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Antinociceptive and Anti-inflammatory Activities of *Viburnum lantana*

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Abstract

Water extract of *Viburnum lantana* L. (Caprifoliaceae) (VL) leaf was investigated for antinociceptive and anti-inflammatory activities in mice and rats. The tail-flick test, acetic acid–induced writhing test, and the carrageenan-induced rat paw edema test were used to determine these effects. Our findings show that VL causes dose-related inhibition in acetic acid–induced abdominal stretching in mice. VL inhibited abdominal stretching at 100 mg/kg, which is similar to that of aspirin, but this extract exhibited stronger antinociceptive activity than aspirin at a 200 mg/kg dose. VL showed powerful antinociceptive activity, which was quantified by a tail-flick test in 100 mg/kg dose. The anti-inflammatory activity of VL was not found to be significantly different at doses of 100 and 200 mg/kg. As a result, VL had shown slight anti-inflammatory activity compared with indomethacin. The LD_{50} of VL was determined as 2.169 g/kg.

Keywords: Anti-inflammatory activity, antinociceptive activity, median lethal dose (LD_{50}), *Viburnum lantana*.

Introduction

The genus *Viburnum* (Caprifoliaceae) is composed of more than 230 species distributed from South America to Southeast Asia, the majority of them being endemic (Lobstein et al., 1999). The plant is represented by four species in the flora of Turkey: *Viburnum opulus* L., *V. orientale* Pallas, *V. lantana* L., and *V. tinus* L. (Davis, 1972; Davis et al., 1988).

In Middle Anatolia, a traditional drink named "gilaboru" has been prepared from the fruits of *V. opulus*. The edible fruit has a dark-red color. The bark of *V. lantana* has been used in folk medicine as a rubticient and antinociceptive (Baytop, 1999). The preventive effect of *V. dilatatum* Thunb. on oxidative damage was found in rats subjected to stress (Iwai et al., 2001) and streptozotocin-induced diabetic rats (Iwai et al., 2004). In addition, the effects of *V. dilatatum* on antioxidant enzymes in plasma, liver, and stomach were examined and the results suggest that ingestion of this plant might contribute to reduce the consumption of antioxidant enzymes, such as superoxide dismutase, catalase, glutathione peroxidase, and glutathione (Kim et al., 2005). The alcohol extracts of *V. erubescens* Wall. have been reported to show antiviral activity (Dhar et al., 1968). Some iridoid aldehydes isolated from *V. Iuzonicum* Rolfe exhibited moderate inhibitory activity against HeLa S3 cancer cells (Fukuyama et al., 2005b).

The genus *Viburnum* is known to contain triterpenoids (Machida & Kikuchi, 1997; Kagawa et al., 1998; Fukuyama et al., 2002), diterpenoids (Kubo et al., 2001; Fukuyama et al., 2005a), sesquiterpenes (Fukuyama et al., 1996), iridoids (Iwagawa & Hase, 1986; Iwagawa et al., 1990; Çaliş et al., 1995; Tomassini et al., 1997), and polyphenols (Machida et al., 1991; Parveen et al., 1998; Lobstein et al., 2003). The biological activities of this plant could be related to these compounds.

The objective of this study is to determine the antinociceptive and anti-inflammatory effects of *V. lantana*. These activities have not been investigated before on this species.

Materials and Methods

Plant material

V. lantana L. was collected in 2005 from flowering plants near Ankara (Turkey). Taxonomic identity of the plant

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was confirmed by Prof. Dr. H. Duman, a plant taxonomist in the Department of Biological Sciences, Faculty of Art and Science, Gazi University, Ankara, Turkey. Voucher specimens were kept in the herbarium of Ankara University, Faculty of Pharmacy (AEF no. 23543).

Preparation of extract

Air-dried and powdered leaves of the plant were extracted with water. The aqueous extract was prepared by macerating 100 g of plant powder in 1000 mL cold distilled water for 1 day. The macerate was evaporated and lyophilized. The extract yield was 13.45% (w/w).

Animals

Male and female Sprague-Dawley rats (200–250 g) and Swiss albino mice (20–24 g) were maintained in the Animal House of Yuzuncu Yil University, Faculty of Medicine. The animals were bred in the university's institutional animal house, but the lineage originally obtained from Ankara Health Protection Institute (a governmental organization). The animals were housed in standard cages (48 cm × 35 cm × 22 cm) at room temperature (22 ± 2°C) with artificial light from 7:00 AM to 7:00 PM, and provided with pelleted food (Van Animal Feed Factory, Van, Turkey) and water *ad libitum*. The protocol for the study was approved by the Ethical Committee of Yüzüncü Yil University Faculty of Medicine Animal Breeding and Research.

Drugs and chemicals

Lambda-carrageenan type IV and indomethacin were obtained from Sigma (Steinheim, Germany), etodolac was obtained from Fako (Istanbul, Turkey), and aspirin was obtained from Bayer (Istanbul, Turkey).

Acute toxicity test

Male and female mice were randomly assigned to nine groups with six animals in each group. The first group was the control group and was treated with isotonic saline solution (ISS; 0.9% NaCl). The other eight groups were treated with *Viburnum lantana* given intraperitoneally (i.p.) in increasing dosages of 0.20, 0.32, 0.40, 0.80, 1.60, 3.20, 4.80, and 6.40 mg/kg body weight. The mortality in each cage was assessed 72 h after administration of *V. lantana*. Regression lines were fitted by the method of least squares, and confidence limits for the LD_1, LD_{10}, LD_{50}, LD_{90}, and LD_{99} values were calculated by the method of Litchfield and Wilcoxon (1949) and Kouadio et al. (2000).

Antinociceptive activity

Acetic acid–induced writhing test

The method of Koster et al. (1959) was used with slight modification. The animals were kept in a temperature-controlled environment (22 ± 2°C) with a 12-h light-dark 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 after the test had been started. The plant extract was tested at 100 and 200 mg/kg i.p. Control animals received the same volume of ISS (0.2 mL). Acetylsalicylic acid at a dose of 300 mg/kg, which is the preferential dose in such studies, was given orally and used as a standard for comparison (Hunskaar et al., 1985). After drug application, pairs of mice were placed in a glass cage measuring 44 cm × 44 cm × 25 cm. The number of stretchings occurring for 15 min immediately after the acetic acid injection was recorded. Six mice were used per group. Animals were sacrificed immediately after each 15-min experiment. The results were evaluated by calculating the mean number of stretchings per group, and they were represented as percent inhibition of stretching movements with the control group (Tanker et al., 1996):

$$\%\ \text{antinociceptive activity} = (n - n')/n \times 100$$

where n is average number of stretchings of control group, and n' is average number of stretchings of test group.

Tail-flick test

Antinociceptive response was assessed with a tail-flick apparatus (LSI Letica LE 7106, Barcelona, Spain) using a method initially described by D'Amour and Smith (1941). 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 5–8 s in control animals. Measuring was terminated if the latency exceeded the end of time (15 s) to avoid tissue damage. In all experiments, mice were tested twice at each time point. The tests were performed 30 min before drug admistration, which served as the baseline latency, and 30, 90, and 150 min after drug administration. Aspirin (150 mg/kg, by mouth) and morphine hydrochloride (10 mg/kg, subcutaneous) were used as reference standard (Parimala et al., 2003; Matsumoto et al., 2004). Only ISS (0.2 mL, i.p.) was given to the control group. *Viburnum lantana* extract 100 mg/kg was given intraperitoneally to *Viburnum lantana* groups.

The data derived from groups for statistical analysis were standardized by using the following formula (Tanker et al., 1996).

$$\%\ \text{antinociceptive activity} = 100 \times (n - n_i)/n_i$$

where n is tail-flick results at the 30th, 90th, and 150th min, and n_i is tail-flick results before drug administration.

Anti-inflammatory activity

The method of Winter et al. (1962) with slight modification was used. Thirty-six rats of either sex were divided into six groups of six 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 to minimize variability in edematous response. Inflammation of the hind paw was induced by injecting 0.05 mL fresh λ carrageenan (phlogistic agent) into the subplantar surface of the right hind paw (Winter et al., 1962). Control group I was given normal saline and control group II was given ethyl alcohol. The third group (reference group I) received indomethacin (3 mg/kg, i.p.) (Rimbau et al., 1999) and the remaining three groups received the extract at doses of 50, 100, and 200 mg/kg, i.p. The doses of the extract utilized in the current study where chosen according to the LD_1 value ($LD_1 = 1.042$ g/kg).

The measurement of foot volume was accomplished by displacement technique using plethysmometer (Ugo Basile 7140 plethysmometer, Comerio [VA] Italy) immediately before and 3 h after the injection. The inhibition percentage of the inflammatory reaction was determined for each animal by comparison with controls and calculated by the formula (Kouadio et al., 2000):

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

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

Results are reported as mean ±SEM (standard error of mean). The total variation was analyzed by performing one-way analysis of variance (ANOVA). Tukey's HSD (honestly significant difference) and Tamhane's T2 tests were used for determining significance. Probability levels of less than 0.05 were considered significant.

Results

Acute toxicity test

Mice were used to determine the i.p. LD_{50} value of *V. lantana*. The LD_{50} value of the extract was found to be 2.169 g/kg in mice. These data enabled us to select the dose to be administrated to rats for assessing its antinociceptive and anti-inflammatory activity.

Antinociceptive activity

Acetic acid–induced writhing test

VL caused dose-related inhibition of the acetic acid–induced abdominal stretching response in mice (Table 1). VL at 100 and 200 mg/kg significantly reduced the acetic acid–induced abdominal pain by 62.25% and 89.64%, respectively. Acetylsalicylic acid inhibited abdominal stretching (44.37%) at a dose of 300 mg/kg. Degree of inhibition of aspinin and both VL groups was significant compared with control group. There was a statistically significant difference between aspirin and the VL treated groups.

Table 1. Results of *V. lantana* on the acetic acid–induced writhing test in mice.

Treatment	Dose (mg/kg)	Abdominal stretching	% inhibition of stretching
Control (ISS)	0.2 mL	17.67 ± 1.67	—
Acetylsalicylic acid	300	9.83 ± 0.60[a]	44.37
V. lantana	100	6.67 ± 3.22[a]	62.25
V. lantana	200	1.83 ± 0.75[ab]	89.64

Values of abdominal stretching are mean ±SE, n = 6, $p < 0.05$ significant. Post hoc LSD (least significant difference test) test.
[a]$p < 0.05$, comparison with control group.
[b]$p < 0.05$, comparison with acetylsalicylic acid group.

Tail-flick test

The results of the VL tail-flick test are shown in Table 2. The VL was tested at 100 mg/kg, i.p. A dosage of 100 mg/kg produced significant antinociceptive effects at all time points.

Antinociceptive effect of aspirin was started for the first 30 min, but it showed significant analgesia at 150 min. Whereas the morphine group showed significant antinociceptive effect at 30 and 90 min, this effect was not observed at the 150th minute. VL extract showed an antinociceptive effect at the dose of 100 mg/kg throughout the study

Table 2. Results of *V. lantana*, acetylsalicylic acid, morphine, and control groups on tail-flick test.

	Antinociceptive activity (%)		
Groups	30th min	90th min	150th min
ISS (control)	2.03 ± 01.45	3.26 ± 04.26	1.21 ± 04.98
Acetylsalicylic acid (300 mg/kg)	22.74 ± 07.24	23.55 ± 08.87	37.29 ± 06.91[a]
Morphine (10 mg kg^{-1})	46.31 ± 10.99[a]	69.38 ± 12.28[ab]	0.16 ± 06.61[b]
V. lantana (100 mg/kg)	30.75 ± 16.05[a]	60.97 ± 21.61[ab]	40.43 ± 18.12[ac]

ISS, isotonic saline solution.
Values are mean ±SE, n = 6, $p < 0.05$ significant. Post hoc LSD (least significant difference test) test.
[a]$p < 0.07$ compared with control (ISS) group.
[b]$p < 0.07$ compared with acetylsalicylic acid group.
[c]$p < 0.05$ compared with morphine group.

Table 3. Anti-inflammatory effect of *V. lantana.*

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	3 mg/kg	0.042 ± 0.015^{ab}	95.70
V. lantana	50 mg/kg	0.767 ± 0.108^{c}	26.44
V. lantana	100 mg/kg	0.559 ± 0.046^{abc}	46.39
V. lantana	200 mg/kg	0.539 ± 0.076^{abc}	48.37

ISS, isotonic saline solution.
Values are mean ± SE, $n = 6$, $p < 0.05$ significant. Post-hoc Tukey's HSD test (Tukey's honestly significant difference test).
[a]$p < 0.05$ compared with control I (SF) group.
[b]$p < 0.05$ compared with control II (ethyl alcohol) group.
[c]$p < 0.05$ compared with indomethacin group.

period. The antinociceptive effect of VL extract had a statistically similar effect at 90 min with the morphine group, and at 150 min with the aspirin group.

Anti-inflammatory activity

Table 3 shows the results on antiedema effect of intraperitoneally administered VL on carrageenan paw edema in rats. VL extract showed a significant anti-inflammatory effect at 100 and 200 mg/kg doses, which peaked at a dose of 200 mg/kg (48.37% inhibition) with a lesser degree of inhibition at 50 mg/kg (26.44%) and 100 mg/kg (46.39%). Compared with the controls, the greatest anti-inflammatory activity was observed in the reference group receiving indomethacin with a 95.7% regression of the inflammation. VL has significantly lower anti-inflammatory effect compared with indomethacin at all doses.

Discussion

The LD_{50} level of the leaf extract of *Viburnum lantana*-was determined to be 2.169 g/kg.

When compared with aspirin, VL extract had similarly inhibited abdominal stretching at 100 mg/kg, but this extract exhibited, stronger antinociceptive activity than aspirin at the 200 mg/kg dose. VL showed powerful antinociceptive activity, which was determined by the tail-flick test at the 100 mg/kg dose.

The antinociceptive activity of *Viburnum tinus* was also studied by Calle et al. (1999). They found that the ethyl acetate extract of *V. tinus* had significant activity at a 250 mg/kg dose using the acetic acid writhing test. The extract reduced the acetic acid–induced abdominal pain by 68.6% at that dose. The results obtained with the antinociceptive activity of *V. lantana* are in agreement with the results of Calle et al. (1999).

VL showed significant anti-inflammatory activity only at 100 and 200 mg/kg doses compared with the control group. No significant difference was found at 100 and 200 mg/kg doses of VL extract. The indomethacin group, which was selected as a reference group, showed significant powerful anti-inflammatory activity compared with VL extract at all doses. As a result, VL extract showed slight anti-inflammatory activity compared with indomethacin.

The antinociceptive and anti-inflammatory effects of the extract may be due to their content of triterpenoids, diterpenoids, sesquiterpenes, and/or polyphenols.

The pain response in the tail-flick test is considered to be mediated at the level of the spinal cord (Yaksh, 1999). Antinociceptive activity of opioid agonists, opioid partial agonists, and nonsteroidal anti-inflammatory agents can be determined using the writhing test (Vogel & Vogel, 1997). Therefore, it is possible that VL extract elicited antinociceptive activity in mice through its action on spinal opioid receptors and/or the inhibition of cyclooxygenase enzyme. The combination of antinociceptive and anti-inflammatory effects of VL extract indicated a likelihood of intervention with prostaglandin synthesis, as prostaglandins have been established as a common mediator in these two responses.

In order to elucidate the mechanism(s) by which VL extract components exhibit the antinociceptive and anti-inflammatory effects, which we demonstrated in this study, further studies with the isolated components will follow.

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