Evaluation of anti-inflammatory potential of *Ricinus communis* Linn leaves extracts and its flavonoids content in Wistar rats

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**ABSTRACT**

The present study was aimed to screen *Ricinus communis* (RC) leaves for anti-inflammatory potential using carrageenan-induced paw edema (Acute model) and cotton pellet induced granuloma models (Sub-chronic model) in Wistar rats. For this 80% methanolic extract (ME) at two dose levels of 250 and 500 mg/kg and total flavonoids fractions (FF) at three different doses 25, 50 and 100 mg/kg, were studied in acute model. The ME (500 mg/kg) and FF 50 mg/kg showed potent anti-inflammatory action in this model. Moreover FF (50 mg/kg) had shown almost same effect as shown by FF at the dose of 100 mg/kg. Hence, ME (500 mg/kg) and FF (50 mg/kg) have been selected for further evaluation in sub-chronic model. The results of ME (500 mg/kg) and FF (50 mg/kg) were at par with diclofenac sodium (20 mg/kg). The study shows the RC leaves have anti-inflammatory potentials and flavonoids are dominating this activity in the extract.

**Keywords:** Anti-inflammatory; Carrageenan; Cotton pellet granuloma; *Ricinus communis*

**INTRODUCTION**

Inflammation is a patho-physiological response of mammalian tissues to a variety of noxious agents including infectious organisms, toxic chemical substances, physical injury etc resulting local accumulation of plasma fluid and blood cells [1]. A systematic study of anti-inflammatory effects of a number of Indian medicinal plants began by Gujral and his associates in 1956.
Ricinus communis Linn (RC) is a monotypic genus [2] commonly known as Castor bean (Euphorbiaceae) has been used as folklore medicine for the treatment of hemorrhoids [3] jaundice [4], ulcer, headache, sores, epilepsy, rheumatism, and sciatica [5]. The leaves are being used to treat inflammation manifestations in north India along with turmeric paste [6]. The roots have been reported to possess anti-inflammatory [7], antioxidant and antidiabetic activities [8]. The leaves had shown antioxidant activities and are reported to contain flavonoids: rutin, quercetin, epicatechin and polyphenols (Gallic and ellagic acid) and gentesic acid [9, 10], these compounds had proven by researchers as potent anti-oxidant and anti-inflammatory agents.

Keeping this in view, it has been assumed to substantiate the ethno pharmacological use and the effectiveness of flavonoids content of RC leaves as an anti-inflammatory agent. Therefore, the present study was designed to investigate the anti-inflammatory effect of RC leaves.

MATERIALS AND METHODS

Chemicals
Carrageenan from Sigma Chemical Co., St Louis, MO, USA; diclofenac from Novartis, India; and thiopental sodium from NEON Laboratories Ltd, India were used. All other chemicals and reagents of analytical grade were used freshly.

Plant Material
The leaves of RC were collected in the month of September-October from Medicinal Garden, ISF College of Pharmacy, Moga, Punjab, India, and got authenticated. A voucher specimen (ISF-12) has been kept in the laboratory for further reference.

Preparation of extract
The leaves were shade dried, coarsely powdered (935 g) and defatted with n-hexane using Soxhlet extractor for 72 h. The marc was extracted with 80 % methanol by soxhletion to obtain methanolic extract (ME) and concentrated under vacuum. The yield of ME was found to be 9.17 % w/w.

Phytochemical screening
Preliminary phytochemical screening of ME revealed the presence of alkaloids, flavonoids and carbohydrates [11].

Isolation of total flavonoids
The ME (80 g) was dissolved in water and fractionated 3 times with chloroform to remove color pigments; and addition of 10 % NaCl was in aqueous phase drop wise in order to precipitate tannins. The resulting solution was subjected to centrifugation, and supernatant was partitioned with ethyl acetate. The ethyl acetate layer was evaporated to get crude flavonoids fraction (FF) [12].

Animals
Wistar albino rats of either sex weighing 180-200 g were procured from Animal house, I.S.F. College of Pharmacy, Moga (Reg. No 816/04/C/CPCSEA). The animals were kept in polypropylene cages at an ambient temperature 25 ± 2°C and relative humidity 55-65%,
respectively. The 12-12 h light and dark cycles were maintained. The rats were fed at commercially available normal chow diet from Aashirwad Industries Ltd., Ropar, Punjab, and water ad libitum.

**Acute toxicity study**
The acute toxicity study of ME was carried out as per organization for economic cooperation and development 423 guidelines, 1987 using Wistar rat of either sex n=3[13].

**Carrageenan-induced rat paw edema:**
The anti-inflammatory activities of ME and FF were evaluated using carrageenan-induced paw edema model in rats [14]. The animals were divided into different groups each comprising six rats as control: 1% w/v CMC p.o. (5 ml/kg) as vehicle; diclofenac sodium (DS) 20 mg/kg as standard; ME 250 and 500 mg/kg; and FF: 25 and 50 mg/kg. Paw edema was induced by injecting 0.1 ml of carrageenan 1% w/v in sub-plantar region of rats. The test drugs were administered orally 30 min prior to the carrageenan injection. The estimation of edema formation was done using plethysmograph at 0, 30, 60, 90 min, 2, 3, 4 and 24 h after carrageenan injection the % paw volume inhibition was measured using formula:

\[
\% \text{ Inhibition} = \frac{(V_o-V_t)}{V_o} \times 100
\]

Where \(V_o\) is the initial paw volume and \(V_t\) is the paw volume after drug treatment.

**Cotton pellet granuloma**
In this model the animals were anaesthetized with thiopental sodium (40 mg/kg, i.p.). The interscapular implantations of sterile cotton pellets (20 mg) were done in both sides to induce chronic inflammation [15]. The test drugs: ME (500 mg/kg), FF (50 mg/kg) and DS (20 mg/kg) were administered orally for 9 consecutive days from the day of cotton pellet implantation. On 10th day, animals were sacrificed; cotton pellets were removed surgically; freed from extraneous tissue; and dried till constant weight achieved. The increment in dry weight of pellets over 20 mg was taken as an index of granuloma formation.

**RESULTS**

**Acute toxicity study**
The ME of leaves RC showed no mortality as well as behavioral changes even up to the dose 2000 mg/kg.

**Effect of test drugs on carrageenan-induced paw edema**
In the carrageenan-induced paw edema model, sub plantar administration of carrageenan caused significant paw edema formation as compared to normal paw in successive hrs up to 24 h. Pretreatment with ME (250 and 500 mg/kg, respectively) showed marked inhibition of paw edema from 30 min to 24 h dose dependently as compared to untreated control rats received carrageenan only. The FF: 25, 50 and 100 mg/kg also showed significant decrease in paw volume from 30 min to 24 h in comparison to control. The maximum effect was attained with FF: 50 mg/kg and 100 mg/kg. However FF: 100 mg/kg had not shown marked difference when
compared with FF (50 mg/kg). DS (20 mg/kg) also showed significant prevention of edema from 30 min to 24 h in comparison to untreated rats receiving carrageenan only (Figure 1).

Figure 1: Effect of ME on carrageenan-induced paw edema. Results are expressed as Mean ± SD; *P<0.05 statistically significant vs control at respective time; [ME: Methanol extract 250 and 500 mg/kg; FF: Flavonoids fraction 25, 50 and 100 mg].

Effect of test drug on cotton pellet-induced granuloma
In this model, inter-scapular implantation of sterile cotton pellets caused significant granuloma tissue formation as indicated by elevated weight of cotton pellet. The ME 500 mg/kg, FF 50 mg/kg and DS 15 mg/kg treated animals showed marked decrease in granuloma tissue formation as compared to control group as shown in table 1.

<table>
<thead>
<tr>
<th>Group</th>
<th>Granuloma weight</th>
<th>% Granuloma weight</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>108.27</td>
<td>100</td>
</tr>
<tr>
<td>DS 15 mg/kg</td>
<td>56.83</td>
<td>52.49 ± 3.71*</td>
</tr>
<tr>
<td>ME 500 mg/kg</td>
<td>82.45</td>
<td>76.15 ± 1.15*</td>
</tr>
<tr>
<td>FF 50 mg/kg</td>
<td>75.67</td>
<td>69.89 ± 1.41*</td>
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</tbody>
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Mean ± SEM, *P<0.05 vs control. ME = Methanolic extract; FF = Flavonoid fraction

DISCUSSION
The present study demonstrated the evidences for the anti-inflammatory effects of ME and its flavonoids content isolated from leaves of RC. In acute toxicity study, the ME was found to be safe up to 2000 mg/kg as evidenced by no mortality as well as behavioral change. The carrageenan-induced rat paw edema model is a well known irritant for investigating or evaluating new drug therapies for acute inflammatory pathological condition [16]. Carrageenan is reported to cause paw edema which is a biphasic event. The initial phase is attributed with the release of histamine and serotonin causing vasodilatation and increased capillary permeability; the second phase is due to release of bradykinin, prostaglandins, protease and lysosomal enzymes regulating the process of adhesion molecules [17], cell migration, activation and degranulation [18-20]. The present study also showed the significant paw edema formation due to sub plantar administration of carrageenan, characterizing the cellular events of acute inflammation. The ME of RC leaves (250 and 500 mg/kg) respectively showed protective effect in prevention of cellular events during edema formation and in all the stages of acute inflammation.
inflammation. This effect of ME was supported by the protective effect of flavonoids against carrageenan-induced paw edema in rats.

Cotton pellet granuloma model is indicative of proliferative phase of inflammation involving macrophages, neutrophils, fibroblast cells and collagen formation resulting in granuloma formation [21]. The ME and flavonoids content of RC leaves confirms their potency over chronic inflammatory conditions.

CONCLUSION

The Phytochemical screenings had shown the presence of flavonoids in ME. The ME had shown the anti-inflammatory activities in both the acute and sub-chronic models in Wistar rats. The activity was potentiated by flavonoids fraction. Hence, it is concluded that anti-inflammatory activity of leaves extract is attributed to the flavonoids. Our findings also support the previous finding regarding the effectiveness of flavonoid as an anti-inflammatory compounds.

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