THE ANTI-INFLAMMATORY ACTIVITY OF EUGENIA CARYOPHYLLATA ESSENTIAL OIL: AN ANIMAL MODEL OF ANTI-INFLAMMATORY ACTIVITY

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Aim: The aim of this study is gas chromatographic analysis of Eugenia caryaphyllata (clove) essential oil and investigation of its anti-inflammatory effects.

Methods: The study involved eight groups; Serum physiologic, ethyl alcohol, indomethacin (3 mg/kg), etodolac (50 mg/kg), cardamom (0.05 mL/kg), EC-I (0.025 mL/kg), EC-II (0.050 mL/kg), EC-III (0.100 mL/kg) and EC-IV (0.200mL/kg). After measuring the volumes of right hind-paws of rats using a plethysmometer, drugs were injected intraperitoneally and lambda-carrageenan were injected subcutaneously into the plantar region. Three hours after the injections the volume measurements of the right hind-paws were repeated and the difference between the volumes were compared.

Results: The composition of the essential oil was as follows: ß-caryophyllen % 44.7, eugenol % 44.2, α-humulen % 3.5, eugenyl acetate % 1.3 and α-copaen % 1.0. It was found that indomethazin reduced the inflammation by 95.70%, etodolac by 43.42 %, EC-I by 46.55 %, EC-II by 90.15 %, EC-III by 66.94 % and EC-IV by 82.78 %. The essential oil of Eugenia caryophyllata had an anti-inflammatory effect matching to that of etodolac at 0.025 and 0.1 mL/kg and to that of indomethacin at 0.05 and 0.2 mL/kg doses.

Conclusion: As a result Eugenia caryophyllata essential oil extract was shown to have an anti-inflammatory effect.

Key words: Eugenia caryophyllata, essential oil, anti-inflammatory activity, rats.

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INTRODUCTION

Eugenia caryophyllata (clove) plant belongs to Myrtaceae families (1). Clove spice is a nail-shaped dried flower bud of Eugenia caryophyllata Thunb. species. It is an ever-green plant of ten-to-twenty centimeters in height with spear-shaped leaves and racemiferous yellowish flowers. It's grown naturally in Moluku Islands of Indonesia and cultivated in Tanzania, Madagascar, Sri Lanka, India, Indonesia, Malaysia, Brazil, Jamaica and Guinea. The plant has a strong phenolic smell and sharp acrid taste. It contains essential oil at 15-20%, tanene 13% and fixed oil 10%. Non-essential ether extract constitutes 6-12%. Essential oil of clove is a colorless or light yellowish fluid extracted from dried flower buds by steam distillation. The main constituents are eugenol (80-90%), β-caryophyllene (9%), eugenyl acetate (7%), α-humulen, ylangen, metoxy benzaldehyde, benzyl alchohol, benzaldehyde and cavychole. Clove is used in cooking, food processing, pharmacy, parfumery and cosmetics (2).

Eugenia caryophyllata (EC) was found to be effective against egg and adult of Pediculus capitis (3). It has antiseptic as well as bacteriostatic and bactericidal activity against several bacteria including Escherichia coli and Staphylococcus aureus (4;5). Growth of Helicobacter pylori being one of the major causes of peptic ulcer disease has been shown to be inhibited by EC (6). Clove oil also showed the acaricidal activity against Dermatophagoides farinae and Dermatophagoides pteronyssinus (7). Its anesthetic and spazmolytic properties have also been reported (5). In mouse macrophage cultures, methanol extract of EC inhibits cyclooxigenase by 80% (8) without affecting

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Table 1. Analysis of Eugenia caryophyllata essential oil

Component	%
ß-caryophyllen	44.7
Eugenol	44.2
α-humulen	3.5
Eugenyl acetate	1.3
α-copaen	1.0

COX-1 enzyme (7). Some components of virus-cell fusion inhibitors were isolated from its extracts (9). Pourgholami and colleagues (10) found the essential oil of EC had anticolvulsive effect in tonic seizures but not in clonic seizures in mice. The terpens, beta-caryophyllene, beta-caryophyllene oxide, alpha-humulene, alpha-humulene epoxide I and eugenol from EC induces glutathione S-transferase enzyme which plays an important role in detoxification in liver and intestines. Induction of glutathione S-transferase has been suggested to inhibit chemical carcinogens, hence these terpens are promising anticarcinogens (11). Özbek et al reported that median lethal dose of EC and Eugenia caryophyllata etheric oil were 0.613 mL/kg and 0.863 mL/kg in mice respectively (12). In traditional public medicine, EC has been used as antipyretic, aphrodisiac, appetizer (13,14), expectorant, antiemetic, anksiolytic, myorelaxant, analgesic, decongestant, anti-inflammatory and hypnotic (15,16).

In this study, we investigated whether EC had an anti-inflammatory effect on the anti-inflammatory animal model as supposed by traditional medicine.

MATERIALS AND METHODS

Animals

Male, outbred, Sprague-Dawley rats were maintained in the Animal House of Yüzüncü Yıl University, Faculty of Medicine. The rats were bred in our institutional animal house but the lineage originally obtained from Ankara Health Protection Institute (a governmental organisation). The animals were reared in standard cages with food and water ad libitum, at room temperature (22±2 ^{0}C) with artificial light from 7.00 am to 7.00 pm, and provided with pelleted food (Van Animal Feed Factory, Van-TURKEY). Ambient temp was 22±2 ^{0}C and RH was % 55-60. The rats housed in groups. The approval of Animal Ethics Committee was obtained.

Chemicals

Lambda-carrageenan Type IV and indomethacin were obtained from Sigma (Steinheim, Germany), and Etodolac from FAKO Pharmacy (İstanbul, Turkey). EC used was purchased at local market from Van in Turkey. It was identified by Dr. Fevzi Özgökçe, a plant taxonomist from Department of Biology in Faculty of Science and Art, Yüzüncü Yıl University. A voucher specimen (B-12) has been kept in our laboratory for future reference.

Isolation of tested material

EC was powdered in a mixer, placed in a distillation flask (50 g) and the oil was collected by steam distillation (100 ^{0}C, 2 hours). The yield of essential oil was 1.06% (v/w). Lambda-carrageenan was prepared in isotonic saline solution (0.9 % NaCl) and indomethacine was prepared in ethyl alcohol just before use.

Analysis of essential oil

Analysis of essential oil was carried out by Anadolu University Research Center for Medical and Aromatic Plants and Drugs, Eskisehir, Turkey. Gas chromatography analysis was carried out on a Shimadzu GC-9A gas chromatograph with FID detector and a Thermon-600 T capillary column (50 mL, 0.25 mm I.D.). The operating condition was as follows. Carrier gas was nitrogen with a split rate of 60:1, the oven temperature for first 10 min was kept at 70°C-10' and then increased at a rate of 2°C:min until 180°C-30'. Injector and detector temperature was set at 250°C.

Anti-inflammatory activity

The method of Winter et al (17) with slight modification was used. Eighty rats of either sex were divided into eight groups of ten animals each. The rats were fasted for 12 h and deprived of water only during the experiment. Deprivation of water was to ensure uniform hydration and minimize variability in edematous response. Inflammation of the hind paw was induced by injecting 0.05 mL fresh lambda carrageenan (phlogistic agent) into the subplantar surface of the right hind paw. The control group I was given normal saline and the control group II was given ethyl alcohol. The third (reference group-I) and fourth (reference group-II) group received indomethacin (3 mg/kg, i.p.) (18) and etodolac (50 mg/kg, i.p.) (19), respectively. The remaining four groups received the extract at doses of 0.025 mL/kg,

Table 2. Symptoms and findings

Groups	Dose	Paw edema (% mL)	Inhibition (%)
Control-I (ISS)	0.1 mL	1.043±0.084	-
Control-II (ethyl alcohol)	0.1 mL	0.988±0.075	-
Indomethacin	3mg/kg	0.042±0.015[b]	95.70
Etodolac	50mg/kg	0.572±0.033[bd]	43.42
E. caryophyllata-I	0.025mL/kg	0.555±0.056[ad]	46.55
E. caryophyllata-II	0.050mL/kg	0.102±0.037[afh]	90.15
E. caryophyllata-III	0.100 mL/kg	0.476±0.090[ac]	66.94
E. caryophyllata-IV	0.200 mL/kg	0.179±0.047[aeg]	82.78
F value		50.151	
p value		0.000	

Data presented as mean ± standard error mean (n=10).
a: p<0.001 compared to control-I (SF) group, b: p<0.001 compared to control-II (ethyl alchohol) group,
c: p<0.05 compared to indomethacin group, d: p<0.001 compared to indomethacin group,
e: p<0.01 compared to etodolac group, f: p<0.001 compared to etodolac group,
g: p<0.01 compared to E. Caryophyllata-I group, h: p<0.001 compared to E. caryophyllata-I group.

0.050 mL/kg, 0.100 mL/kg, and 0.200 mL/kg, i.p. These doses of the extract utilized in the current study has been chosen accordingly LD_1 value (LD_1: 0.20014 mL/kg) (12).

The measurement of foot volume was accomplished immediately by displacement technique using the plethysmometer (Ugo Basile 7140 plethysmometer, Italy) before the drug injection and three hours after the carrageenan injection.

The inhibition percentage of the inflammatory reaction was determined for each animal by comparison with controls and calculated by the formula (20): I%: [(1-(dt/dc)]x100dt is the difference in paw volume in the drug-treated group and dc the difference in paw volume in the control group.

Statistical analysis

Results of the paw edema of the rats were reported as mean±standard error of mean (SEM). The total variation was analyzed by performing one-way analysis of variance (ANOVA). Tukey's HSD test (Tukey's honestly significant difference test) was used for determining significance. Probability levels of less than 0.05 were considered significant.

RESULTS

Analysis of essential oil

The results of gas chromatographic analysis was shown in Table 1.

Anti-inflammatory activity

Table 2 shows antiedema effect of intraperitoneally administered EC on carrageenan paw oedema in rats. Essential oil of EC extract showed significant anti-inflammatory effect at doses of 0.05 mL/kg (90.15% inhibition) and 0.200 mL/kg (82.78% inhibition). However, the activity at doses of 0.05, 0.100 and 0.200 mL/kg were similar (p>0.05). Therefore the order of anti-inflammatory activity was not 0.05>0.200>0.100 mL/kg. It can be say the order of anti-inflammatory activity were 0.05=0.100=0.200 mL/kg. Compared to the controls, strong anti-inflammatory activity was observed in indomethacin group with a 95.7 % regression of the inflammation.

Etodolac, the second reference agent showed significant but weaker anti-inflammatory activity with 43.42 % regression of edema. Essential oil extract of EC has significantly lower anti-inflammatory effect compared to indomethacin at 0.025 mL/kg and 0.100 mL/kg doses and comparable effect at 0.050 mL/kg and 0.200 mL/kg doses.

When compared to etodolac the extract had statistically similar effect at 0.025 mL/kg and 0.100 mL/kg and higher activity at 0.050 mL/kg and 0.200 mL/kg.

Essential oil extract of EC had significantly lower anti-inflammatory activity at 0.025 mL/kg compared to the other doses of EC.

DISCUSSION

EC has been used in traditional public medicine to relieve nasal obstruction and musculoskeletal pain which imply anti-inflammatory activity for the plant (16). Analgesic, anesthetic, spasmolytic and antibacterial effects of EC were demonstrated by several scientific studies (3-6). Molecular evidence for its anti-inflammatory activity comes from the studies indicating COX-2 inhibition without affecting COX-1 in mice macrophage cell cultures (8,21). On the other hand in vitro studies are validated

only with consistent in vivo studies as many compensatory mechanisms may interfere with the drug effects resulting in weaker physiological response.

The current study clearly demonstrated anti-inflammatory effect of EC essential oil in vivo, which equals to that of etodolac at 0.025 mL/kg and 0.100 mL/kg doses and to that of indomethacin at 0.050 mL/kg and 0.200 mL/kg doses.

Anti-inflammatory effect of EC was thought to be due to COX-2 inhibition (7,8). However, the effective molecule of the extract has to be identified by testing each of the constituents after being purified. Eugenol which makes up 44.2 % of the extract is sold in pharmacies as an analgesic. Analgesic effect of the eugenol may imply anti-inflammatory effect as well. In an experimental model of arthritis in rats induced by deactivated Mycobacterium tuberculosis bacilli, eugenol suppressed the inflammation significantly when administered at a dose of 33 mg/kg, orally for 26 days (22). It was also shown to inhibit COX-2 enzyme in mouse macrophage RAW264.7 cell lines induced by lipopolisaccaride (21). These studies favor eugenol as the effective molecule of EC extract providing an anti-inflammatory activity comparable to indomethacin. However, all other constituents listed in Table 1 may contribute to that activity. These are mixed together in the essential oil extract so they may have some enhancing or interfering effects on one another. Therefore, in the next step of investigation of therapeutic potential of EC, each constituent molecule should be tested for its anti-inflammatory activity and undergone all necessary stages of modern drug development process.

The current study proves the anti-inflammatory activity of EC in vivo besides its antibacterial, analgesic, spasmolytic and anesthetic actions. EC appears to be a promising agent for drug research.

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