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Short communication

Comparative study of the anti-edematogenic effects of anethole and estragole

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Abstract:
Background: Anethole and estragole are monoterpene position isomers and constituents of essential oils from aromatic plants and were used in this study with the aim of analyzing their anti-inflammatory activity.
Methods: The anti-edematogenic effects of anethole and estragole were evaluated through plethysmometry in Swiss mice.
Results: Anethole inhibited carrageenan-induced edema at doses of 3, 10 and 30 mg/kg from 60 to 240 min after induction. However, the inhibitory effects of estragole were observed only from 60 to 120 min at the two highest doses. Anethole and estragole similarly inhibited edema elicited by substance P, bradykinin, histamine and TNF-α but were different in the inhibition of serotonin-elicited edema. In addition, only estragole inhibited sodium nitroprusside-induced edema.
Conclusions: Anethole and estragole showed different profiles in the anti-inflammatory response to substance P, bradykinin, histamine, serotonin and TNF-α. NO is involved only in the inhibition mechanism of estragole.

Key words:
anethole, estragole, anti-inflammatory, paw edema, mice

Introduction

Phytotherapic compounds belonging to the terpene group have been suggested to be anti-inflammatory because they can inhibit TNF-α [6, 9, 25] and IL-2 production [33]. Anethole and estragole are monoterpene position isomers (Fig. 1) and constituents of essential oils from aromatic plants that are used in a variety of foods. They have been used for flavoring, alcoholic beverage production and pharmaceutical formulations [10], and their safety certification has been issued by the United States Food and Drug Administration (FDA-US) [19]. Experimentally, anethole and estragole show no toxicity in mice that consume food containing these compounds at low doses [5, 29].

Anethole exhibits anticarcinogenic [6], antigenotoxic [1], gastroprotective and antioxidative [12], antithrombotic [31], vasoactive [30] and antimicrobial and antiviral [4] properties. Estragole exhibits myorelaxant [3], anticonvulsant and anesthetic [8], bradycardic [28], vasoactive [30] and antioxidative and antimicrobial [27] properties.

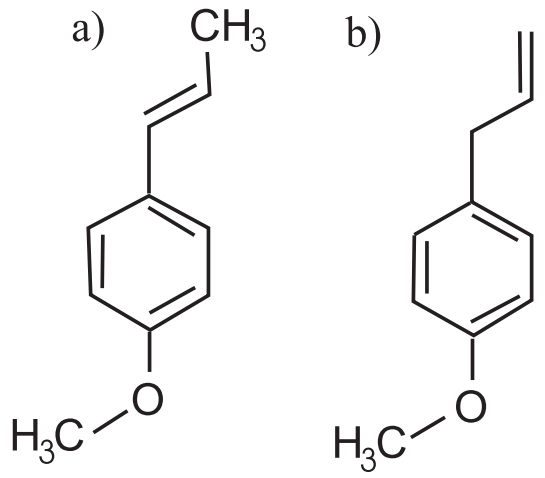

Fig. 1. Chemical structures of (**a**) anethole, (**b**) estragole

Based on the large human consumption and structural similarity of anethole and estragole, and also on the potential anti-inflammatory effects of diterpenoids *in vitro* and anethole *via* NF-κB pathway [6], we compared the anti-edematogenic effects of the monoterpenes anethole and estragole in a mouse model of paw edema. We also evaluated the toxicity of these compounds in mice.

Materials and Methods

Animals

Swiss mice (25–35 g) were maintained at 25°C, under a 12 h/12 h light/dark cycle, receiving food and water *ad libitum*. All animals were cared for in compliance with the Guide for the Care and Use of Laboratory Animals, published by the US National Institutes of Health (NIH Publication 85–23, revised 1996). The experimental protocols were approved by the Institutional Animal Care and Use Committee (UECE no. 10130208-8/40).

Chemicals

Anethole, estragole, histamine, λ carrageenan, serotonin, bradykinin, TNF-α and sodium nitroprusside were obtained from Sigma-Aldrich, USA. Substance P was from Alexis Biochemicals, Sweden. Most drugs were solubilized in sterile saline (0.9% NaCl), except for anethole and estragole that were solubilized in 0.1% Tween-80.

Acute paw edema

Edema was measured by plethysmometry (Panlab-LE-7500) before (zero time) and at 30 min and from 60 to 240 min after a 50 µl subcutaneous (*sc*) intraplantar injection of inflammatory stimuli. Negative controls received sterile saline (0.9% NaCl; *sc*) in the contralateral paw. Edema was calculated by the difference in paw volume displacement (µl) between the different time periods and the zero time or by the percent edema reduction based on the area under the curve (AUC) (arbitrary units) [14]. The anti-edematogenic activity of anethole and estragole at 3, 10 and 30 mg/kg was assessed by oral administration (*po*) (0.1 ml/10 g) at 60 min before *sc* induction of paw edema with carrageenan (1%). Control animals received 0.1% Tween 80 (*po*).

Anethole and estragole at a dose of 10 mg/kg were also injected into animals before the induction of paw edema with modulators or inflammatory mediators that participate in carrageenan-induced edema, such as bradykinin (3 nmol), histamine (100 nmol), serotonin (100 µg), substance P (20 nmol), sodium nitroprusside (10 µmol) or TNF-α (5 ng).

Toxicity assay

Mice (n = 8) were treated daily *po* with anethole and estragole in a single dosage scheme (10 mg/kg) for 14 days. Negative controls received 1% Tween-80. Animals were weighed at the beginning and at the end of the treatment. Peripheral blood was collected for biochemical analysis of urea (nephrotoxicity) and alanine amine transferase (ALT) and aspartate aminotransferase (AST) activity (hepatoxicity). Biochemical analyses were performed using enzymatic and colorimetric tests.

Statistical analysis

Results were expressed as the mean ± SEM (n = 5–8) and analyzed by ANOVA, followed by Bonferroni's test. A value of $p < 0.05$ was set to indicate significance.

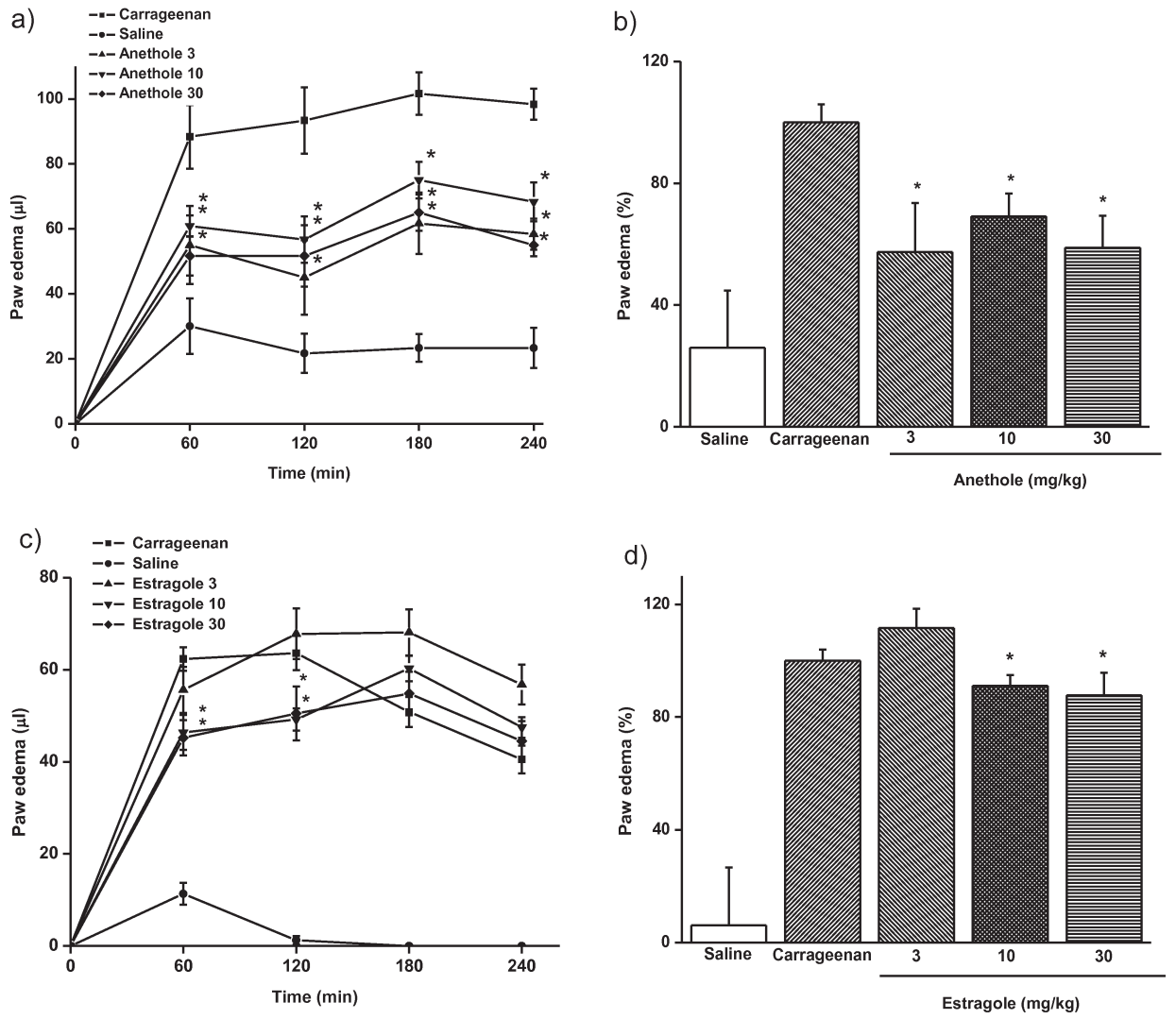

Fig. 2. Inhibitory effect of anethole and estragole on the paw edema induced by carrageenan. (**a**, **b**) anethole, (**c**, **d**) estragole (3, 10 and 30 mg/kg) or Tween 80 (0.1%) were administered *po*, 60 min before induction of paw edema by carrageenan (1% *sc*). Negative control received saline (0.9% *sc*) in the contra lateral paws. Edema was measured before (zero time) and at 60–240 min after induction and calculated by the difference in paw volume (µl) measured between the different time periods and zero time. The mean ± SEM (n = 6–8), ANOVA and Bonferroni test; * $p < 0.05$ compared to carrageenan

Results

Effects of anethole and estragole on mouse paw edema

Anethole inhibited the edematogenic effect induced by carrageenan at all doses tested, an effect that started in the first 60 min and lasted until 240 min (Fig. 2a). At 3, 10 and 30 mg/kg, the inhibitory percentages of the AUC, were 43, 31 and 42%, respectively (Fig. 2b). Estragole was also inhibitory, but only at higher doses (10 and 30 mg/kg), and showed a short-lasting effect from 60 to 120 min, compared to anethole (Fig. 2c). At 10 and 30 mg/kg, the inhibitory percentages were 8 and 12%, respectively (Fig. 2d). Since the dose of 10 mg/kg was the lowest dose showing better activity, it was chosen to be used in the mechanism of action and toxicity studies.

Anethole and estragole exhibited similar percent inhibition responses to edema induced by the following substances: substance P (anethole: 64%, estragole: 67%);

bradykinin (anethole: 41%, estragole: 42%); histamine (anethole: 70%, estragole: 72%); TNF-α (anethole: 34%, estragole: 44%) (Fig. 3a, b, c, d) and compound 48/80 (anethole: 23%, estragole: 21%). However, the percent inhibitions exhibited by these monoterpenes were differentiated from each other in serotonin-induced edema (anethole: 55%, estragole: 30%) (Fig. 3e). Moreover, only estragole (22%), but not anethole, exhibited an inhibitory effect to the sodium nitroprusside edematogenic response (Fig. 3f).

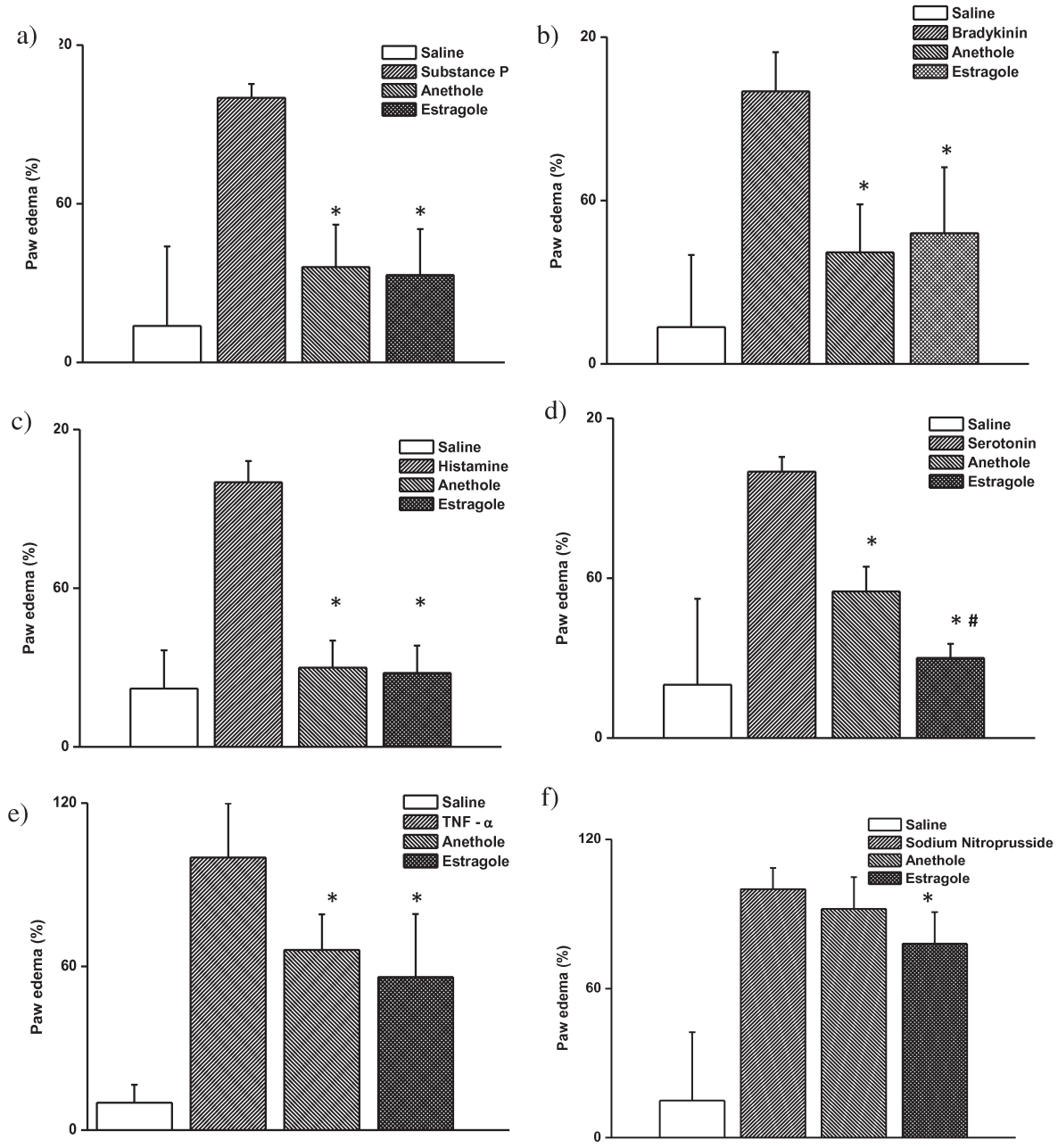

Fig. 3. Effects of anethole and estragole on the paw edema induced by inflammatory agents. Reduction (%) of paw edema induced by (**a**) substance P (20 nmol); (**b**) bradykinin (3 nmol), (**c**) histamine (100 nmol); (**d**) serotonin (100 μg) (**e**) TNF-α (5 ng) and (**f**) sodium nitroprusside (100 μM) in animals pre-treated *po* with anethole and estragole at 10 mg/kg or Tween 80 (0.1%). Negative control received saline (0.9% *sc*) in the contralateral paws. Edema was measured before (zero time) and at 30, 60–240 min after induction. The mean ± SEM (n = 6–8), ANOVA and Bonferroni test. * $p < 0.05$ compared to inflammatory agents, # $p < 0.05$ compared to anethole

Tab. 1. Sub-chronic treatment with anethole and estragole at 10 mg/kg does not alter the animal body weight and biochemical parameters of renal and liver function

Parameters	Treatment[a]		
	Tween 80	Anethole	Estragole
Initial body weight (g)	[b]30.9 ± 0.6	29.4 ± 0.7	38.1 ± 0.4
Final body weight (g)	27.4 ± 1.3	27.9 ± 0.5	39.7 ± 0.6
ALT (U/l)	174.4 ± 8.0	185.1 ± 15.6	177.0 ± 17.5
AST (U/l)	62.3 ± 4.8	54.3 ± 2.3	53.1 ± 3.6
Urea (mg/dl)	61.7 ± 2.9	60.5 ± 1.9	55.4 ± 1.8

[a] mice received a single daily dose of anethole and estragole at 10 mg/kg (*po*) and 0.1% Tween 80 for 14 days, n = 6–8 animals per group, [b] mean ± SEM

Anethole and estragole are devoid of toxicity

Anethole and estragole at 10 mg/kg did not cause significant changes in animal body weight when compared to the control group (Tab. 1). Macroscopic evaluation of the spleen, heart, liver and kidneys showed normal morphology. Biochemical analysis revealed that the urea levels and kinetics of the kidney and liver homeostasis markers, aspartate aminotransferase (AST) and alanine aminotransferase (ALT), were not significantly different from controls (Tab. 1).

Discussion

This study demonstrates that the position isomers anethole and estragole exhibit different response profiles in the acute inflammatory model of mouse paw edema. The two isomers inhibited paw edema induced by carrageenan, however, anethole showed a better potency, efficacy and duration of response than estragole. Such differences have already been described with respect to the long-lasting cardiovascular effects (bradycardia and lowering of heart rates) [28] and to the vasorelaxant responses in aortic rings [30] shown by anethole in comparison to estragole in rats.

Many studies have shown that mouse paw edema induced by carrageenan produces a characteristic biphasic response that involves a cascade of early-phase inflammatory mediators such as amine derivatives and tachykinins [11, 32], and late-phase mediators such as nitric oxide (NO), prostaglandins [23] and cytokines [13].

In this study, we compared the anti-edematogenic effects of anethole and estragole and observed the following results: a similar percentage of inhibition of edema elicited by substance P, bradykinin, histamine and TNF-α; a different percentage of inhibition of edema elicited by serotonin; and markedly different responses to edema elicited by sodium nitroprusside, where only estragole was inhibitory. Moreover, neither anethole nor estragole inhibited edema elicited by compound 48/80.

The inhibitory effects of anethole and estragole were more pronounced in edema induced by bradykinin and substance P, peptides that cause vasodilation, increased vascular permeability and nociception [15, 16, 22]. Along this line, the antinociceptive effect of the essential oil of *Croton zehntneri*, which is rich in anethole and estragole [21], supports these data and suggests the involvement of these compounds in the control of pain and inflammation. It is well known that the vascular effects of substance P are mediated in part *via* prostaglandin I_2 (PGI_2) and NO production [22]. However, the relaxant response of anethole [30] does not appear to involve PGI_2. Additionally, unpublished data from our laboratory indicate that the essential oil of *C. zehntneri* does not inhibit the PGE_2-mediated edematogenic response. Thus, prostaglandins do not appear to be involved in the anti-edematogenic action of anethole.

Our data also showed the inhibitory response of estragole, but not of anethole, on paw edema elicited by the NO donor, sodium nitroprusside. NO, a modulator of inflammatory reactions, may act either as anti- or pro-inflammatory, depending on the cell type and stimulus [2, 17], and can activate the cyclooxygenases pathway, increasing prostaglandin production

[26]. Based on this information, it can be speculated that late-phase mediators are the main targets of estragole action, directly *via* inhibition of NO, and indirectly *via* inhibition of prostanoid production, thereby preventing vasodilation and edema. Accordingly, Soares et al. [30] demonstrated that the relaxation effect of anethole on vascular smooth muscle *in vitro* does not involve NO.

Additionally, this study demonstrates that anethole and estragole reduce histamine-elicited edema by similar percentages and serotonin-elicited edema by different percentages. Histamine and serotonin (5-HT) are amine mediators released by inflammatory cells that mediate the vascular events of acute inflammation and the immune response [18, 32]. Moreover, 5-HT_1 and 5-HT_3 receptors have been associated with serotonin's autocrine effect and the modulation of mast cell degranulation elicited by substance P [24] and compound 48/80 [20]. Therefore, based on the lack of inhibitory effect of anethole and estragole on edema elicited by compound 48/80, it can be suggested that their anti-inflammatory effects involve a direct antagonism of histamine and 5-HT receptors. It appears that estragole exhibits a higher affinity for the 5-HT receptor.

Studies show that anethole is a potent inhibitor of TNF-α *via* the suppression of IκBα phosphorylation and NF-κB expression and the inhibition of other transcription factors such as AP-1, c-Jun-N-terminal and MAPK kinases in human myeloid leukemia cells [6] and fibrosarcoma cells [7]. In agreement with these findings, our data demonstrate the inhibitory activity of anethole and estragole on paw edema induced by TNF-α. The inhibitory effects of terpenoids on the TNF-α pathway have been already described [6, 25] along with the critical role of TNF-α in edema formation, mechanical allodynia and neutrophil migration elicited by carrageenan administration in mice [24].

A significant aspect of this work was the difference in profile of the anti-edematogenic responses of anethole and estragole. These variations in activity and mechanism can be explained, at least in part, by the difference in the position of the double bond in the propenyl side chains, which is coupled to the aromatic ring in anethole but not in estragole (Fig. 1). These molecular changes could possibly lead to different stabilities and biological effects. Accordingly, Shahat et al. [27] have already observed differences in the antioxidative effects of anethole and estragole.

The anti-inflammatory effects demonstrated in this work for anethole and estragole, associated with its large consumption and low toxicity, since the compounds do not cause changes in animal body weight or in markers of renal and liver function, make these compounds promising herbal medicines.

The conclusions of this study are as follows: 1. Anethole shows better potency, efficacy and duration of anti-inflammatory response in the mouse model of carrageenan-induced paw edema in comparison to estragole; 2. The mechanism of the anti-edematogenic effects of anethole and estragole involves different participation of substance P, bradykinin, histamine, serotonin and TNF-α. NO is involved only with the mechanism of estragole; 3. Anethole and estragole show no toxicity at the dose tested.

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