Screening of Anti-Inflammatory and Analgesic Activity of Cassia grandis Linn

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Abstract: This work has done for the investigation of the anti-inflammatory and analgesic activity of methanolic and petroleum ether extracts of dried leaves of Cassia grandis Linn. by oral administration at dose of 125, 250 and 500 mg/kg/day of body weight to healthy animals. Both extracts were studied for their anti-inflammatory activity by using carrageenan-induced hind paw edema in rats and the mean increase in paw volume and % inhibition in paw volume were measured plethysmometrically at different time intervals after carrageenan (1% w/v) injection. Both extract were also evaluated for analgesic activity using Eddy’s hot plate method and tail-flick method in albino rats. The methanolic extract of Cassia grandis showed significant (P<0.05) reduction in the carrageenan-induced paw edema in comparison to petroleum ether extract and analgesic activity evidenced by increase in the reaction time by Eddy’s hot plate method and tail-flick method. The methanolic extract showed a greater anti-inflammatory and analgesic effect when compared with the standard drugs, indomethacin and diclofenac sodium respectively. Results of present studies suggest that methanolic extract of Cassia grandis significant (P<0.05) anti-inflammatory and analgesic activity in dose dependent manner whereas petroleum ether extract exhibited feeble effect in the treatment of inflammation and pain. 

Key words: Carrageenan-induced paw edema, Indomethacin, Diclofenac sodium, Inflammation, Pain

INTRODUCTION

Inflammation is a complex biological response of vascular tissues to harmful stimuli, such as pathogens, damaged cells, or irritants. It is a defensive mechanism of the organism to remove the injurious stimuli as well as to initiate the healing process for the tissue. In absence of inflammation, wounds and infections would never heal and progressive destruction of the tissue would compromise the survival of the organism [1]. Many components are involved in the inflammation process to name few are oedema formation, leukocyte infiltration and granuloma formation are widely noticeable [2]. The formation of edema in paw results a synergism between various inflammatory mediators which increases the vascular permeability and the blood flow [3]. Carrageenan induced paw edema is used widely for determining the acute phase of inflammation. Histamine, 5-hydroxytryptamine and bradykinin are used as the first detectable mediators in the early phase of carrageenan induced inflammation [4] and prostaglandins are used in the late phase of inflammation[5]. Medicinal plants, those having a wide variety of chemicals, from which anti-inflammatory agents could be derived, can be scientifically proven for their efficacy and safety parameters.

Cassia grandis Linn. (Family: Leguminosae) is a deciduous or semi deciduous spreading tree. It is well known as a Pink shower. The phytochemical studies revealed the presence of flavonoids, anthraquinones and sterols. Several studies on the various parts of this plant have been reported as in-vitro antioxidant, purgative and in treatment of skin disorders etc. The pulp from the pods is very strong smelling with a bitter and astringent taste, which has laxative properties. It is sometimes used in veterinary practices also hence known as Horse Cassia. The juice from the pods is reported to strengthen the blood[6-13].

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MATERIALS AND METHODS

Indomethacin, Micro Labs, Bangalore; Carrageenan, Sigma Chemicals, USA and Diclofenac sodium, Apex Labs, Chennai, were used in the experiment. All other chemicals were used of analytical grade.

Collection of Plant: The leaves of *Cassia grandis* were collected from Nasik, India, in the month of July. The plant was identified with the help of available literature and authenticated by Department of Pharmacognosy, N. D. M. V. P. Samaj’s College of Pharmacy, Nasik.

Preparation of Extract: The powdered leaves (500 g) were packed in soxhlet apparatus. The drug was defatted with petroleum ether (60-80°C) for about 30-35 complete cycles. Defatted material was extracted with two liters of petroleum ether by soxhlet apparatus and then extracted material successively extracted with methanol followed by maceration at room temperature and then these extracts were dried by rotary vacuum dryer. The petroleum ether and methanol extracts of *Cassia grandis* was designated as PEBCG and MECG and the percentage yield were found to be 6.82% and 12.75% respectively.

Toxicity Studies: The methanolic and petroleum ether extracts were given at the dose of 125, 250 and 500 mg/kg/day of body weight per day were selected range from 1/6 to 1/15 of LD₅₀ based on the preliminary study conducted at our laboratory and data are not shown in this paper.

Animals: Wister albino rats (120-200 g) and Swiss albino mice (20-30 g) of either sex were used. The animals housed under standard laboratory conditions maintained at 25±1°C and under 12/12 h light / dark cycle and fed with standard pellet diet (Gold Mohur brand, Lipton India Ltd.) and water *ad libitum*. Animal experiments were approved by the Institutional Animal Ethical Committee.

Anti-Inflammatory Activity Study: The albino rats of either sex were divided into eight groups consisting of six animals in each group. Group I received 0.2 ml of 2% w/v carboxy methyl cellulose suspension orally as a control group, group II received 125 mg/kg; p. o. of methanolic extract of *Cassia grandis* (MECO-I), group III received 250 mg/kg; p. o. of methanolic extract of *Cassia grandis* (MECO-II), group IV received 500 mg/kg; p. o. of methanolic extract of *Cassia grandis* (MECO-III), group V received 125 mg/kg; p. o. of petroleum ether extract of *Cassia grandis* (PEBCG-I), group VI received 250 mg/kg; p. o. of petroleum ether extract of *Cassia grandis* (PEBCG-II), group VII received 500 mg/kg; p. o. of petroleum ether extract of *Cassia grandis* (PEBCG-III) and group VIII received 10 mg/kg; i. p. of indomethacin as a standard drug. Acute inflammation was induced in all groups by injecting 0.1 ml of 1% w/v carrageenan into the sub-plantar region of the right hind paw of rats. Mean paw volume was measured 1 h prior to carrageenan injection using plottymeter and at 0, 15, 30, 60, 120 and 180 min after the carrageenan injection [14-15]. Mean increase in the paw volume was measured and percent inhibition was calculated by using following formula:

\[
\text{Percent inhibition} = 100 \times \left( \frac{Vc - Vt}{Vc} \right)
\]

Where,

- \(Vc\) = Edema volume in control
- \(Vt\) = Edema volume in test / standard compound.

Analgesic Activity Study

Analgesic Activity by Tail Flick Method: The albino mice were divided into eight groups consisting of six animals in each group. Group I received 0.2 ml of 2% w/v carboxy methyl cellulose suspension orally as a control group, group II received 125 mg/kg; p. o. of methanolic extract of *Cassia grandis* (MECO-I), group III received 250 mg/kg; p. o. of methanolic extract of *Cassia grandis* (MECO-II), group IV received 500 mg/kg; p. o. of methanolic extract of *Cassia grandis* (MECO-III), group V received 125 mg/kg; p. o. of petroleum ether extract of *Cassia grandis* (PEBCG-I), group VI received 250 mg/kg; p. o. of petroleum ether extract of *Cassia grandis* (PEBCG-II), group VII received 500 mg/kg; p. o. of petroleum ether extract of *Cassia grandis* (PEBCG-III) and group VIII received 1.0 mg/kg; i. p. of diclofenac sodium as a standard drug. The reaction time was recorded using tail flick analgesiometer at 0, 30, 60, 120 and 180 min time interval after the drug administration [16]. The temperature was maintained at 50-55°C and data are represented in Figure 1.

Analgesic Activity by Eddy’s Hot Plate Method: Mice were divided into eight groups consisting of six animals in each group and treatments were given as per tail-flick method. Animals were placed on the Eddy’s hot plate maintained at 55±1°C. The reaction time in control and treated animals was recorded at 0, 30, 60, 120 and 180 min after the treatment [17] and data are represented in Figure 2.
Statistical Analysis: Results were expressed as Mean±SEM, statistical significance was calculated by applying one way ANOVA. P<0.05 was considered as significant.

RESULTS

The results of anti-inflammatory activity by carrageenan-induced paw edema methods showed in Table 1. Methanolic extract of Cassia grandis; MECG-I, MECG-II and MECG-III were found to be significant (P<0.05) effective in reduction of paw volume after 3 h i.e. 57.86%, 64.77% and 72.96% respectively when compared with the control group and Indomethacin; which indicate that the effect of methanolic extract of Cassia grandis exhibited in dose dependent manner against acute inflammation. The percent inhibition in paw edema by indomethacin was 79.87 % after 3 h.

In case of petroleum ether extract of Cassia grandis; PEECG-I, PEECG-II and PEECG-III reduced the paw volume were found to be 02.51 %, 06.29% and 13.20 % respectively after 3 h so petroleum ether extract of
Table 1: Effect of Cassia grandis on carrageenan-induced paw edema

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dose (mg/kg)</th>
<th>0 min.</th>
<th>15 min.</th>
<th>30 min.</th>
<th>60 min.</th>
<th>120 min.</th>
<th>180 min.</th>
<th>Percent Inhibition after 180 min.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>Saline</td>
<td>0.78±0.02</td>
<td>1.02±0.15</td>
<td>1.48±0.14</td>
<td>1.79±0.16</td>
<td>1.88±0.17</td>
<td>1.59±0.06</td>
<td>---</td>
</tr>
<tr>
<td>MECG-I</td>
<td>125</td>
<td>0.77±0.16</td>
<td>1.18±0.20</td>
<td>1.57±0.41</td>
<td>1.29±0.17</td>
<td>0.95±0.15</td>
<td>0.67±0.42</td>
<td>57.86</td>
</tr>
<tr>
<td>MECG-II</td>
<td>250</td>
<td>0.76±0.11</td>
<td>0.99±0.18</td>
<td>1.18±0.80</td>
<td>1.01±0.12</td>
<td>0.82±0.14</td>
<td>0.56±0.20</td>
<td>64.77</td>
</tr>
<tr>
<td>MECG-III</td>
<td>500</td>
<td>0.75±0.21</td>
<td>0.85±0.18</td>
<td>0.92±0.30</td>
<td>0.81±0.54</td>
<td>0.61±0.24</td>
<td>0.43±0.32</td>
<td>72.96</td>
</tr>
<tr>
<td>PEECG-I</td>
<td>125</td>
<td>0.78±0.64</td>
<td>1.09±0.24</td>
<td>1.41±0.42</td>
<td>1.67±0.19</td>
<td>1.71±0.14</td>
<td>1.55±0.28</td>
<td>02.51</td>
</tr>
<tr>
<td>PEECG-II</td>
<td>250</td>
<td>0.77±0.63</td>
<td>1.18±0.17</td>
<td>1.47±0.15</td>
<td>1.76±0.10</td>
<td>1.65±0.23</td>
<td>1.49±0.18</td>
<td>06.29</td>
</tr>
<tr>
<td>PEECG-III</td>
<td>500</td>
<td>0.76±0.19</td>
<td>1.24±0.22</td>
<td>1.53±0.41</td>
<td>1.48±0.14</td>
<td>1.55±0.19</td>
<td>1.38±0.41</td>
<td>13.20</td>
</tr>
<tr>
<td>Indomethacin</td>
<td>10</td>
<td>0.74±0.04</td>
<td>0.77±0.30</td>
<td>0.81±0.20</td>
<td>0.71±0.30</td>
<td>0.52±0.14</td>
<td>0.32±0.02</td>
<td>79.87</td>
</tr>
</tbody>
</table>

n = 6, Values are expressed as mean ± SEM, *P < 0.05 When compared with control.

Cassia grandis does not possess significant anti-inflammatory activity when compared with control and Indomethacin treated animals (Table 1).

For the determination of analgesic activity, we used two methods i.e. tail-flick method and Eddy’s hot plate method. The analgesic activity profile of methanolic extract at different doses (MECG-I, MECG-II and MECG-III) showed significant (P < 0.05) analgesic activity when compared with control and Diclofenac treated animals but in case of PEECG-I, PEECG-II and PEECG-III don’t exhibit significant analgesic activity (Figure 1 and 2). Thus MECG-I, MECG-II and MECG-III extract exhibited marked central analgesic effect as evidenced by significant increase in reaction time when compared with control and diclofenac sodium in both methods.

**DISCUSSION**

Inflammation has different phases the first phase is caused by an increase in vascular permeability, the second one by infiltrate of leucocytes and the third one by granuloma formation. We determined anti-inflammatory activity by using inhibition of carrageenan-induced inflammation which is one of the most feasible methods to screen anti-inflammatory agents. The development of carrageenan-induced edema is bi-phasic; the first phase is attributed to the release of histamine, serotonin and kinins and the second phase is related to the release of prostaglandins and bradykinins [18-20]. We observed that methanolic extract of Cassia grandis at the given different doses possess significant inhibition against carrageenan-induced paw edema in rats whereas petroleum ether extract exhibited feeble effect in the treatment of acute inflammation and pain. This response tendency of the extracts in carrageenan-induced paw edema revealed good peripheral anti-inflammatory properties of the extracts. The anti-inflammatory effect of methanolic extract of Cassia grandis may be due to the presence of flavonoids. It has been reported that a number of flavonoids possess anti-inflammatory [21] and analgesic [22] activities. Flavonoids are known to inhibit the enzyme prostaglandin synthetase, more specifically the endoperoxidase [23] and reported to produce anti-inflammatory effects. [24] Since, prostaglandins are also involved in the pain perception, inhibition of their synthesis.

We used eddy’s hot plate and tail-flick methods for the determination of analgesic activity. The methanolic extract of Cassia grandis possess good analgesic activity by increase in the reaction time (increase threshold potential of pain) may be due to the above mentioned mechanism where as petroleum ether extract don’t show significant analgesic effect.

Thus, we concluded that the crude methanolic extract of leaves of Cassia grandis produces significant anti-inflammatory and analgesic activities in dose dependent manner.

**ACKNOWLEDGEMENT**

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**REFERENCES**