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Anti-inflammatory evaluation and acute toxicity of three food supplements that contain *Moussonia deppeana*

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ABSTRACT

Objective: To identify the anti-inflammatory activity through two murine models and in the median Lethal Dose (LD_{50}) of three dietary supplements that contain *Moussonia deppeana*.

Methods: The anti-inflammatory activity of three dietary supplements (Cicatrisan/Gastricus®, Gastinol®, and Gastrovita®) EtOH extracts was evaluated by TPA and by carrageenan murine models; also, median Lethal Dose (LD_{50}) was determined. Verbascoside was quantified by High-Performance Liquid Chromatography. β-sitosterol, stigmasterol and the mixture of ursolic and oleanolic acids were identified in all supplements by TLC; however, none of these dietary supplements contain verbascoside.

Results: For the TPA model, Cicatrisan/Gastricus® generated a notable effect with 38.24% inhibition. While in the carrageenan model, it also exhibited noteworthy anti-inflammatory activity of ear edema with 66.39% of paw edema inhibition at 150 mg/kg, followed by Gastinol® and Gastrovita® with ≈50% at 300 mg/kg. Finally, LD_{50} was >2 g/kg for all supplements, when was administered intragastrically and Body Weight (BW) gain in mice was not altered after 14 days.

Conclusions: Of the three food supplements containing *M. deppeana*, only the EtOH extract from Cicatrisan/Gastricus® formulation (tablets) showed significant anti-inflammatory activity in both experimental models and the LD_{50} was >2 g/kg.

1. Introduction

Currently, worldwide commercialization and use of medicinal plants and their derivatives for treatment of several human illnesses is in expansion. The majority of these products, obtained from vegetal species, are labeled and distributed as food or dietetic supplements [1,2], thus avoiding the need for scientific evidence of their alleged pharmacological benefits or possible toxic effects. Therefore, companies decrease production costs but maintain high sales destined to a growing sector of the population who are in the search of more economical and accessible alternatives to conventional allopathic medicine [3,4].

Due to this high demand, many countries (mostly developed ones) have propagated countless strategies to regulate and support the use of medicinal plants and their derivatives, first through regional institutions funded by federal governments, and second, by the World Health Organization (WHO). This is due to majority of herbal remedies are registered for sale under a very lax legal framework, in which production quality is required but not the effectiveness and scientific verification of their pharmacological effects [2,3].

In this context, legislation and regulation of herbal remedies in Mexico is behind compared with those of developed countries such as the U.S. and Germany. The Federal Commission of Protection against Health Risks in Mexico defines herbal remedies as follows: 'an herbal remedy only has to prove its safety (sometimes this parameter is not even evaluated), and should not, under any circumstances, guarantee its therapeutic efficacy against a specific disease'. Due to this definition, there are gaps

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in the registration process of many products containing medicinal plants in their formulation [5].

Mexico City and some Mexican states are the main consumers of medicinal plants and their derivatives; it is estimated that daily, nearly 250 species are sold (fresh or dehydrated) in markets, these species are delivered from the center and south of the country, while the products elaborated with them, such as food supplements, are distributed and sold in naturist stores and pharmacies [4].

Due to that wide sales without regulation of these polyherbal remedies (mixture of 5–6 medicinal plant extract powders), in addition to supporting their ethnomedicinal use, it is also of great importance to study their possible toxic effects in order to ensure the safety of their consumption by the population. There are many medicinal plants that give rise to severe adverse effects, mainly hepatic and renal damage, such as chaparral (*Larrea divaricata*) or the purple passionflower (*Passiflora incarnata*); these cause tachycardia and ataxia when both are consumed for long periods of time [6,7]. Another example of how food supplements may cause adverse effects is described by Höllerhage *et al*. [8], who demonstrated that the intake of dietary supplements containing the plant materials of *Annonaceae* species (*Annona muricata* L., *Annona squamosa* L., *Annona mucosa* Jacq., *Annona squamosa* x *cherimola* Mabb.) could provoke neurotoxicity. For example, the Ethyl Acetate (EtOAc) extract from these supplements generated 67% of cellular death of human mesencephalic neurons *in vitro*.

One of the well-known medicinal plants in Mexican traditional medicine, and one that is widely used in the formulation of many food supplements and polyherbal remedies, but with few scientific studies, is *Moussonia deppeana* (Schldl. & Cham) Hanst; syn. *Kohleria deppeana*, *Gesneria deppeana*, or *Moussonia elongata*, commonly known as *tlachichinole*. This plant is frequently employed for the treatment of arthritis, colon and intestinal inflammation, stomach ache, kidney failure, vaginal infection, ulcers, diarrheas, burns and flu [9–11]. Some ethnopharmacological uses have been demonstrated through scientific research with some extracts obtained from its aerial parts or from the whole plant, such as antioxidant and anti-inflammatory [12,13], antimycobacterial [14], anti-*Helicobacter pylori* [15,16], antiprotozoal [17], and antitrypanosomal properties [18]. Also, in mouse, acute and sub-acute toxicity of the Ethanolic (EtOH) extract from aerial parts has been described; in this case, this extract demonstrated no adverse effects or lethality. In addition, verbascoside, a main metabolite, was identified in the EtOH extract and can be utilized as a marker in polyherbal formulations and dietary supplements that contain *M. deppeana* [13].

Although *M. deppeana* is a medicinal plant used widely as a food supplement, to date, the pharmacological from this plant alone has been reported, and not for food supplements that contains it. In this paper, we described the anti-inflammatory activity determined in two murine models and the median Lethal Dose (LD_{50}) of three dietary supplements in which this medicinal plant comprises part of their formulation.

2. Materials and methods

2.1. Description of food supplement formulation

The commercial name, the composition of each supplement, and the main use are described as follows:

Cicatrisan/Gastricus®: *Medicago sativa* (Fabaceae), *Malva sylvestris* (Malvaceae), *Kohleria deppeana* (Gesneriaceae), *Conyza filaginoides* (Asteraceae), and *Matricaria recutita* (Asteraceae), for stomach and intestinal inflammation.

Gastinol®: *Amphipterygium adstringens* (Julianaceae), *Salvia hispanica* L. (Lamiaceae), *Trigonella foenum-graecum* (Fabaceae), *Calendula officinalis* (Asteraceae), *Kohleria deppeana* (Gesneriaceae), and *Aloe barbadensis* (Xanthorrhoeaceae), for gastritis and stomach inflammation.

Gastrovita®: *Gentiana lutea* (Gentianaceae), *Croton niveus* (Euphorbiaceae), *Amphipterygium adstringens* (Julianaceae), *Mentha piperita* (Lamiaceae), and *Kohleria deppeana* (Gesneriaceae), for intestinal inflammation and gastric ulcers.

Formulation of three food supplements that contain *Moussonia deppeana* are as follows: Net content/pharmaceutical presentation of Cicatrisan/Gastricus®, Gastinol® and Gastrovita® are 353 mg/50 tablets, 220 mg/40 capsules and 500 mg/40 capsules, respectively; *M. deppeana* formulation dose of Cicatrisan/Gastricus® and Gastinol® are 37.5 mg/tablet, and 20.0 mg/capsule, respectively. Food supplements are used 3 times/day each meal.

The polyherbal preparations were purchased in naturist stores in Mexico city.

2.2. Ethanolic extract preparation

The capsules were emptied until reaching 20 g of each Gastinol® and Gastrovita® supplements, while Cicatrisan/Gastricus® tablets were ground into powder (20 g). Each sample was macerated with EtOH (200 mL) for 1 week under constant shaking at room temperature. Final extracts were filtered and concentrated at 40 °C using a rotary evaporator (Buchii RE-11) coupled to a vacuum system (BuchiiVac V-153) and a cooling system (ECO 20). The extracts were maintained under conditions of darkness until their use. Yield was calculated for each compared with its respective dried-powder initial weight.

2.3. Qualitative phytochemical analysis and verbascoside quantification through HPLC

In the EtOH extracts of each sample, β-sitosterol, stigmasterol, ursolic acid, and oleanolic acid were detected by Thin Layer chromatography (TLC) utilizing as mobile phases and chromogenic agents described previously [13]. For the identification of verbascoside, EtOAc:EtOH:H_2O (100:13.5:10) or EtOAc:Formic acid:Acetic acid:H_2O (10:1.1:1.1:0.3) as mobile phase were employed and were detected with 2-aminoethylester diphenyl boric acid 1% in MeOH with polyethylene glycol 5% in EtOH as chromogenic agent. β-sitosterol, stigmasterol, ursolic acid, and oleanolic acid were compared with Sigma pure standards and standard verbascoside was obtained previously from the *M. deppeana* EtOH extract [13].

For verbascoside identification and quantification, High-Performance Liquid Chromatography (HPLC) analysis was carried out in Waters equipment (Waters, USA) comprising a 600E multi-solvent delivery system with a 486 UV detector, as described by Gutiérrez-Rebolledo *et al*. [13]. Equipment control, data acquisition, and the processing and management of HPLC information were performed by Empower 2 software (Waters). Analytical conditions were employed: column ZORBAX Eclipse XDB-C18 (5 μm, 4.6 × 250 mm i.d.) with pre-column

(Agilent Technologies); the mobile-phase linear gradient of 0.0125N aqueous-acetic acid (eluent A) and CH_3CN (eluent B), starting from 95% A at 50% in 20 min and returning to 95% for 20 min–25 min, was maintained for 35 min. Flow rate was 0.7 mL/min and injection volume was 20 μL; peaks were detected at 280 nm, and this condition was utilized for each EtOH extracts.

2.4. Animal in vivo assays

Balb/c male mice (25 ± 5) g was obtained from the Bioterium CMN-SXXI, IMSS, Mexico City, Mexico. Experiments were performed according to the statutes of the International Committee for the Care and Use of Laboratory Animals (IACUC) and Mexican Official Norm (NOM-062-ZOO-1999) revised in 2016 [19]. The project (CNIC-IMSS R-2013-785-053) was approved by the National Commission of Scientific Investigation.

2.5. Acute toxicity (LD_{50})

LD_{50} was determined following the methodology describe by the Organization for Economic Co-operation and Development and Test Guideline (OECD TG 423) [20] and Gutiérrez-Rebolledo *et al.* [13], the assay was performed in two independent tests. The EtOH extracts at 1 and 2 g/kg were administered intragastric (i.g.) via after a 12-h fasting period and controls received vehicle (Tween 80-H_2O, 1:9), in a volume not exceeding 10 mL/kg Body Weight (BW). Mice were observed during 14 days and their BW gain was registered at days 3, 7, 9, and 14.

2.6. In vivo anti-inflammatory evaluation

2.6.1. 12-O-Tetradecanoyl Phorbol 13-Acetate (TPA)-induced mouse ear edema

This assay was conducted as described by Gutiérrez-Rebolledo *et al.* [13]. The experimental groups (n = 7) received TPA (0.1 μg/μL in acetone), and 30 min later were treated with the EtOH extracts or Indomethacin (2 mg/ear) in the right ear. Anti-inflammatory activity was calculated according to the weight difference between the ear sections (6 mm) at 6 h, compared with that of the control group, using the formula previously described [13].

2.6.2. Carrageenan-induced mouse paw edema

The assay was performed as described by Gutiérrez-Rebolledo *et al.* [13]. Treated groups (n = 7) received Indomethacin (10 mg/kg) or EtOH extracts (150 and 300 mg/kg) by i.g. route and 1 h prior to the injection of carrageenan (20 μL, 2%). Percentage of inhibition was calculated using the formula previously described [13,21].

2.7. Statistical analysis

Sigma Plotter ver. 12.0 statistical software (2011–2012) was utilized. Data was presented as Standard Error of the Mean (SEM). BW gain values in the acute toxicity test and the development of paw edema in the carrageenan model were analyzed with bifactorial ANalysis Of VAriance (ANOVA) and with a post hoc Student–Newman–Keuls (SNK) test. Results of $P < 0.05$ were considered statistically significant. For TPA assay one-way ANOVA was employed with a post hoc SNK test, in which $P < 0.05$ was considered significant.

3. Results

3.1. Phytochemical analysis

The EtOH extract of each dietary supplement was prepared via maceration process and was analyzed by TLC and HPLC. The Dry Weight (DW) of each sample was 3.85, 2.14, and 4.04 g for Gastrovita®, Gastinol®, and Cicatrisan/Gastricus®, respectively, with a yield of 14.5, 7.0, and 10.8% with respect to DW. TLC analyses of each extract demonstrated the presence of β-sitosterol and stigmasterol with Retention factor (R_f) values of 0.43 and 0.49, respectively, utilizing Hex: $CHCl_3$ (7:3) as an elution system. Also, the mixture of ursolic and oleanolic acids with [R_f = 0.47 in Hex:EtOAc (6:4) or 0.52 in CH_2Cl_2:MeOH (96:4)] was detected. These compounds were identified by comparison of the R_f with their Sigma commercial reference. Only Cicatrisan/Gastricus® exhibited traces of an unidentified polyphenol that may be a derivative of caffeic acid or chlorogenic acid. In addition, all extracts were analyzed by HPLC, and the chromatograms showed that none of the samples contained verbascoside (Figure 1). Standard verbascoside demonstrated an R_t = 9.20 min with UltraViolet (UV) spectra (λ_{max}): 219 and 330 nm. The Gastrovita® sample showed four peaks between 8.93 and 9.89 min; the peak at an R*t* of 9.19 min was very similar to that of standard verbascoside, but the UV spectrum (λ_{max} at 214 and 327 nm) was different. In addition, the Gastrovita® sample exhibited, in the chromatogram, two peaks with an R*t* at 28.88 and 28.66 min, these absorbed at 208, 267 and 349 nm (Figure 2), which perhaps can be a luteolin derivative or a *p*-coumaric acid ester. We are currently in the process of isolating these compounds to determine their structure.

3.2. Acute toxicity

The LD_{50} value for each supplement was >2 g/kg when was administered orally. The single i.g. administration of EtOH extracts from the three supplements did not generate lethality during the observation period (14 days). At the end of the experimental period, BW gain did not change with respect to the control group, the major organs exhibiting no macroscopic abnormalities after extraction (data not shown).

3.3. In vivo anti-inflammatory activity

3.3.1. TPA model

Only the EtOH extract from Cicatrisan/Gastricus® tablets at 2 mg/ear demonstrated a statistically similar effect (38.24%) to that of Indomethacin (43.29%), while Gastrovita® and Gastinol® supplements generated statistically lower anti-inflammatory activity compared with the reference drug, with inhibition values of 20.48% and 33.21%, respectively. Although topical administration is not the common way that these supplements are used, it is noteworthy that the most effective supplement when applied topically was Cicatrisan/Gastricus®: thus, the mixture of medicinal plants in this dietary supplement formulation generates notorious, local anti-inflammatory activity similar to that of Indomethacin (Table 1).

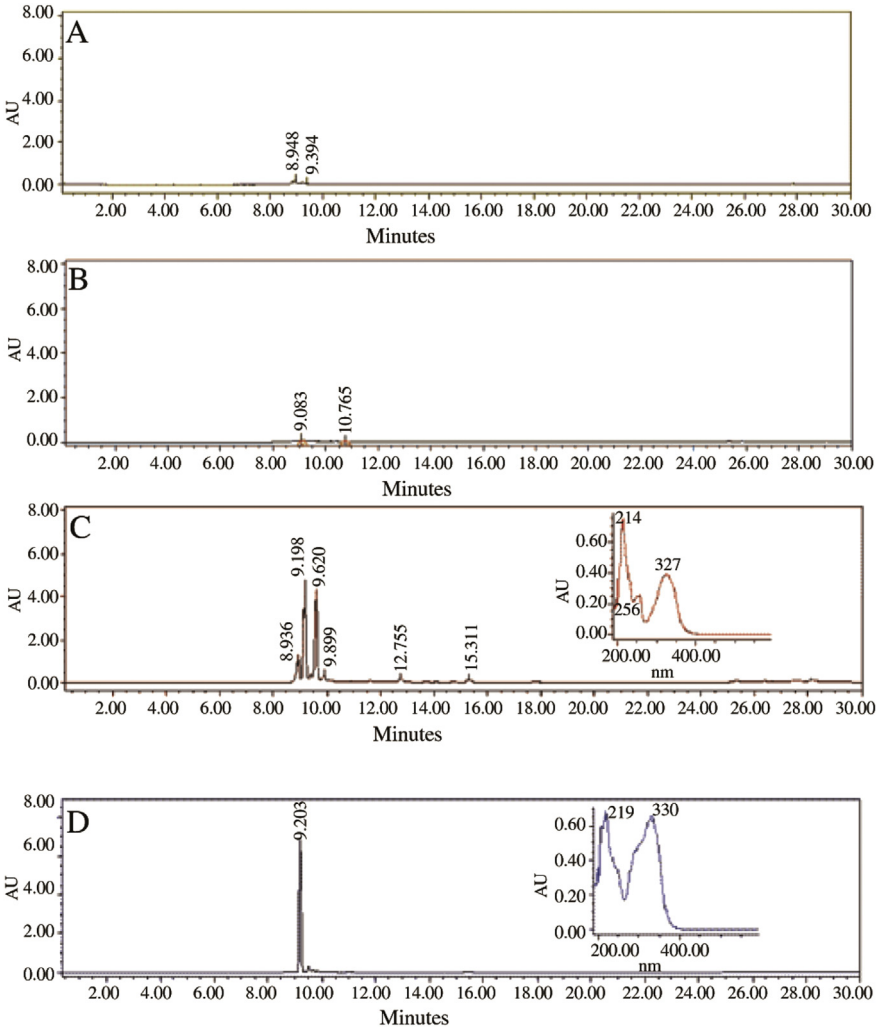

Figure 1. High-performance liquid chromatography chromatogram of verbascoside quantification in food supplements that contain *Moussonia deppeana*. A: Gastinol®, B: Cicatrisan/Gastricus®; C: Gastrovita®; D: Standard verbascoside.

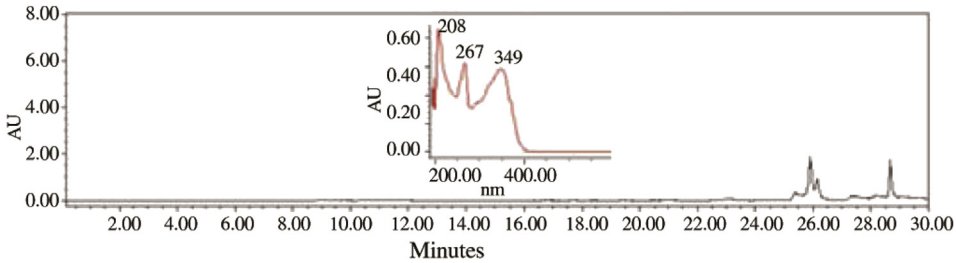

Figure 2. High-performance liquid chromatography chromatogram of the Gastrovita® supplement.
R_t = 25.88 min, possible correspondence to luteolin derivative, and R_t = 28.60 min, corresponds to coumaric acid ester.

3.3.2. Carrageenan model

The EtOH extract from Gastinol® exerted the statistically similar anti-inflammatory effect at both doses (150 and 300 mg/kg) 5 h after carrageenan injection (43.77% and 51.14%, respectively); however, these values were lower than that generated by Indomethacin at the same time (61.34%). Only supplement that demonstrated a dose-dependent effect was the Gastrovita® EtOH extract, due to that the lower dose of 150 mg/kg inhibited paw edema formation in 35.60%, while at 300 mg/kg, this inhibited 49.59%; this values were less than that of Indomethacin at same time. Finally, the Cicatrisan/Gastricus® EtOH extract at both doses exhibited effects of nearly 66.39 and 56.10%, respectively; both values were statistically similar to that observed by Indomethacin in the same time. The EtOH extracts obtained from Cicatrisan/Gastricus® tablets generated similar anti-inflammatory activity to that of Indomethacin and the same behavior as in TPA-induced ear edema, while Gastinol® and Gastrovita® EtOH extracts had the statistically same effect at 300 mg/kg, but this was below that of Indomethacin inhibition (Table 2).

Table 1
Anti-inflammatory activity of *Moussonia deppeana* food supplements on ear edema induced by TPA.

Treatment	Auricular edema formation (mg)	Inhibition (%)
Control	14.70 ± 1.40	–
Indomethacin	8.34 ± 0.72[a]	43.39
Gastrovita®	11.72 ± 0.84[ab]	20.48
Cicatrisan/Gastricus®	9.10 ± 0.37[a]	38.24
Gastinol®	9.84 ± 0.87[ab]	33.21

Data are expressed as mean ± SEM. Inhibition was calculated with respect to the TPA control group. One-way ANOVA, post hoc Student–Newman–Keuls (SNK), [a]$P \leq 0.05$ compared with TPA control group, [b]$P \leq 0.05$ compared with Indomethacin group; $n = 7$; Doses: 2 mg/ear.

Table 2
Anti-inflammatory activity of *Moussonia deppeana* food supplements on carrageenan assay.

Treatment	Dose (mg/kg)	Paw edema formation (mm)	Inhibition (%)
Carrageenan	–	0.41 ± 0.03	–
Indomethacin	10	0.16 ± 0.01[ab]	61.34
Gastinol®	150	0.20 ± 0.01[ab]	43.77
	300	0.19 ± 0.02[ab]	51.14
Gastrovita®	150	0.27 ± 0.01[abd]	35.60
	300	0.21 ± 0.01[abe]	49.59
Cicatrisan/Gastricus®	150	0.14 ± 0.01[ace]	66.39
	300	0.20 ± 0.01[ace]	56.31

Data are expressed as mean ± SEM. One-way ANOVA, post hoc Student–Newman–Keuls (SNK), [a]$P < 0.05$ compared with carrageenan control, [b]$P < 0.05$ compared with Indomethacin, [c]$P < 0.05$ compared with Gastinol® 150, [d]$P < 0.05$ compared with Gastinol® 300, [e]$P < 0.05$ compared with Gastrovita® 150; $n = 7$; Time: 5 h.

4. Discussion

The presence of β-sitosterol, ursolic and oleanolic acids mixture, and verbascoside in an EtOH extract from aerial parts of *M. deppeana* through Nuclear Magnetic Resonance (NMR) ^{1}H and TLC analysis, were previously described [13], and verbascoside has been identified as a chemical fingerprint for this medicinal species. The lack of verbascoside in all three supplements may indicate that perhaps none of these really contain *Moussonia deppeana* powder. On the other hand, luteolin has been identified previously as a main compound in all *Gentiana* genus [22]; in addition, the coumaric acid ester (gentianin) has been identified and isolated from *Gentiana verna* flowers and in other plants of the Gentianaceae family [23]. *Gentiana lutea* is a medicinal plant that is present in the Gastrovita® supplement formulation.

In addition, the three dietary supplements show a LD_{50} values of >2 g/kg and are considered a Category 5 substance according to the OECD TG 423 [13], none of the three supplements caused lethality at the high dose tested (2 g/kg), nor an alteration on BW gain, nor macroscopic lesion in major organs.

These supplements showed a moderated anti-inflammatory activity in the TPA model respect to Indomethacin. Previous works describe the topical anti-inflammatory effect exerted by different extracts of *M. deppeana*. Domínguez-Ortiz *et al.* [12], for example, detailed that successive hexanic, EtOAc and EtOH extracts of *M. deppeana* leaves at 2 mg/ear dose inhibited ear edema formation in 39%, 28%, and 4%, respectively, while Gutiérrez-Rebolledo *et al.* [13] described that the direct EtOH extract from *M. deppeana* aerial parts generated an inhibition of ≈60% in both male and female mice at the same dose. This observed topical anti-inflammatory activity is due to the presence of metabolites such as ursolic and oleanolic acids, which have been described as anti-inflammatory compounds that suppress the prostaglandin biosynthesis pathway [24–28].

The TPA assay in mice is very utilized to evaluate any anti-inflammatory compound or extract that can be applied topically, evaluating the capacity of topical agents to inhibit inflammatory mediators resulting from Protein Kinase C (PKC) activation pathway in which phospholipase A_2 is stimulated and leads to the biosynthesis of prostaglandins and leukotrienes from arachidonic acid, both involved in the inflammation acute process [29].

Medeiros *et al.* [30] demonstrated that the EtOH extract of Brazilian polyherbal formulation (*Eucalyptus globulus* Labill, *Peltodon radicans* Pohl and *Schinus terebinthifolius* Raddi) administered by i.g. route was effective in reducing ear edema in the TPA-induced ear edema model, showing 49% of inhibition in rats. Other studies, described that the herbal remedies or formulations evaluated by topical administration showed that when 0.2 g of a gel containing 1% w/w herbal MeOH extract of leaves from *Eupatorium adenophorum* (Asteraceae) was rubbed on the hind paw 1 h prior to carrageenan injection, it generated 20% inhibition in paw edema formation at hour 5 [31]. In addition, Jyothi and Koland [32] described the topical application of 0.2 g of gel containing *Trigonella foenum-greacum* EtOH extract exhibited significant reduction of paw edema (57.78%) when was compared with the control topical-baseline group 3 h after carrageenan injection.

From the three food supplements containing *M. deppeana*, only the EtOH extract from the Cicatrisan/Gastricus® formulation (tablets) showed significant anti-inflammatory activity in both experimental models. This activity was comparable to that of the standard drug Indomethacin when administered by topical and i.g. routes, supporting its common use as a food supplement for treatment of acute inflammation-related diseases. Any food supplement contained verbascoside (this compound is a main metabolite present in *M. deppeana*) when was analyzed by HPLC, this metabolite was considered as fingerprint by *M. deppeana* [13]. Gastinol and Gastrovita exhibited a moderate systemic anti-inflammatory activity at 150 and 300 mg/kg. In a previous paper, the EtOAc extract from *M. deppeana* leaves at 100 mg/kg dose showed good inhibition (43%) at 1 h, but poor effect (17%) at 5 h [12]. On the other hand, the EtOH extract from *M. deppeana* aerial parts at 300 and 450 mg/kg generated ≈45% and ≈53% inhibitions, respectively, in paw edema formation in both male and female mice at hour 5. Doses of 150 and 600 mg/kg generated poor anti-inflammatory activity at the same time (≈30% and ≈25%, respectively) in both sexes for the carrageenan model [13]. The systemic anti-inflammatory effect was demonstrated by EtOH extracts in the carrageenan model to its main identified metabolites, such as verbascoside, ursolic and oleanolic acid, apigenin, and hesperetin [13], which are well-known COX-2 inhibitors [33–35]. In addition, Cao *et al.* [22] also described the anti-inflammatory effect of *Gentiana striata* Maxim EtOAc leaf extract during experimental arthritis in rat through inhibition of prostaglandin biosynthesis decreasing paw edema development;

it is noteworthy that this extract exhibited high levels of luteolin, a compound identified in the Gastrovita® supplement.

Deorukhakar *et al.* [36] demonstrated the anti-inflammatory effectiveness of the oral administration of polyherbal formulation Entox® constituted of fruits of *Terminalia chebula* (Combretaceae) and *Embelica officinalis* (Euphorbiaceae) fruits, *Punica granatum* (Punicaceae) fruit rinds, *Terminalia arjuna* (Combretaceae) bark, *Rubia cordifolia* (Rubiaceae), *Withania somnifera* (Solanaceae), *Tinospora cordifolia* (Menispermaceae) roots, and *Curcuma longa* (Zingiberaceae) rhizomes, which revealed 51.61% and 54.84% inhibition of paw edema in Wistar rats (carrageenan model) at doses of 300 and 600 mg/kg, respectively, at 3 h, using as Indomethacin (10 mg/kg) as reference drug, which inhibited paw edema in 61.29% at the same time. Another study described the anti-inflammatory activity of Dazzle® capsules [*Boswellia serrate* (Burseraceae), *Vitex negundo* (Lamiaceae), and *Withania somnifera* (Solanaceae)] in carrageenan-induced paw edema in rats, which were administered in oral doses of 90 and 180 mg/kg, demonstrating 39% inhibition on edema development at hour 5. According to the authors, this effect occurs through inhibition of prostaglandin synthesis by major metabolites identified in Dazzle® capsules, such as alkaloids, flavonoids, and tannins [37].

The anti-inflammatory activity of each of the constituents of the Cicatrisan/Gastricus® formulation, which includes *Medicago sativa* (Fabaceae) [38], *Malva sylvestris* (Malvaceae) [39], *Kohleria deppeana* or *M. deppeana* (Gesneriaceae) [13], *Conyza filaginoides* (Asteraceae) [40], and *Matricaria recutita* (Asteraceae) [41], has been reported in the scientific literature, in which the authors relate their anti-inflammatory effect with the presence of polyphenols.

Despite demonstrating the anti-inflammatory activity of each of the species present in the food supplements evaluated, the effect observed was not significant, nor was it greater than that of the reference drug (Indomethacin). This could be because the amount of each vegetable species in the supplement is very low, or that the food supplements are adulterated; therefore, it is advisable to conduct this type of investigation to confirm its use.

Carrageenan assay is widely employed to identify orally active agents during an acute inflammation phase [42]. In this model, compounds or extracts that could inhibit the release of vasodilators, such as histamine, to an inactivation of the induced COX-2 enzyme are also evaluated [13,24].

Conflict of interest statement

The authors declare that they have no conflict of interest.

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