, where σ is the standard deviation of the response and S is the slope of the calibration curve (Silva et al., 2014).

2.3. Animals

Male Swiss mice of adult age, weighing 20–30 g from the Central Animal Laboratory of the Faculty of Medicine of Juazeiro – FMJ were used. The animals were maintained in collective cages made of polyethylene with stainless steel cover, lined with autoclaved wood shavings. Room temperature and quantity of noise were maintained at ideal conditions. The photoperiod had a light/-dark cycle of 12/12 h daily. The animals were kept in solid fasting for a period of 8–12 h before the experiment and the filtered water was supplied by *ad libitum*. Animals were handled according to procedures for the scientific use of animals established by Law No. 11.794, of October 8, 2008 and in accordance with the Ethical Principles for the Use of Laboratory Animals recommended by COBEA (Brazilian College of Animal Experimentation) and submitted to and approved by the Ethics Committee on the use of animals of the Regional University of Cariri - URCA with process under No. 00198/2014.1.

2.4. Investigation of acute oral toxicity and hippocratic screening

The mice were separated randomly, weighed and divided in six groups (n = 3) and left in fasting for a period of 6 h with free access only to water, ration was allowed 3 h after administration. The extracts were dissolved in saline solution at 0.9% in the concentrations of 5000, 2000, 500, 175, 55 and17.5 mg/kg v.o. (single dose), each animal received a different concentration with a volume of 0.1 mL/10 g. Initially, administration of 5000 mg/kg v.o. resulted in the occurrence of death, further decreasing doses were performed. The body weight of the all animals were verified on the day of administration, in the seventh and fourteenth day post-administration. At the end of the 14-day trial, the animals were euthanized by CO_2 inhalation and its organs (spleen, heart, liver, lungs and kidneys) were excised and weighed (OECD, 2012).

The results were registered in an independent and systematic manner. The observed parameters after administration of the drugs, relating to stimulatory actions, are: scratching of nose, tonic clonic seizure, exophthalmos, increased respiratory rate, licking paws, tail biting, increased motility, stereotyped movements, pilo-erection, fine and coarse tremors; relating to depressing actions, are: media alienation, analgesia (by the animal's tail compression test), anesthesia (pulling the hair and suspending the animal), ataxia, decreased paw withdrawal, catatonia, dyspnea, exophthalmos, decreased respiratory rate, decreased motility, decreased corneal reflex, dorsal tone, ptosis and sedation; other observed parameters are: aggressiveness, contortion, urine coloration, pupil diameter, diarrhea, tail erection, twitching, grunting, increased or decreased urination, mydriasis, ear (cyanotic, hyperemic or pale) passivity, flight reaction, drooling, tail tremor, tearing, sweating, coma and death (Malone and Robichaud, 1962). During the experimental period the animals were examined daily for the clinical aspects mentioned above.

2.5. Evaluation of the anti-inflammatory response of the hydroalcoholic extract of leaves from Tocoyena formosa (Cham. & Schlecht.) K. Schum

2.5.1. Carrageenan-induced paw edema test

The animals were divided in 5 experimental groups (n = 6), treated orally with vehicle (saline 0.9% 10 mL/kg), HELTF (400, 200 and 100 mg/kg), and indomethacin (10 mg/kg as positive control), respectively. The baseline volume of the hind paws was determined before the administration of any drug. The determination of the baseline volume is given by 3 measurements for each animal. The tested substances were administered one hour prior to the intraplantar injection of carrageenan 1% (20 μl/paw) in right hind paw and saline vehicle in the left hind paw (20 μL/paw). Paw volume was registered at 1, 2, 3, 4 h with an electric hydroplethysmometer, where for each hour 3 measurements were also performed. The results were presented as mean change (Δ) of the volume difference (mL) of the right and left paw in relation to baseline values; and the difference in weight (mg) of the right and left paw in relation to baseline values (Winter et al., 1962).

2.5.2. Dextran-induced paw edema test

The animals were divided in 5 experimental groups of six mice, treated orally with vehicle (saline, 0.9% 10 mL/kg), HELTF (400, 200 and 100 mg/kg) and with indomethacin (10 mg/kg, as positive control), respectively. The hind paw baseline volumes were determined prior to administration of any drug. The determination of baseline volume is given by 3 measurements for each animal. The tested substances were administered one hour prior to intraplantar injection of Dextran 1% (20 μL/paw) in the right hind paw and vehicle saline (20 μL/paw) in the left paw. Paw volume was registered at 30, 45, 60, 90 and 120 min with an electric hydroplethysmometer, where for each hour 3 measurements were also performed. The results are presented as mean change (Δ) of the volume difference (mL) of the right and left paw in relation to baseline values; and the difference in weight (mg) of the right and left paw in relation to baseline values (Wirtz et al., 2007).

2.5.3. Vascular permeability test

In the day prior to the test, the back of the animals were shaved using a razor. The animals were divided in 3 experimental groups (n = 6), treated orally with vehicle (saline 0.9% 10 mL/kg), HELTF (200 mg/kg) and with indomethacin (10 mg/kg as positive control), respectively. The animals were anaesthetized with ether and received an intravenous injection (lateral tail vein) of 25 mg/kg of Evans blue (5% in saline, filtered 2× in filter paper). Soon after, 4 intradermal injections in the shaved dorsal region of 20μL of carrageenan (2% in saline solution) were performed, 1 h after they were anaesthetized, exsanguinated, and the colored dorsal skin was removed, and then discs were removed with leather cutter (10 mm diameter) and weighed. Afterwards, each disc was cut in small fragments and placed in 4 ml formamide and maintained in 37 °C/24 h to extract the dye, they were then centrifuged at 2500 rpm/30 min. The colored supernatant is estimated colorimetrically, utilizing a wavelength of 600 nm. Absorbance reading of

the total dye extravasation is taken as a quantity of the protein extravasation(Saria and Lundberg, 1983; Udaka et al., 1970).

2.5.4. Croton oil-induced ear edema test

The animals were divided in 5 experimental groups of 6 mice, treated topically with vehicle (saline 0.9% 10 mL/kg), HELTF (400, 200 and 100 mg/kg) and with Dexamethasone (2 mg/kg as positive control), respectively. One hour post treatment, topical application of 20 μL of Croton oil (5% in acetone) in the right ear and 20 μL of acetone in the left ear was performed. Four hours later, the animals were euthanized by cervical dislocation and 6 mm diameter discs were removed from their left and right ears with a metallic push, these being weighed on an analytical balance (Gábor, 2003; Tubaro et al., 1986).

2.5.5. Statistical analysis

We used the GraphPad Prism Software version 6.0 program, where the parametric data is expressed as mean ± standard error of the mean (SEM). To verify the statistical differences between the groups a One- or Two-way analysis of variance (ANOVA) is performed according to the experimental protocol, followed by Tukey's multiple comparison test. For all analyzes a p value of <0.05 will be considered significant.

3. Results

3.1. Phytochemical prospecting

Phytochemical analysis of leaves from *Tocoyena formosa* (Cham. & Schlecht.) K. Schum (HELTF) allowed the identification of secondary metabolites, as shown in Table 1. HPLC fingerprinting of HELTF, just previously published, revealed the presence of the gallic acid, catechin, chlorogenic acid, caffeic acid, ellagic acid, rutin, quercetin and luteolin (Cesário et al., 2018), however, Chlorogenic acid (21.73 mg/g), Caffeic acid (13.86 mg/g), Quercetin (17.54 mg/g) and Luteolin (25.09 mg/g) are major compounds present in extract. This result confirms a secondary metabolite identified in qualitative phytochemical prospecting showed in Table 1.

3.2. Investigation of the acute oral toxicity and hippocratic screening

In the acute toxicity test regarding the behavioral assessment of mice, the HELTF presented low toxicity in the performed tests when administered orally. The following symptoms were noted in the treated animals: increased motility, piloerection, tail biting, ataxia, loss of corneal reflexes, passivity and change in urine color. All these alterations were observed in the dose of 5000 mg/kg of HELTF in comparison to the control group, in it was revealed that the LD50 is greater than 5000 mg/kg v.o. However, there were no deaths in the treated animals. During the entire experimental period, the animals were examined daily for the above clinical aspects and did not show significant changes compared to the control groups. The average body weight at intervals (0, 7, 14 days) did not show significance in weight of the treated animals with 5000 mg/kg HELTF, when compared to the control (Table 2).

During macroscopic analysis of the organs, animals treated with HELTF in the dose of 5000 mg/kg did not exhibit alterations in organ color, volume and texture, when compared with the control group, and did not have any significant organ weight differences (spleen, heart, liver, lungs and kidneys) when compared to control. The results demonstrate that the essential organs, such as the liver and kidneys, were not adversely affected during the treatment. The choice of (100, 200, 300, 400 and 500 mg/kg) doses were investigated for anti-inflammatory activity, which were doses equivalent to a maximum of 10% of the LD50, in which the value of LD50 is greater than 5000 mg/kg which classifies it as a compound of low toxicity.

3.3. Evaluation of the anti-inflammatory response of the hydroethanolic extract from leaves of Tocoyena formosa (Cham. & Schlecht.) K. Schum

3.3.1. Carrageenan-induced paw edema test

The results show that the extract (100, 200 and 400 mg/kg, v.o.) significantly reduced paw edema induced by carrageenan after 2, 3, 4 and 5 h of its administration, with the most effective dose (200 mg/kg) reducing edema by 55.03%, 42.84%, 57.47%, 42.03%, respectively. Indomethacin reduced edema at 1, 2, 3, 4 and 5 h by 58.23%; 61.66%; 65.56%; 79.59% and 80.31%, respectively, in relation to control (Fig. 1A). The doses of 100 and 200 mg/kg did not alter edema of animals subjected to the test in the first hour. Indomethacin manifested its effect in the first hours post its administration, maintaining a reduction of edema up to the fifth hour, as well as the extract (100 and 200 mg/kg). Weighing of the paws was performed, where antiedematogenic activity of the extract was confirmed, especially in the doses of 400 and 200 mg/kg which presented with significance in relation to the control (Fig. 1B).

3.3.2. Dextran-induced paw edema test

The data indicates that the HELTF (200 and 400 mg/kg) was significantly effective in reducing paw edema volume induced by dextran compared to control. A dose of 200 mg/kg was capable of preventing the development of edema in 35.60%, 30.76%, 33.32%, 33.32% and 39.64%, while a dose of 400 mg/kg promoted an inhibition of 45.45%, 41.95%, 56.81%, 54.54% and 43.86%, while indomethacin was capable of reducing edema by 78.78%, 72.02%,

Table 1
Classes of secondary metabolites found in the hydroethanolic extract of leaves from *Tocoyena formosa (Cham. & Schlecht.) K. Schum.*

Metabolites classes	(+) Presence (−) Absence
Phenolic acids	+
Tannins pirogalic	−
Condensed tannins	+
Anthocyanins	−
Anthocyanidins	−
Flavones	+
Flavonols	+
Flavonones	+
Flavononols	+
Xanthones	+
Chalcones	+
Aurones	+
Leucoanthocyanidins	+
Catechins	+
Alkaloids	−
Steroids	+
Pentacyclic triterpenoids	+
Saponins	+

Table 2
Average body weight of animals treated with 5000 mg/kg of HELTF and the control group.

Days	Control	HELTF 5000 mg/kg
Day 0	28.0 ± 1.5 (n = 3)	29.0 ± 2.9 (n = 3)
Day 7	29.0 ± 2.7 (n = 3)	30.0 ± 4.0 (n = 3)
Day 14	29.0 ± 5.2 (n = 3)	30.0 ± 4.6 (n = 3)

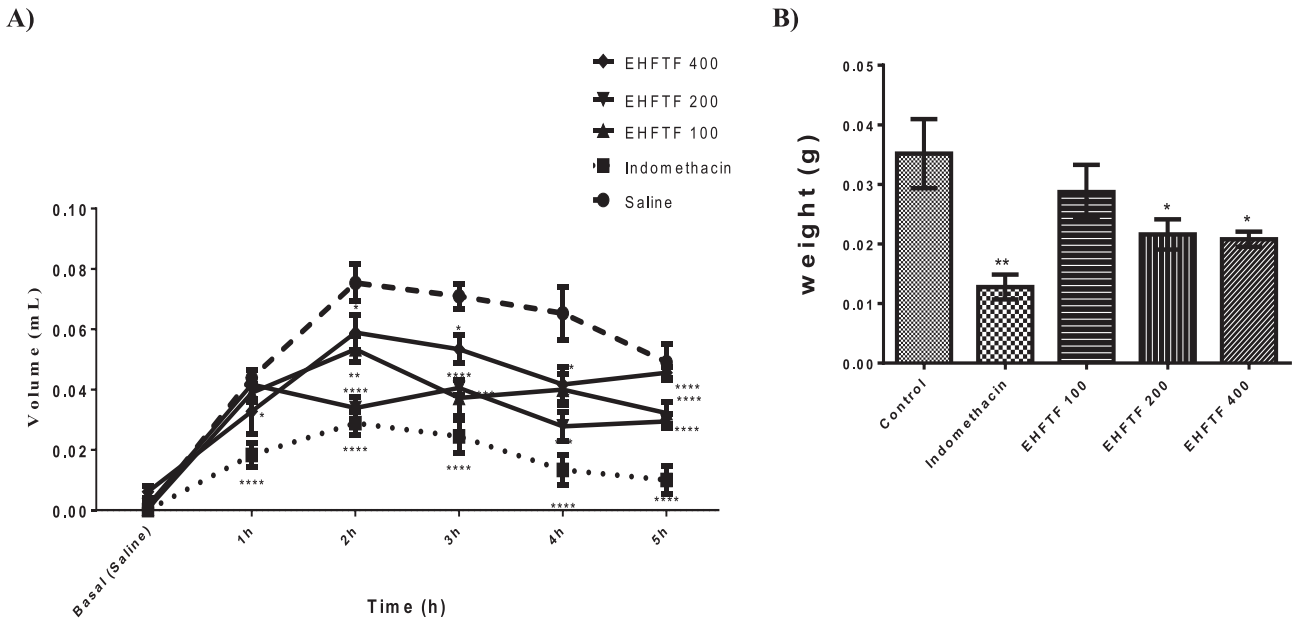

Fig. 1. Effect of oral administration of the extract on carrageenan-induced paw inflammation (edema), 1% (i.pl.), in the left hind paw and saline in the right hind paw. The results are presented as A: the symbols and lines indicate the mean ± standard error of the mean of change (Δ) of volume differences (ml) of the left and right paw compared to baseline values; B: The bars and lines indicate the mean ± standard error of the mean of weight difference (g) of the left and right paw compared to baseline values. **** p < .0001, *** p < .001, ** p < .01, * p < .05, when compared to negative control (Saline), One-way ANOVA followed by Tukey's test.

71.21%, 69.72% and 68.09% at 30, 45, 60, 90 and 120 min intervals, respectively (Fig. 2). A dose of 200 and 400 mg/kg exhibits a significant anti-inflammatory effect with initial action at 30 min of administration and throughout the test, with maximal effects at 120 min, on the contrary, the dose of 100 mg/kg had delayed action with significant results only after 90 min. Indomethacin expressed its maximum effect in the first 30mins and maintained a reduction of edema until 120 min. To rectify the plethysmometer data, weighing of the swollen paws was performed, where HELTF at a 200 and 400 mg/kg dose showed a significant reduction in the inflammatory process in relation to saline.

3.3.3. Vascular permeability test

To verify the participation of the vascular permeability process in the potential anti-inflammatory action of the extract, the smallest effective dose of the extract (200 mg/kg) was selected for this test according to the tests above. The values illustrate the effect of the extract on inflammation induced by intradermal carrageenan in the back of animals, on which the HELTF effect on protein/cellular migration (exudate) was compared and analyzed from the swollen tissue weight (Fig. 3A), as well as the concentration of Evans Blue in the supernatant (Fig. 3B), in relation to the control. HELTF and indomethacin significantly reduced edema (g) by

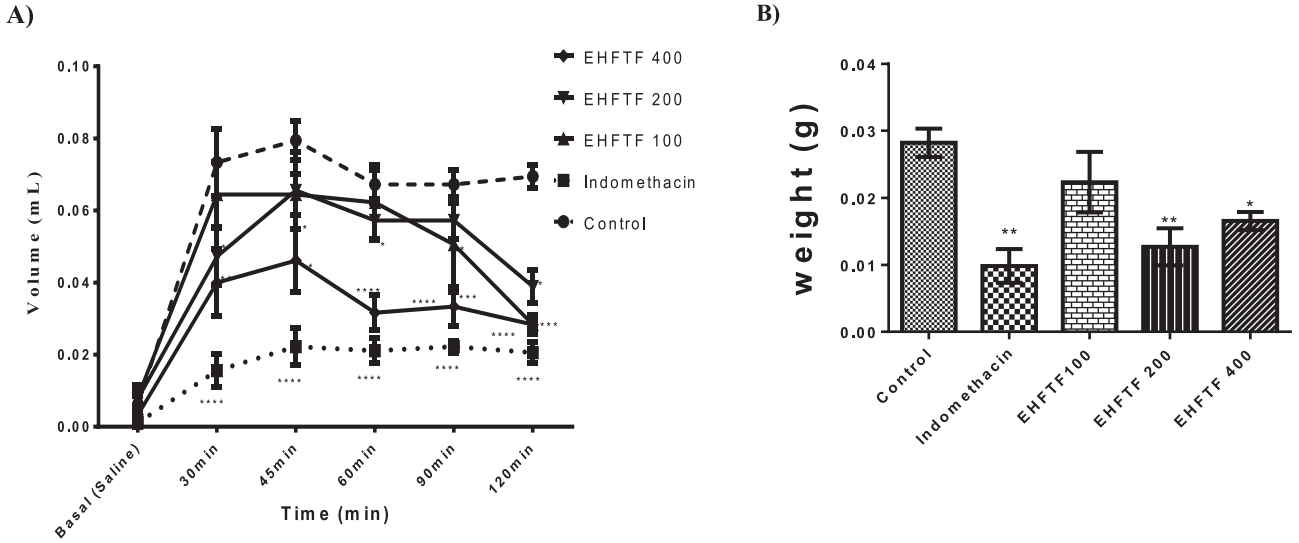

Fig. 2. Effect of oral administration of the extract on dextran-induced paw inflammation (edema), 1% (i.pl) in the left hind paw and saline in the right. The results are presented as: A: The symbols and lines indicate the mean ± standard error of the mean of change (Δ) of volume differences (ml) of the left and right paw compared to baseline values; B: The bars and lines indicate the mean ± standard error of the mean of weight difference (g) of the left and right paw compared to baseline values. **** p < .0001, *** p < .001, ** p < .01, * p < .05, when compared to negative control (Saline), One-way ANOVA followed by Tukey's test and paired T test.

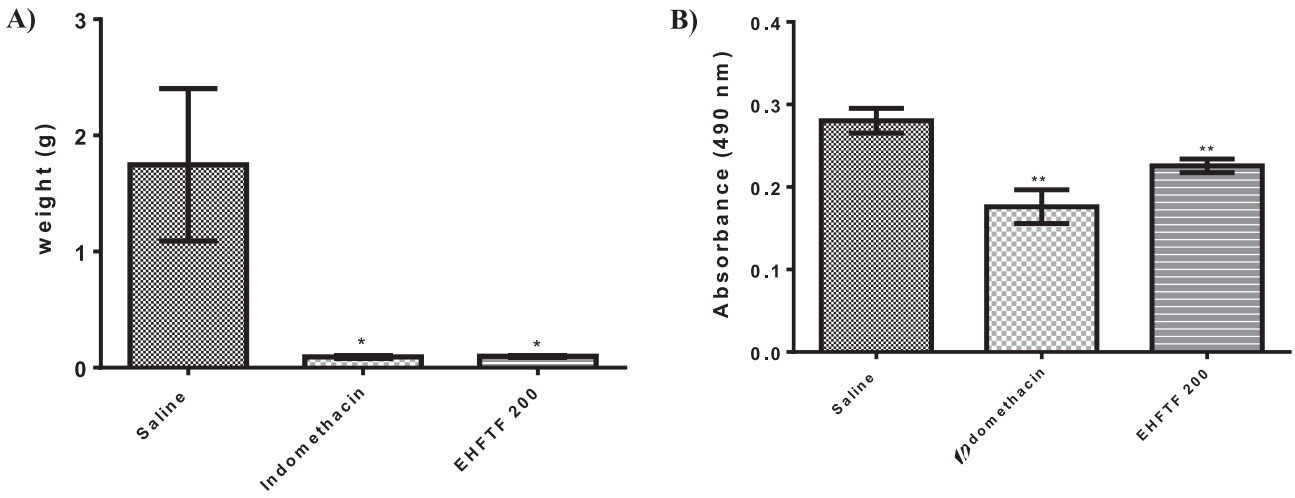

Fig. 3. Effect of oral administration of the extract on carrageenan-induced paw inflammation (edema), 1% (i.d.), on the back of mice. The bars and lines indicate the mean ± standard error of the mean of weight difference (g) of the left and right paw compared to control (Saline); B: the bars and lines indicate the mean ± standard error of the mean of absorbance (nm) of Evans Blue overflowed with the protein and leukocyte content compared to control (saline). **** $p < .0001$, ** $p < .01$, * $p < .05$, when compared to negative control, One-way ANOVA followed by Tukey's test.

94.32%, 94.56%, respectively. As for the evaluation of leukocyte extravasation exudate (nm), HELTF and indomethacin, produced an inhibition of 19.47%, 37.17%, respectively.

3.3.4. Croton oil-induced ear edema test

The data shows that the HELTF did not promote reduction in the inflammatory process and consequently of ear edema at the topical level, where the values presented were not significant in relation to the control group. Dexamethasone, exerted its antiedematogenic effect topically in an effective and significant manner (Fig. 4).

4. Discussion

This new study chemically characterized the Hydroalcoholic Extract of Leaves from *Tocoyena formosa* (Cham. & Schlecht) K. Schum. via a phytochemical *screening* and evaluated its toxic and anti-inflammatory activity, in experimental models *in vivo*. The reason for choosing the solvent Hydroalcoholic, besides the ease of extraction and purification by means of this solvent, is due to the fact that the number of active compounds carried is much larger.

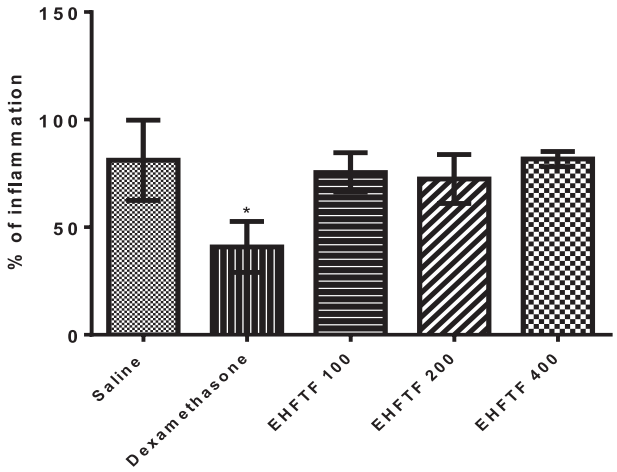

Fig. 4. Effect of topical administration of the extract on Croton oil-induced ear inflammation (edema) on the right ear and acetone on the left. The results are expressed as means and standard errors of the percentage difference of weight in milligrams between the left and right ear (Δ). * $p < .05$, when compared to negative control (Saline), One-way ANOVA followed by Tukey's test.

There are some reports in the literature about the chemical composition of *Tocoyena formosa* species just previously published by Cesário et al. (2018). They were isolated chemical constituents as saponin (3-O-beta-D-glucopyranosyl-28-O-b-D-glucopyrano syl-quinovic); flavonoid (3-O-beta-D-ramnosyl-3-O-methylquerce tin); iridoids (α and β Gardiol and genipin) (Da S. Bolzani et al., 1997; Hamerski et al., 2005). This result corroborated with compounds present in this work showed in Table 1.

The data shows that the HELTF presented significant anti-edematogenic efficacy especially at a 200 mg/kg dose, this effect was estimated using the paw edema test induced by carrageenan, as it is a method that considers the early stages of the acute inflammatory process (Gábor, 2003). This anti-edematogenic activity reinforces the indication that anti-inflammatory mechanisms are involved in the activity exerted by the extract in this test, as the inhibition promoted by HELTF in each time period in this test shows that the same has a non-selective antagonistic effect over the release or synthesis of mediators. In the first hour post carrageenan administration, the increase in vascular permeability is mediated by histamine and serotonin, in the second hour by kinins and in the third hour by prostaglandins and nitric oxide (Di Rosa et al., 1971), this therefore indicates that, HELTF is reducing or inhibiting mainly the release of kinins, prostaglandins (PG's) and nitric oxide (NO) and this effect may be related with caffeic acid, that demonstrates to act by this way (Shin et al., 2004), since the extract only begins acting after the second hour of carrageenan administration, giving evidence that the constituents of the extract have antiedematogenic action by these inflammatory pathways (Morris, 2003).

The results revealed that the extract also presents significant anti-inflammatory efficacy in the paw edema test induced by dextran, having a smallest effective dose of 200 mg/kg, however, with a significant difference compared to the previous test. One of the possible explanations is that the formation of this edema is mediated by the release of vasoactive amines, serotonin and histamine, followed by kinins and prostaglandins, causing osmotic edema (Dawson et al., 1991), corroborating with the edema test induced by carrageenan, because in the first hour of the carrageenan induction the inflammatory mediators are also serotonin and histamine and the extract did not present significant effect, this way, the

possible action of the extract in this test may have been through the inhibition of the kinin and prostaglandin pathway.

To further confirm the hypothesis of the extract as an anti-inflammatory, the smallest effective dose (200 mg/kg) was selected for evaluation of cellular migration. Taking into account that the inhibitory process of leukocyte migration is also one of the characteristic actions of compounds with anti-inflammatory actions, the vascular permeability test induced by carrageenan evaluated the action of HELTF in this migration. Drugs with anti-inflammatory effect potentially reduce and/or inhibit leukocyte transmigration by blocking the synthesis and/or release of chemotactic mediators or blocking a phase in the migration process (Muller, 2002). In the cited study, administration of the extract promoted significant reduction in leukocyte and neutrophil migration by blocking some of the phases to induced tissue damaged. This result reveals the specificity of the action promoted by the HELTF in influencing the establishment of the inflammatory process by reduction of leukocyte migration (Hebeda et al., 2011).

This reduction, encouraged by the extract, can be mediated by blocking the prostaglandin pathway, since in this test the migration process can be inhibited by diverse AINEs, prostaglandin inhibitors, and appear to be mainly related to with the reduction/inhibition of $PGF_{2\alpha}$ (de Menezes et al., 2005). As demonstrated above in the previous tests, the extract possibly presents action over prostaglandins, which may be inhibiting leukocyte extravasation in this way, as well as via NO, for this is also responsible for the interference of leukocyte recruitment (Moncada et al., 1991). This suggests and reinforces that the mechanism of action is related to the inhibition of PG's and/or NO observed in carrageenan assay.

The development of ear edema induced by croton oil is mediated mainly by serotonin and histamine (Gábor, 2003; Tubaro et al., 1986). Topical treatment with HELTF did not present a significate inhibition of the edema, with a similar profile to that obtained with the negative control test. This is a possible explanation for the inefficacy of the same via topical administration, furthermore, the extract may possess compounds with low cutaneous absorbance, giving evidence that the extract does not possess anti-inflammatory and anti-edematogenic activity at the topical level, only at the systemic level (v.o.), but to prove this it is essential to carry out other models of skin inflammation or of ear edema induced by various irritants.

This study demonstrated, from the data, that the class substances present in this extract, such as: tannins, flavonoids, triterpenes and saponins, are responsible for its anti-inflammatory therapeutic potential, since the literature on these class of substances, have confirmed this effect as shown in studies by de Jesus et al. (2012), Kim et al. (2004), Safayhi and Sailer (1997), Sparg et al. (2004), respectively. Rutin, Caffeic acid and quercetin, compounds identified in finger print of HELTF, present excellent antioxidant activity and this activity may be promote the reduction in the formation of free radicals, which are responsible for promoting, facilitating or co-participating in the development of inflammatory processes and tissue damage (Afanas' ev et al., 1989; Son and Lewis, 2002).

The extract in turn did not promote a significate toxicity effect, presenting with little effects over the systemic and CNS. In view of the results, it is observed that the acute oral toxicity of HELTF is greater than 5000 mg/kg, according to adopted experimental criteria. Thus, HELTF has a relative margin of safety for use as a therapeutic agent, however other studies need to be performed to establish its efficacy without the promotion of side effects, especially after repeated exposure in low doses, therefore, subchronic or chronic toxicity studies need to be performed to determine its risk-benefit.

This study illuminates propaedeutic perspectives on the use of the extract from *Tocoyena formosa* (Cham. & Schlecht.) K. Schum. as an anti-inflammatory agent, with similar characteristic in some tests to indomethacin, in which the evidence of this event requires further investigation, as well as new studies for the detection and clarification of the possible mechanism of action of the substance under study.

5. Conclusion

The first report establishing the chemical composition and anti-inflammatory activity of the extract obtained from the leaves of *Tocoyena formosa* (Cham. & Schlecht.) K. Schum. Our study demonstrated the presence of important secondary metabolites class, as flavonoid and tannin and identified presence of luteolin, chlorogenic acid, quercetin and caffeic acid as majority compounds can be responsible for anti-inflammatory activity. The results present in this work show a low acute toxicity and significate systemically inflammatory activity. However, this results showed possible action over the prostaglandin and/or nitric oxide pathway, but, new insights into the related molecular mechanisms.

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Declaration of interest

Declared for all due purposes, that there is no personal and financial relationships with other people or organizations that could inappropriately influence the publication of this article, and not create conflicts of interest.

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