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Original Article

Antinociceptive Activity of *Ballota glandulosissima* Hub. -Mor & Patzak

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Abstract. We aimed to investigate antinociceptive activity and median lethal dose (LD_{50}) of water extract of *Ballota glandulosissima* Hub.-Mor&Patzak in mice. In this study, water extract of *Ballota glandulosissima* was investigated for antinociceptive activity using acetic acid-induced "writhing" and "tail-flick" tests. Acetyl salicylic acid and morphine were used as the reference drug. Mice were injected *Ballota glandulosissima* extract intraperitoneally in doses of 100 mg/kg and 200 mg/kg, respectively. The extract of *Ballota glandulosissima* caused dose related inhibition in the acetic acid-induced ab dominal stretching response in mice. The extract of *Ballota glandulosissima* also showed significant changes in the nociceptive threshold of the tail-flick test. The motor coordination of mice treated with the water extract was evaluated by using the "rotarod" test and was found to be not impaired in comparison with that of control mice.The results obtained in the present study indicate that the water extract of *Ballota glandulosissima* has promising antinociceptive activity. The LD_{50} of *Ballota glandulosissima* was determined as 8.885 g/kg.

Keywords: *Ballota glandulosissima*, lamiaceae, antinociception, writhing test, tail-flick test, rotarod test.

1. Introduction

Ballota glandulosissima Hub.-Mor & Patzak (BG), is a member of *Lamiaceae* and is found in South Anatolia (1). *Ballota* L. species have been used in Turkish folk medicine as antiulcer, antispasmodic, diuretic, choleretic, antihaemorrhoidal and sedative agent (2-5). *Ballota nigra* L. is used externally, in the treatment of wounds and burns. It is used internally to supress coughs and upper respiratory inflammation (6-8). Vural *et al*. reported that *Ballota nigra* L. subsp. *anatolica* P.H. Davis and *Ballota larendana* Boiss & Heldr. have antidepressant activity. *B. larendana* has also anxiolytic activity (4). Antimicrobial activities of some flavonoids isolated from BG have been reported (9, 10).

The main components of the *Ballota* species are flavonoids, labdane diterpenoids and phenyl propanoids (11). In our previous studies, three Diterpenoids hispanolone, ballonigrine, dehydrohispanolone) and ten flavonoids (kumatakenin, pakipodol, 5-hydroxy 7, 3',4'trimethoxyflavone, velutin, corymbosine, 5-hydroxy 3,7,4'trimethoxy flavone, retusin, 5-hydroxy 7, 4'dimethoxy flavone, flindulatine, ladanein) were isolated, chemically characterized and analysed by HPLC in dif ferent species of *Ballota* (3, 10-12).

This paper is a part of our on-going studies on thisgenus (3, 9-12). BG is known as *karınca somureağı* in Silifke. We aimed to investigate the antinociceptive activity of the water extract of BG. To our knowledge no data is available with respect to antinociceptive activity of this plant.

2.Material and method

2.1.Plant material

Ballota glandulosissima was collected in 1998 from flowering plants near Antalya (Turkey). Voucher specimens were kept in Herbarium of Ankara University, Faculty of Pharmacy (AEF No. 19900).

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Table 1

Effect of aqueous extract of *B. glandulosissima* on rotarod test in mice (n=10).

Treatment	Time(min.)	The time of animals remained without falling (mean ± SEM*)
Control (ISS)	0	120 ± 0
	15	120 ± 0
	30	120 ± 0
	60	-
B.glandulosissima(100mg/kg)	0	120 ± 0
	15	120 ± 0
	30	113 ± 4.4
	60	-
B.*glanduloisssima*(200 mg/kg)	0	120 ± 0
	15	120 ± 0
	30	115 ± 3.16
	60	-

*SEM : Standard Error of the Mean.

2.2.Preparation of extract

Air-dried and powdered aerial parts of the plant were extracted with water. The aqueous extract was prepared by maserating 200 g of powder in cold distilled water (300 mL) for 1 day and then evaporated and lyophilized (13).

2.3.Animals

The protocol for the study was approved by the Ethical Committee of Yüzüncü Yil University Faculty of Medicine. Male Swiss albino mice weighing 22±2.5 g were used for the study. All animals were housed in standart cages (48x35x22 cm) at room temperature (20±2 ^{0}C), with artificial light from 7.00 a.m. to 7.00 p.m. and provided with pelleted food (Van Animal Feed Factory) and water *ad libitum*.

2.4.Acute toxicity

Male white albino mice were randomly divided into 7 groups (T1, T2, T3, T4, T5, T6 and T7) with 8 animals in each group. One group was (T1) treated with normal saline and considered as control and the other six groupswere treated with the aqueous extract given i.p. in increasing dosages of the extract (50, 100, 200, 400, 800, 1600 mg/kg body weight). The maximum volume was 0.2 mL. The animals were returned to their home cages and given free access to food and water. The mortality in each cage was assessed 24 h, 48 h and 72 h after administration of the extract. The percentage mortalities were converted to probits and plotted against the $\log_{10}$ of the dose of extract. Regression lines were fitted by the method of least squares and confidence limits for the LD_{50} values were calculated by the method of Litchfield & Wilcoxon and Abdel-Barry et al (14, 15).

Table 2

Effect of the aqueous extract of *B. glandulosissima* on abdominal writhing test (n=10).

Treatment	Abdominal stretching (mean±SEM)	% Inhibition of stretching
Control (ISS)	41.16 ± 2.52	-
Acetylsalisylic acid (300 mg/kg)	25.16 ± 3.41 [b]	38.87
B.glandulosissima (100 mg/kg)	30.00 ± 1.57 [a]	27.11
B. glandulossima (200 mg/kg)	26.40 ± 1.56 [b]	35.86

a $p < 0.05$ significantly different from control value (ANOVA followed by Tukey's test).
b $p < 0.01$ significantly different from control value (ANOVA followed by Tukey's test).

2.5.Antinociceptive activity

a) *Acetic acid-induced writhing test:* The method of Koster *et al.* was used with slight modification (16). The animals were kept in a temperature controlled environment (22±2$_0$C) with a 12 h lightdark cycle. Food and water were freely available. Abdominal writhing was introduced by intraperitoneal injection of acetic acid (6%,60 mg/kg). Animals were pretreated with the aqueous extract through intraperitoneal administration, 30 min prior to acetic acid injection and 5 min thereafter the test has been started. The plant extract was tested at 100 and

Table 3

Effect of aqueous extract of *B. glandulosissima* on tail flick latency in mice.

Treatment	Measurements (Mean ± S.E.M.)		
Groups	15th min	30th min	60th min
Morphine (10 mg/kg)	11.45±0.91	15.74±1.40	15.26±1.50
Control (ISS)	4.82±0.33^{a}	4.80±0.36^{a}	4.50±00.28^{a}
B. glandulossima (100 mg/kg)	7.44±0.72^{ab}	7.55±0.45 ^{ab}	6.47±00.21^{ab}
B. glandulossima (200 mg/kg)	5.80±0.19^{a}	5.16±0.24^{a}	5.50±00.20^{a}

ANOVA followed by Tukey's test:
a: $p<0.05$ significant different from corresponding Morphine value.
b: $p<0.05$ significant different from corresponding control (ISS) value

200 mg/kg intraperitoneal (i.p.). These doses of BG utilized in the current study has been chosen accordingly LD_{10} value (LD_{10} = 0.276 mg/kg). Control animals received the same volume of isotonic saline solution (ISS) (5 ml/kg). Acetylsalicylic acid at a dose of 300 mg/kg, which is a preferential dose in such studies, given orally was used as a standard for comparison (17). After challenge, pairs of mice were placed in a glass cage measuring 44x44x25 cm. The number of stretching occurring for 15 min immediately after the acetic acid injection was recorded. Ten mice were used per group. Animals were killed immediately after each 15 min experiment. The results were evaluated by calculating the mean number of stretching per group and they were represented as % inhibition of stretching movements with the control group (18).% analgesic activity = (n-n')/n) x 100 n: average number of "stretching" of control group n': average number of "stretching" of test group

b) Tail-flick test: Nociceptive response was assessed with a tail-flick apparatus (LSI Letica LE 7106, Spain) using a method initially described by D'Amour and Smith (19). The animals were gently immobilized by using a glove, and the radiant heat was focused on a blackened spot 1-2 cm from the tip of the tail. Beam intensity was adjusted to give a tail flick latency of 2-3 sec in control animals. Measuring was terminated if the latency exceeded the end of time (20 sec) to avoid tissue damage. In all the experiments mice were tested three times, 60 and 30 min before drug administration in the baseline latency determined and 30 min after drug administration. The BG extract was tested at 100 and 200 mg/kg i.p. Morphine at a dose of 10 mg/kg given subcutaneously (s.c.) was used as a standard for comparison (20).

2.6.Rotarod test

Motor coordination of the mice were evaluated by using a rotarod apparatus (Dei_im, Turkey) consisting of a bar with a diameter of 5.6 cm, subdivided into five compartments by a disc 19 cm in diameter. The bar rotated at a constant speed of 8 rpm. The motor coordination was assessed on the basis of the endurance time of the animals on the rotating rod. Every day before the test, the animals were trained twice. On the day of the test only the mice able to stay balanced on the rotating rod between 60 and 120 sec (cut-off time) were selected. The performance time was measured before and at 15, 30, 60 and 120 min after treatment (21). The test was conducted between 9 and 13 a.m. ISS was given orally and BG was given intraperitoneally.

2.7.Statistical analysis

All data expressed as mean ± standard error of the mean (SEM) of were analyzed by the analysis of variance (One-way ANOVA), post-hoc Tukey's procedure for multiple comparisons with a single control group. *P* values of less than 0.5 were considered to be significant (22).

3. Results

The aqueous extract of *Ballota glandulosissima*, when administered i.p. in the dose range of 50-1600 mg/kg to mice, did not produce any significant change in the autonomic

or behavioral responses during the observation period. No mortality was observed up to the 3 days of monitoring. Median lethal dose (LD_{50}) of the water extract of BG was 8.885 g/kg.

The motor coordination of mice treated with aqueous extract (100 mg/kg and 200 mg/kg per mouse i.p.) was evaluated by using the rotarod test performed starting from 15 min and 30 min after the i.p. injection of the extract. The motor coordination of mice treated with the extract was not impaired in comparison with that of control mice, since each group progressively reduced its number of falls. The overall results of the rotarod test at the different time points (15_{th} and 30_{th} min) were not found statistically significant when compared to control group (Table 1).

The water extract of BG caused dose related inhibition of the acetic acidinduced abdominal stretching response in mice (Table 2). When the abdominal stretching values of the extract of BG at both doses compared to that of acetylsalicylic acid, no significant differences were observed. Thus, the extract of BG is likely to have similar potency as acetylsalicylic acid.

The results of the nociceptive threshold of tail flick test of water extract of BG are shown in Table 3. At all time points examined the extract of BG, at both doses produced significant analgesic effects compared to that of control group. The analgesic effect of the extract was lower than Morphine group.

4. Discussion

This plant is used as an infusion by public (2). Accordingly, as a preliminary study we decided to prepare water extract of the plant. At this stage, however, we do not know the reason/s behind these findings. Nevertheless, it could be due to flavonoid and phenylpropanoid content of the extract (8,10-12, 23-26). These chemicals have been reported to have analgesic properties (27, 28). However, further studies are needed to clarify this issue. In conclusion, the present study revealed that the water extract of *Ballota glandulosissima* possesses promising antinociceptive activity centrally or peripherally and median lethal dose(LD_{50}) of the water extract of BG is 8.885 g/kg.

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