

# INVESTIGATION OF ANTI-INFLAMMATORY ACTIVITY OF BERGAMOT OIL

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**Aim:** Essential oil of Bergamot (BO) was investigated for anti-inflammatory activity using carrageenan-induced rat paw oedema test.

**Methods:** For the anti-inflammatory activity measurement six different groups were established and BO was administered in three different doses: 0.025, 0.05 and 0.10 mL/kg. Indomethacin was used as a reference agent.

**Results:** It was found that reduction in the inflammation was 95.70% with indomethacin, 27.56% with 0.025 mL/kg BO, 30.77% with 0.05 mL/kg BO and 63.39% with 0.10 mL/kg BO. Indomethacin showed the strongest anti-inflammatory activity among the drugs used. The strongest anti-inflammatory activity of BO was seen with 0.10 mL/kg dosage. Median effective dose (ED50) value of BO was found to be 0.079 mL/kg.

**Conclusion:** The results showed that BO possesses promising anti-inflammatory effect.

**Key words:** Citrus aurantium subsp. bergamia, Bergamot oil, anti-inflammatory activity, carrageenan-oedema.

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## INTRODUCTION

Citrus aurantium subsp. bergamia is a member of the Rutaceae. In Turkish folk medicine, Citrus species have several uses such as for their appetizing, diaphoretic, antiseptic, analgesic and anti-inflammatory effects (1). The Bergamot oil (BO) is a roughly pear-shaped citrus fruit. The fruit is produced in Italy, East Africa, Ivory Coast, Argentina, Brazil, Turkey and some other Mediterranean countries. The fruit is sour, and its aromatic peel is used to produce an essential oil that is used in Earl Grey tea, in perfumery, in candy-making (2). It is known empirically that inhalation of essential oils causes physiological and psychological changes in humans (3). Graham et al reported that aromatherapy during radiotherapy with bergamot oil and some other kinds of plant oil was not beneficial (4). The BO oil has been linked to several negative side-effects such as photosensitivity (5-7). The essential oil of BO is extracted from the peel of the fruit by a cold-pressing procedure or steam distillation. It consists of a volatile fraction, whose main components are, in approximate percentages, limonene (40%), linalool (8%), linalyl acetate

(28%), bergapten, citropten, bergamottin, gamma-terpinen, alpha-pinene, beta-pinene, and a non-volatile fraction (4-7%) formed essentially by coumarins and psoralens (2,8). It has been reported that bergamot oil exhibited antifungal activity against some dermatophytes and antibacterial activity against *Campylobacter jejuni*, *Escherichia coli* O157, *Listeria monocytogenes*, *Bacillus cereus* and *Staphylococcus aureus* (9,10).

Some of the components of BO, limonene, linalool, linalyl acetate and alpha-pinene, were shown to have anti-inflammatory effects (11-13). Therefore it is likely that BO has anti-inflammatory activity.

In this study, essential oil extract of Bergamot oil was investigated for its anti-inflammatory activity using carrageenan-induced rat paw oedema test (14).

## MATERIALS AND METHODS

### *Preparation of BO essential oil extract*

Citrus aurantium L. var bergamia was collected in February, 2006 near Antalya (Turkey) and voucher specimens are kept in Yuzuncu Yil University, Faculty of Medicine (Specimen Nr: B-14).

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**Table 1. Effects of intraperitoneal treatment with the essential oil extract of Bergamot orange on carrageenan-induced hind paw oedema in rats (n:6)**

Groups	Paw oedema at 3 h (mL ± SEM)*	Percentage inhibition (%)
Control-I (ISS**)	1.043±0.127	-
Control-II (etil alcohol)	0.988±0.113	-
Indomethacin (3 mg/kg)	0.042±0.023 <sup>ab</sup>	95.70
Bergamot orange (0.025 mL/kg)	0.753±0.072 <sup>abc</sup>	27.56
Bergamot orange (0.050 mL/kg)	0.720±0.073 <sup>abc</sup>	30.77
Bergamot orange (0.10 mL/kg)	0.381±0.087 <sup>abcde</sup>	63.39

\*SEM: Standard error of mean.

\*\*ISS: Isotonic saline solution (0.9% NaCl).

a:  $p < 0.05$  with respect to control-I group.

b:  $p < 0.05$  with respect to control-II group.

c:  $p < 0.05$  with respect to indomethacin group.

d:  $p < 0.05$  with respect to Bergamot orange (0.025 mL/kg) group.

e:  $p < 0.05$  with respect to Bergamot orange (0.05 mL/kg) group

The plant samples were kept at room temperature until they were processed. The fruits were peeled and the fresh pericarp of the fruit was sliced into small pieces and boiled in Clevenger apparatus (Ildam, TURKEY) to obtain essential oil of the *Citrus aurantium* var. bergamia (BO). BO was kept in test tubes and the yield was determined as 0.7%.

#### Drugs and chemicals

The following drugs were used; Lambda-Carrageenan and indomethacin (Sigma, Steinheim, Germany). Indomethacin was dissolved in ethyl alcohol (96 %).

#### Animals

Male and female Sprague-Dawley rats (180-220 g) were used in these experiments. The animals were housed in standard cages (48 cm x 35 cm x 22 cm) with food and water ad libitum, at room temperature (20 ± 2 °C) with artificial light from 7.00 am to 7.00 pm. The animals kept under controlled environment following the standard operating procedures of the animal house facility of the Faculty of Medicine, and provided with pelleted food Animal Feed Factory, Van-Turkey). The protocol for the study was approved of Yüzüncü Yıl University, Faculty of Medicine Animal Breeding and Research. Prior to administration of the drugs, the rats were fasted for 12 h with free access to tap water.

#### Carrageenan-induced rat paw oedema

Thirty-six rats of either sex were divided into six groups of six animals each. Group I, which served as control-I, received 0.2 mL

isotonic saline solution (ISS), Group II, which served as control-II, received 0.2 mL ethanol. Group III received 3 mg/kg, indomethacin (14), group IV received 0.025 mL/kg BO, Group V received 0.05 mL/kg BO. Group VI received 0.100 mL/kg BO. All agents were injected intraperitoneally.

The inhibitory activity of BO on carrageenan-induced rat-paw oedema was determined according to the method of Winter et al, with slight modification (15). The drugs were administered 1 h before the injection of 0.05 mL of carrageenan (1%) into the subcutaneous tissue of right hind paw. Since the hydration state of the animals can modify the intensity of swelling, the rats were fasted 12 h before the experiment with water ad libitum. The degree of oedema was measured 30 minutes before and 3 h after the injection of carrageenan. The difference between the volume of the paw before and after the injection of the oedema provoking agent indicated the severity of oedema. Volumes of right hind paw of the animals were measured with a plethysmometer (model 7140, Ugo Basile, Italy).

The percentage inhibition of the inflammatory reaction was determined for each animal by comparing with controls and calculated by the formula (16):

$$I \% = [(1 - (dt/dc))] \times 100$$

where dt is the difference in paw volume in the drug-treated group and dc the difference in paw volume in the control group.

#### Statistical analysis

All data were represented as mean ± standard error of mean (SEM) or as

percentages. The analysis of variance (ANOVA) was used for the statistical analysis of data. LSD test (Least significant difference test) was used for determining significance. Results with  $p < 0.05$  were considered as statistically significant.

## RESULTS

Table 1 shows the results on antioedematous effect of intraperitoneally administered Bergamot oil on carrageenan paw oedema in rats. At doses of 0.025, 0.05 and 0.100 mL/kg BO caused a significant reduction in paw oedema (27.56%, 30.77% and 63.93%, respectively). In this model, indomethacin, a commonly used anti-inflammatory drug, produced a significant inhibition by 95.70%. As seen in Table 1, all doses of BO showed anti-inflammatory activity higher than that of the control groups, but it was not as strong as indomethacin. Anti-inflammatory activity of 0.100 mL/kg BO was stronger than those of 0.025 and 0.05 mL/kg BO.

## DISCUSSION

Bergamot oil (*Citrus aurantium* subsp. *bergamia*) essential oil has been used for its diaphoretic, appetizing, antiseptic, analgesic and anti-inflammatory effects (1).

Anti-inflammatory effect of Bergamot oil shown in this study confirms its use as an anti-inflammatory agent in traditional medicine. Median effective dose (ED<sub>50</sub>) value of BO was found to be 0.079 mL/kg. It was reported that main components of the volatile fraction of BO essential oil are limonene, linalool, linalyl acetate, bergapten, citropten, bergamottin, gamma-terpinen, alpha-pinene and beta-pinene (2,8). Anti-inflammatory effect of BO essential oil could be due to one or several of these components. Linalool, linalyl acetate, limonene and alpha-pinene were shown to have strong anti-inflammatory effects (11-13), which supports the results obtained in the current study. More studies are needed to show the mechanisms of the anti-inflammatory effects of these agents. Bergapten, citropten, bergamottin, gamma-terpinen and beta-pinene, which are also found in BO essential oil, should be further studied for their anti-inflammatory effects.

The results of the present study showed that BO had anti-inflammatory effect and provided support to the traditional usage of BO in inflammation.

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