Research Article

Anti-inflammatory Activity of the Essential Oil of Croton zehntneri and Its Main Constituent Estragole

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ABSTRACT

The essential oil of *Croton zehntneri* (EOCz) has largely been used in traditional medicine. There are many studies regarding the biological activities of this essential oil, however, few studies have been performed to determine its main constituents and evaluate their biological effects. Furthermore, its anti-inflammatory activity remains to be determined. In this paper, we evaluated the anti-inflammatory activity of EOCz and its main constituent, the estragole (EST). In this regard, a series of experiments was conducted to determine the effect of the test compounds on carrageenan-induced paw edema and carrageenan-induced pleurisy. Our data showed that EOCz reduced significantly the cellular migration and edema in carrageenan-induced pleurisy, after oral administration. Also, we observed that EST promoted a significant anti-inflammatory activity by decreasing the carrageenan-induced paw edema. In conclusion, we propose that both EOCz and EST modulate the inflammatory response after oral administration, in rodents. Then, the EOCz anti-inflammatory effect is partially related to EST, its main constituent. It constitutes a promising therapeutic profile meriting further investigations.

Keywords: Inflammatory response; essential oil; *Croton zehntneri*; estragole.

1. INTRODUCTION

Since ancient times, medicinal plants have been used by the human population in order to treat diseases and recovering health. Nowadays, considering that part of the population still has low access to resources of traditional medicine, the use of natural products has a great significance in maintaining health. Furthermore, natural compounds offer one of the most important sources of biologically active molecules in the development of new drugs[1]].

The aromatic plant *Croton zehntneri* (Euphorbiaceae) has been used in the folk medicine as sedative, appetite stimulant and antiseptic[2,3]. The essential oil extracted from *Croton zehntneri* (EOCz) is rich in monoterpenes, sesquiterpenes, and arylpropanoids[4]. Some studies have demonstrated that EOCz presents relaxant and antispasmodic activities and improves cutaneous wound healing[5–7]. Also, cardiovascular effects and antibacterial activity were demonstrated for this essential oil[8,9]. Thus, the study of its biological activities, chemical composition, and toxic effects has a great significance.

One interesting compound contained in EOCz is the estragole (EST), a phenylpropanoid largely used as a flavoring agent by the food and beverage industry[8]. It is also found in essential oils from many aromatic plants commonly used in traditional medicine, such as *Artemisia dracunculus* (Asteraceae), *Ocimum basilicum* (Lamiaceae), *Pimpinella anisum* (Apiaceae), *Illicium anisatum* (Illiciaceae) and *Foeniculum vulgare* (Apiaceae)[9]. According to recent studies, EST exerts many pharmacological and biological effects, such as anticonvulsant[10], myorelaxation[2], bradycardia[8], antimicrobial[11], and immunomodulatory activity[12].

However, there are just a few reports describing the biological effects exerted by EOCz or EST as a neat compound. Furthermore, their anti-inflammatory activity has been superficially evaluated and constitutes the focus of this report.

In this regard, we conducted a series of experiments aimed at evaluating the potential anti-inflammatory activity exerted by EOCz

and EST on carrageenan-induced paw edema and carrageenan-induced pleurisy.

2. MATERIALS AND METHODS

2.1. Plant material and constituents of the essential oil

The leaves of *Croton zehntneri* were commercially purchased in Maringá, Paraná, Brazil. The essential oil was extracted by conventional steam distillation using a Clevenger-type apparatus for 2 h. The obtained essential oil was dried over sodium sulfate and stored at 4 °C in dark vials until tested. The main constituent EST (98% purity) was purchased from Sigma (St. Louis, MO, USA).

2.2. Analysis of the Essential Oil and Compound Identification

2.2.1. Gas Chromatography-Mass Spectrometry

Gas chromatography (GC) was performed with a Thermo Electron Corporation Focus GC model under the following conditions: DB-5 capillary column (30 m × 0.32 mm, and 0.50 mm); column temperature, 60 ºC (1 min) to 180 ºC at 3 °C/min; injector temperature, 220 ºC; detector temperature, 220 ºC; split ratio, 1:10; carrier gas, He; flow rate, 1.0 mL/min. The volume injected (1 µL) was diluted in chloroform (1:10). The GC/mass spectrometry (MS) analysis was performed with a Quadrupole mass spectrometer (DSQ II model, Thermo Electron Corporation) that operated at 70 V. The identification of the individual compounds was based on comparisons of their GC retention indices (RI) on an apolar column and comparisons with the mass spectra of authentic standards purchased from Sigma-Aldrich and literature data.

2.2.2. Nuclear Magnetic Resonance

The Nuclear Magnetic Resonance (NMR) was used to prove the chemical structure of the essential oil constituents identified by gas chromatography – mass spectrometry (GC-MS). ^{1}H (300.06 MHz) and ^{13}C nuclear magnetic resonance (NMR; 75.45 MHz) spectra were recorded in a deuterated chloroform ($CDCl_3$) solution using a Mercury-300BB spectrometer, with δ (ppm) and spectra referenced to $CDCl_3$ (δ 7.27 for ^{1}H and δ 77.00 for ^{13}C) as the internal standard.

2.3. Animals

Male Wistar rats (180-220 g) and male mice Swiss (25-35 g) were used. The animals were obtained from the Central Biotherium of the State University of Maringá and housed under standard conditions receiving food and water *ad libitum* before experimentation. The experimental protocol was approved by the Ethical Committee in Animal Experimentation of the State University of Maringá (CEAE/UEM 126/2010).

2.4. The anti-inflammatory activity of EOCz

2.4.1. Carrageenan-induced pleurisy

According to Vinegar et al.[13], male Wistar rats were orally treated with EOCz (125, 250 or 500 mg/kg), indomethacin (5 mg/kg) or saline (Control group). Thirty minutes later, the animals received an intrapleural carrageenan injection (200 µg/animal) or an equal volume of saline. Four hours after stimuli, the animals were anesthetized and euthanized, the pleural exudates were collected, the volume was determined, and the pleural harvested by introducing 2 mL of phosphate buffered saline (PBS) with ethylenediaminetetraacetic acid (EDTA). Then, counts were performed in order to determine the total number of cells per cavity. The results were expressed as the pleural exudate volume and the number of leucocytes per cavity.

2.5. The anti-inflammatory activity of the main constituent EST

2.5.1. Carrageenan-induced paw edema

Paw edema was induced in the right hind paw of the mice by a subplantar injection of carrageenan suspension (200 µg/paw) in sterile saline. The contralateral paw was injected with saline (negative control). EST (125, 250 or 500 mg/kg) or indomethacin (5 mg/kg) were administered orally 30 min before carrageenan administration. The degree of edema was measured 1, 2, and 4 h after carrageenan injection using plethysmography (Ugo-Basile, Comerio, Italy). Edema was expressed in terms of the increase in paw volume, subtracting the rates of the paw injected with saline (control paw) from the rates of the paw injected with carrageenan.

2.6. Statistical analysis

Results were expressed as mean ± standard error of the mean (SEM). Data were subjected to analysis of variance (ANOVA) followed by Tukey's post hoc test. Values of $P < 0.05$ were considered statistically significant.

3. RESULTS AND DISCUSSION

The chemical composition of EOCz was investigated using GC-MS and NMR. The results of the GC-MS analysis (Figure 1) showed a predominance of EST (87.5%). The percentages of the major components and retention time are summarized in Table 1.

The pleurisy model is used to investigate the mechanisms involved in acute inflammation and to evaluate the effectiveness of drugs with anti-inflammatory properties[17]. Acute inflammation is the response of the tissue to injury and is characterized by edema formation, fluid exudation and recruitment of inflammatory cells. Immediately after the injury occurs local production of inflammatory mediators, such as nitric oxide (NO), prostaglandin E2 (PGE2), interleukin (IL)-1β, IL-6 and tumor necrosis factor (TNF-α), that promote an increase in capillary permeability and also chemotaxis. These factors act together, leading to cellular and vascular events of inflammation[14,15]. Thus, drugs that modulate the production these inflammatory mediators may provide an interesting therapeutic option for acute inflammation treatment.

Our results showed that intrapleural injection of carrageenan induced an acute inflammatory response characterized by an increase in the pleural exudate volume and an intense cell migration to the pleural cavity. Oral administration of EOCz (125, 250 and 500 mg/kg) reduced significantly the inflammatory edema volume when compared to the Control group (Table 2), but was not able to reduce the leukocyte migration to the pleural cavity. In other studies performed by our research group, the EST (EOCz major compound) was able to reduce the *in vivo* and *in vitro* leukocyte migration[16]. This discrepancy might be related to the antagonistic effect of the other constituents of this essential oil[17,18]. These effects were also observed with others aromatic plants such as anise (*Piper auritum*), fennel (*Foenicullum vulgare*), clove (*Syzygium aromaticum*), and ginger (*Zingiber officinale Roscoe*)[19,20].

Intradermal injection of carrageenan in the hind paw of rats induced a progressive edema which was measurable 1 h after the injection, reaching its maximum at 4 h after administration. As observed in Figure 2, a single oral dose of EST promoted a significant decrease in the paw thickness of rats at all doses tested, an effect observed 4 h after the administration of this phlogistic agent. However, only the treatment with EST 500 mg/kg reduced the paw edema 2 h after carrageenan injection.

Data from the literature report that non-steroidal anti-inflammatory drugs are effective against edema formation, mainly in the late phase in which prostanoids derivatives are involved[21]. In this work, in a manner similar to indomethacin, oral administration of EST showed an inhibitory effect on paw edema at 4 h after the carrageenan administration. Recently, a study performed by our research group showed that the EST was able to reduce PGE2 levels in the paw tissue of the animals after carrageenan injection[22]. This effect could be related to the decreasing in the paw edema. However, it is not discarded the possibility of EST is acting in other stages of carrageenan-induced inflammatory process.

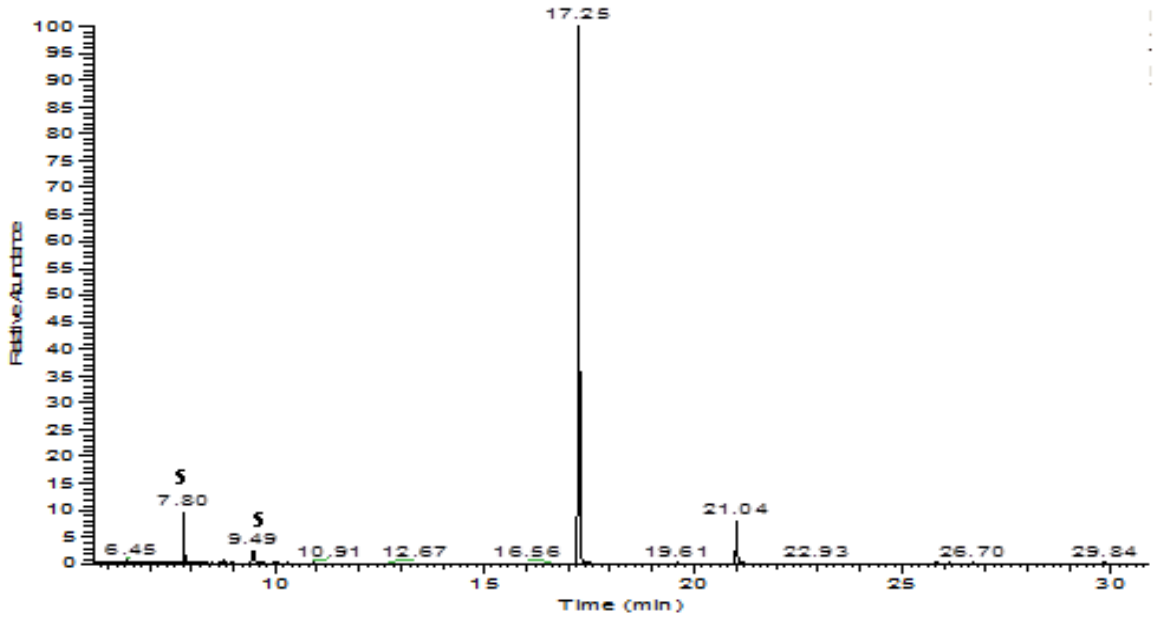

Fig. 1: Chromatogram of *Croton zehntneri* essential oil. Percentage data were obtained by gas chromatography – mass spectrometry (CG-MS)

Table 1: Percentual chemical composition of *Croton zehntneri* essential oil

Retention Time	Compound	Percentual (%)	Identification
6,98	α-pinene	0,5	MS, NMR
8,78	β-myrcene	0,2	MS,NMR
10,04	p-cymene	0,3	MS, NMR
10,30	1,8-cineole	0,5	MS, NMR
17,25	estragole	87,5	MS, NMR
19,61	anisaldehyde	0,3	MS, NMR
21,04	anethole	7,7	MS, NMR
26,70	β-caryophyllene	0,3	MS
25,01	methyl eugenol	0,3	MS
29,88	xantoxilina	0,4	MS, NMR

Table 2: Effect of oral treatment with *Croton zehntneri* essential oil (EOCz) on exudate volume and cellular migration after intrapleural carrageenan injection in rats

Group	Exudate volume (mL)	Leukocytes ($x10^3/\mu L$)	Edema inhibition (%)
Control	0.74 ± 0.03	51.7 ± 3.7	-
Indomethacin	0.40 ± 0.03*	45.7 ± 4.2	45.9
EOCz 125 mg/kg	0.59 ± 0.05*	49.7 ± 3.0	20.0
EOCz 250 mg/kg	0.57 ± 0.05*	47.7 ± 3.0	22.9
EOCz 500 mg/kg	0.57 ± 0.05*	46.7 ± 3.6	22.9

Data were expressed as mean ± SEM (n = 8).* $P < 0.05$ compared to the Control group.

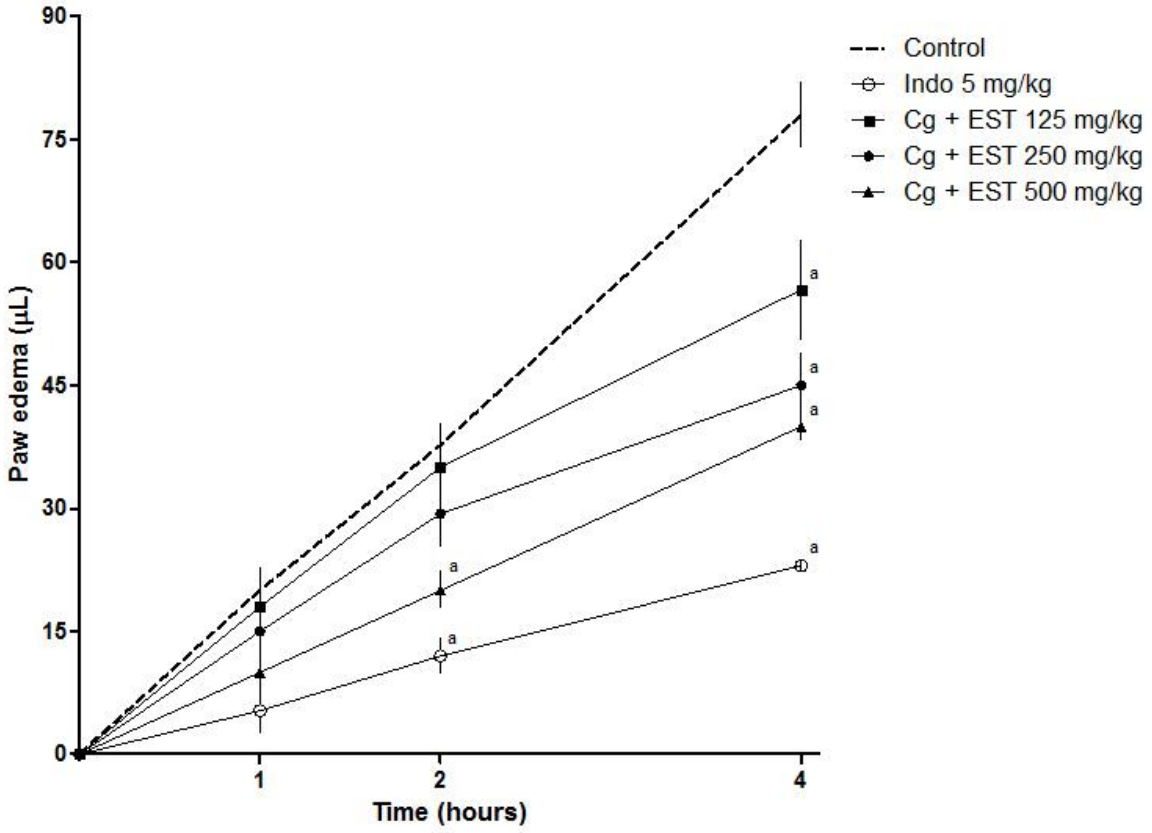

Fig. 2: Effect of oral treatment with estragole (EST) or indometacin (Indo) on carrageenan-induced paw edema in rats (n = 8). Data were expressed as mean ± SEM. [a] P < 0.05 compared to the Control group

4. CONCLUSION

In conclusion, we propose that both EOCz and EST modulate the inflammatory response after oral administration, in rodents. Then, the EOCz anti-inflammatory effect is partially related to EST, its main constituent. It constitutes a promising therapeutic profile meriting further investigations.

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