Antinociceptive and anti-inflammatory activities of Viburnum opulus

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Abstract
Water extract of Viburnum opulus L. (Caprifoliaceae) (VO) 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 VO causes dose related inhibition in acetic acid-induced abdominal stretching in mice. VO inhibited abdominal stretching at 100 and 200 mg/kg. VO showed antinociceptive activity, which was quantified by the tail-flick test at doses of 100 and 200 mg/kg. However, VO did not have an anti-inflammatory effect at these doses. The LD₅₀ of VO was determined as 5.447 g/kg.

Keywords: Anti-inflammatory activity; antinociceptive activity; median lethal dose (LD₅₀); Viburnum opulus

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 “gilaburu” 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 rubefacient and analgesic (Baytop, 1999). The preventive effect of V. dilatatum Thunb. on oxidative damage was found in rats subjected to stress (Iwai et al., 2001) and in streptozotocin 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. luzonicum 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; Çalış 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 was to determine the antinociceptive and anti-inflammatory effects of V. opulus. These activities have not been previously investigated for this species.

Materials and methods

Plant material
V. opulus L. was collected in 2005 from flowering plants near Kayseri (Turkey). Taxonomic identity of the plant...
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 have been kept in the Herbarium of Ankara University, Faculty of Pharmacy (AEF No 23696).

**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 22.4% (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 the Yüzüncü Yıl University Faculty of Medicine. The animals were bred in the University’s institutional animal house, but the lineage was originally obtained from Ankara Health Protection Institute (a government 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 a.m. to 7.00 p.m., 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 Yıl 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 opulus* given 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. opulus*. Percentage mortalities were converted to probits. Regression lines were fitted by the method of least squares, and confidence limits for the LD₁, LD₁₀, LD₂₀, LD₅₀, and LD₉₀ values were calculated by the methods 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 i.p. injection of acetic acid (6%, 60 mg/kg). Animals were pretreated with the aqueous extract through i.p. 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 a preferential dose in such studies, was given orally and used as a standard for comparison (Hunskaar et al., 1985). After challenge, 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 percentage inhibition of stretching movements compared with the control group (Tanker et al., 1996):

\[
\% \text{ antinociceptive activity} = \left( \frac{n - n'}{n} \right) \times 100
\]

where \(n\) is the average number of stretchings of the control group, and \(n'\) is the average number of stretchings of the 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 using a glove, and 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 time (15 s), to avoid tissue damage. In all experiments, mice were tested twice at each time point. The test was performed 30 min before drug administration, which served as the baseline latency, and 30, 90, and 150 min after drug administration. Aspirin (150 mg/kg, p.o.) and morphine hydrochloride (10 mg/kg, s.c.) were used as references (Parimala Devi et al., 2003; Matsumoto et al., 2004). Only...
isotonic saline solution (ISS) (0.2 mL, i.p.) was given to the control group. Viburnum opulus extract 100 and 200 mg/kg was given i.p. to Viburnum opulus groups. The data derived from all groups were standardized by using the following formula (Tanker et al., 1996) for statistical analysis:

\[
\% \text{ antinociceptive activity} = 100 \times \left(1 - \frac{n - n_i}{n_i}\right)
\]

where \(n\) represents tail-flick results at the 30th, 90th, and 150th min, and \(n_i\) represents 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 \(\lambda\) 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 extract utilized in the present study were chosen according to the LD\(_{1}\) value (LD\(_{1}\) = 2.345 g/kg).

The measurement of foot volume was accomplished by a displacement technique using a plethysmometer (Ugo Basile 7140; Comerio, 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):

\[
I \% = [(1 - (d_t/d_c))] \times 100
\]

where \(d_t\) is the difference in paw volume in the drug-treated group and \(d_c\) is the difference in paw volume in the control group.

**Statistical analysis**

Results are reported as mean ± SEM (standard error of the mean). The total variation was analyzed by performing one-way analysis of variance (ANOVA). Tukey’s HSD (honestly significant difference), LSD (least significant difference), and Dunnett 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 \(V.\) opulus. The LD\(_{50}\) value of the extract was found to be 5.447 g/kg in mice. These data enabled us to select the dose to be administered to rats for assessing its antinociceptive and anti-inflammatory activity. The results for lethal doses are shown in Table 1.

**Antinociceptive activity**

**Acetic acid-induced writhing test**

\(V.\) opulus (VO) caused dose-related inhibition of the acetic acid-induced abdominal stretching response in mice (Table 2). VO at 100 and 200 mg/kg (p.o.) significantly reduced the acetic acid-induced abdominal pain by 56.59 and 63.21%, respectively.

Acetylsalicylic acid inhibited chemical pain (44.37%) at a dose of 300 mg/kg. The degree of inhibition for each of the three groups was significant compared to the control group. Comparison of the aspirin and VO groups did not show a statistically significant difference.

**Tail-flick test**

Results for the water extract of VO in the tail-flick test are shown in Table 3. VO was tested at 100 and 200 mg/kg i.p.

The antinociceptive effect of aspirin was started by the first 30 min, but it showed significant analgesia at 150 min. On the other hand, the morphine group showed a significant antinociceptive effect at 30 and 90 min; this effect was not observed at the 150th min.

<table>
<thead>
<tr>
<th>Lethal dose</th>
<th>Dose (g/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>LD(_{1})</td>
<td>2.345</td>
</tr>
<tr>
<td>LD(_{10})</td>
<td>3.424</td>
</tr>
<tr>
<td>LD(_{50})</td>
<td>5.447</td>
</tr>
<tr>
<td>LD(_{90})</td>
<td>8.665</td>
</tr>
<tr>
<td>LD(_{99})</td>
<td>11.463</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dose (mg/kg)</th>
<th>Abdominal stretching</th>
<th>% Inhibition of stretching</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (ISS)</td>
<td>0.2 mL</td>
<td>17.67 ± 1.67</td>
<td>—</td>
</tr>
<tr>
<td>Acetylsalicylic acid</td>
<td>300</td>
<td>9.83 ± 0.60*</td>
<td>44.37</td>
</tr>
<tr>
<td>(V.) opulus</td>
<td>100</td>
<td>7.67 ± 2.68*</td>
<td>56.59</td>
</tr>
<tr>
<td>(V.) opulus</td>
<td>200</td>
<td>6.50 ± 3.89*</td>
<td>63.21</td>
</tr>
</tbody>
</table>

\(F\) value / \(p\) value: 3.974/0.023

Values for abdominal stretching are mean ± SE, \(n = 6\), \(p < 0.05\) significant. Post-hoc LSD (least significant difference) test.

\(p < 0.05\), comparison with control group.
VO extract showed an antinociceptive effect at the doses of 100 and 200 mg/kg at all time periods, but the effect was not statistically significant at the 30th min. The VO extract had a similar antinociceptive effect at the 90th min to that of the morphine group, and at the 150th min to that of the aspirin group. Both doses of VO extract created a similar analgesic effect.

### Anti-inflammatory activity

Table 4 shows the results for the anti-edema effect of intraperitoneally administered VO on carrageenan paw edema in rats. The VO extract showed no significant anti-inflammatory effect at any of the test doses. Compared with the controls, the greatest anti-inflammatory activity was observed in the reference group receiving indomethacin, with 95.7% regression of inflammation.

<table>
<thead>
<tr>
<th>Group</th>
<th>Dose</th>
<th>Paw edema (% mL)</th>
<th>Inhibition (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control I (ISS)</td>
<td>0.1 mL</td>
<td>1.043 ± 0.084</td>
<td>—</td>
</tr>
<tr>
<td>Control II (ethyl alcohol)</td>
<td>0.1 mL</td>
<td>0.988 ± 0.075</td>
<td>—</td>
</tr>
<tr>
<td>Indomethacin</td>
<td>3 mg/kg</td>
<td>0.042 ± 0.015</td>
<td>95.70</td>
</tr>
<tr>
<td>V. opulus</td>
<td>50 mg/kg</td>
<td>0.747 ± 0.044</td>
<td>28.40</td>
</tr>
<tr>
<td>V. opulus</td>
<td>100 mg/kg</td>
<td>0.745 ± 0.130</td>
<td>28.49</td>
</tr>
<tr>
<td>V. opulus</td>
<td>200 mg/kg</td>
<td>0.732 ± 0.108</td>
<td>29.78</td>
</tr>
</tbody>
</table>

The extract reduced the acetic acid-induced abdominal pain by 68.6% at that dose. The results obtained for the antinociceptive activity of *V. opulus* are in agreement with the results of Calle et al. (1999).

In our previous study (Sever Yılmaz et al., 2007), antinociceptive and anti-inflammatory activities of the *Viburnum lantana* (VL) leaf extract had a similar effect at the 100 mg/kg dose. However, it had a stronger antinociceptive activity compared to aspirin at the 200 mg/kg dose. VL showed powerful antinociceptive activity determined by tail-flick test at a 100 mg/kg dose.

Except for inhibition of chemical painful stimuli, antinociceptive and anti-inflammatory activity results for VO extract are in agreement with the results of Sever Yılmaz et al. (2007).

The reason for the stronger antinociceptive activity of VL extract at doses of 200 mg/kg compared with that of the VO extract may be related to the phytochemical contents of the plants.

In our previous studies, *Viburnum opulus* and *V. lantana* extracts were also analyzed for their salicin, chlorogenic acid, and amentoflavone contents using high performance liquid chromatography (HPLC) with a water, tetrahydrofurane, and orthophosphoric acid (97.7:1.8:0.5, v/v/v) solvent system (Altun & Sever Yılmaz, 2007). Our results showed that VO extract was enriched in salicin and chlorogenic acid. However, the VL extract contained more amentoflavone than the VO extract.

As we indicated before, *Viburnum* species contain triterpenoids, diterpenoids, sesquiterpenoids, iridoids, and polyphenols. A synergism could therefore be possible among these substances. The antinociceptive and anti-inflammatory activities of these chemicals have been reported before for some *Viburnum* species (Calle et al., 1999). The methanolic extracts and the extract in ethyl acetate from *Viburnum toronis* decreased the
writhings in mice induced by 1% acetic acid at doses of 250 mg/kg. The antinociceptive activity has been attributed to 2-methyl butenoic, 2-methyl-2-butoenoic, 3-methylbutenoic acid, and 4-hydroxy-4-methyl-pentane isolated from the methanolic extract in ethyl acetate.

Another study reported that the methanolic extract and the extract in butanol from Viburnum sargentii had activity in inflammation and writhing tests (Youn et al., 2007).

The VO extract had no significant anti-inflammatory activity at any of the tested doses compared to the control group. The indomethacin group, which was selected as a reference group, showed significant anti-inflammatory activity compared with the VO extract at all doses. As a result, no anti-inflammatory effect was observed for the VO extract. This was in good agreement with our previous study where we reported no anti-inflammatory activity with the VL extract (Sever Yilmaz et al., 2007).

In conclusion, the present study reveals that the water extract of Viburnum opulus possesses promising antinociceptive activity. At this stage we do not know the reason(s) behind these observations. Nevertheless, it could be due to glycosides, terpenes, and polyphenols within the extract (Machida et al., 1991; Fukuyama et al., 1996, 2002, 2005a, Lobstein et al., 2003).

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

**Declaration of interest:** The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

**References**


