Antinociceptive activity of *Ricinus communis* L. leaves

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**ABSTRACT**

**Objective:** To evaluate the antinociceptive activity of the methanol extract of *Ricinus communis* leaves (MRCL). **Methods:** Antinociceptive activity was evaluated using acetic acid induced writhing test, formalin induced paw licking and tail immersion method in mice at doses of 100, 125 and 150 mg/kg bw.

**Results:** The results indicated that MRCL exhibited considerable antinociceptive activity against three classical models of pain in mice. Preliminary phytochemical analysis suggested the presence of saponin, steroids and alkaloids. **Conclusions:** It can be concluded that MRCL possesses antinociceptive potential that may be due to saponin, steroids and alkaloids in it.

1. Introduction

*Ricinus communis* Linn (Euphorbiaceae) (*R. comolunis*) is a soft-wooded small tree widespread throughout tropics and warm temperature regions of the world. In the Indian system of medicine, the leaf, root and seed oil of this plant have been used for the treatment of inflammation and liver disorders[1]. It is reported that this plant possesses hepatoprotective[2,3], antidiabetic[4], laxative[5], and antifertility[6] activities. Methanol extract of root shows anti-inflammatory and free radical scavenging activity[7]. The gallic acid, quercetin, gentisic acid, rutin, epicatechin and ellagic acid are the major phenolic compounds isolated from leaves possessing antioxidant activity[8]. Flavonoids kaempferol–3–O–beta–d–rutinoside and kaempferol–3–O–beta–d–xylopyranoid[9,10] and tannins[11] have been isolated from the leaves. Indole–3–acetic acid has been extracted from the roots[12]. In the present study efforts were made to evaluate the antinociceptive activity of methanol extract of *R. communis* leaves (MRCL).

2. Material and methods

2.1. Plant material

Fresh leaves of *R. communis* were collected from Baramati localities, Pune district (Maharashtra), and dried in the shade at room temperature. The plant was authenticated by Professor Deshmukh RB, Head of Botany Department, Shardaibai Pawar Mahila Mahavidyalaya, Shadanagar, Baramati. The voucher specimen (PASR–115) was deposited in herbarium.

2.2. Preparation of extract

Dried and powdered leaves (100 g) were extracted by petroleum ether in Soxhlet extractor, and remaining marc was extracted by cold maceration with methanol for 48 h. After filtration, the filtrate was evaporated to dryness at (35–40) °C to yield MRCL of 9.5% w/w.

2.3. Animals

Albino mice (25–30) g of either sex were housed under standard laboratory conditions. The animals had free access to food and water.

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to food and water. The Animal Ethical Committee of the Institute approved all the protocols of the study (Registration No.1214/ac/08/CPCSEA).

2.4. Preliminary phytochemical screening

To determine the chemical constituents, qualitative phytochemical screening of MRCL was carried out for alkaloids, flavonoids, saponins, steroids and glycoside following standard procedure[13,14].

2.5. Antinociceptive activity

2.5.1. Acetic acid induced writhing

Mice were divided into five groups with five in each group. Control group was intraperitoneally treated with 1% Tween–80 solution (5 mL/kg), test groups received MRCL at doses of 100–150 mg/kg, and standard group received diclofenac sodium at a dose of 50 mg/kg. After 30 min, all groups were administered with 0.6% acetic acid at a dose of 10 mL/kg intraperitoneally. The number of writhing for each mouse was counted for 20 min starting 10 min after injection of acetic acid and the percent inhibition of writhing was calculated[15].

2.5.2. Formalin induced paw licking

The mice were divided into four groups with five mice in each group. Control group was intraperitoneally treated with 1% Tween–80 solution (5 mL/kg), test groups received MRCL at doses of 100–150 mg/kg, and standard group received diclofenac sodium at a dose of 50 mg/kg. 30 min after administration of test and standard drugs, all groups received 10 μL of 2.5% formalin in the sub–plantar region of right hind paw using a micro syringe. The number of paw licking was monitored 0-5 min (phase–I) and 20-25 min (phase–II) after injection of formalin. Percent inhibition of paw licking was calculated by comparing test group with control group[16].

2.5.3. Tail immersion method

The mice were divided into five groups with five in each group. Control group was intraperitoneally treated with 1% Tween–80 solution (5 mL/kg), test groups received MRCL at doses of 100–150 mg/kg, and standard group received aspirin at a dose of 50 mg/kg. 1–2 cm of the mice tail was immersed in warm water and kept constant at 55 °C. The reaction time was the time taken by the mice to deflect their tails. The first reading was discarded and the reaction time was recorded as a mean of the next three readings. A latent period of 20 sec was defined as complete algesia and the measurement was then stopped to avoid injury to mice. The latent period of the tail–flick response was determined before and 0, 30, 60 and 90 min after the administration of test drugs.

2.6. Statistical analysis

All observations were presented as mean±SEM. The data were analyzed by one way ANOVA and followed by Newman Keuls’ test. P<0.05 was considered as significant.

3. Results

3.1. Preliminary phytochemical test

Preliminary phytochemical test showed the presence of alkaloids, saponins and steroids.

3.2. Acetic acid induced writhing

Control group showed maximum writhing (70.200±8.108) while MRCL reduced acetic acid induced writhing significantly (P<0.001) in the test group. MRCL at doses of 100, 125 and 150 mg/kg inhibited writhing in a dose-dependent manner as shown in Figure 1.

![Figure 1. Effect of MRCL on acetic acid induced writhing test in mice.](image)

3.3. Formalin induced paw licking test

MRCL showed analgesic effect on phase–I and phases–II of formalin induced pain. The phases corresponded to neurogenic and inflammatory pains, respectively. MRCL inhibited significantly neurogenic and inflammatory phase in a dose–dependent manner (Figure 2). MRCL at dose of 150 mg/kg showed significant (P<0.001) inhibition of neurogenic and inflammatory phase comparable to standard drug.

![Figure 2. Effect of MRCL on formalin induced paw licking test in mice.](image)

3.4. Tail immersion method
MRCL showed significant increase in latent time after 90 min at a dose of 150 mg/kg as shown in Table 1.

Table 1
The effect of MRCL on tail immersion method (Mean±SEM).

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dose (mg/kg)</th>
<th>Tail flick time in sec</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>After 0 min</td>
</tr>
<tr>
<td>Control</td>
<td>–</td>
<td>2.00±0.198</td>
</tr>
<tr>
<td>MRCL (i.p.)</td>
<td>100</td>
<td>2.33±0.189</td>
</tr>
<tr>
<td></td>
<td>125</td>
<td>2.61±0.202</td>
</tr>
<tr>
<td></td>
<td>150</td>
<td>2.40±0.205</td>
</tr>
<tr>
<td>Aspirin (i.p.)</td>
<td>30</td>
<td>2.83±0.069</td>
</tr>
</tbody>
</table>

$^aP<0.05; ^bP<0.01; ^cP<0.001$ when compared with control group.

4. Discussion

Results of the present study indicated that MRCL possesses significant antinociceptive activity. The acetic acid induced abdominal contraction and the tail immersion methods elucidated peripheral and central activity, while the formalin test investigated both$^7$. Drugs that act primarily on the central nervous system inhibit both phases equally while peripherally acting drugs inhibit the late phase$^8$. The mechanism of analgesic effect of MRCL could probably be due to inhibition of the effect or release of endogenous substances that induces pain nerve endings similar to that of NSAIDs. In the formalin test, the pain in the early phase was due to the direct stimulation of the sensory nerve fibers by formalin, whereas the pain in the late phase was due to the inflammatory mediators, like histamine, prostaglandin, serotonin, and bradykinin$^9$. It is reported that NSAIDs reduce both phases of the formalin test$^{10}$. MRCL inhibits significantly acetic acid induced writhing and formalin induced paw licking in both neurogenic and inflammatory pain in a dose–dependent manner. Tail immersion test is a chronic pain model which is sensitive to centrally acting analgesic agents. MRCL significantly increases latent time after 90 min of drug treatment.

In conclusion, the present study revealed that MRCL possesses antinociceptive activity that may be due to the presence of alkaloids and steroids.

Conflict of interest statement

We declare that we have no conflict of interest.

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