Evaluation of antidiarrheal and antiinflammatory activity of *Aegle marmelos* on albino wistar rats

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ABSTRACT

*Aegle marmelos* (Linn) is a plant of the Rutaceae family, well known in India as bael/bilva leaves. This research explains about *Aegle marmelos*, which has widespread use in India due to its various medicinal properties. In the present study, evaluation of physicochemical characteristics; preliminary phytochemical parameters and pharmacological activities of ethanolic extract of dried leaves of *Aegle marmelos* Linn has been carried out. The preliminary phytochemical studies of the ethanolic extract of the leaves showed the presence of various phytochemical constituents such as alkaloids, tannins, flavonoids, steroids and saponins. The aim of the present study was to carry out phytochemical screening and to evaluate the anti-inflammatory & antidiarrhoeal activity of ethanolic extract of *Aegle marmelos*. Extract was evaluated for antiinflammatory activity by using carrageenan-induced hind paw edema in albino 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. Ethanolic extract of *A. marmelos* shows the presence of some flavonoids and terpenoids & used for the investigation of antidiarrheal activity by castor oil induced diarrhoea method. The ethanolic extract showed antiinflammatory and antidiarrheal effect in dose dependent manner when compared with the control and standard drug diclofenac sodium (10mg/kg, p.o). These inhibitions were statistically significant (P < 0.001). Thus our investigation suggests a potential benefit of *Aegle marmelos* in treating conditions associated with inflammatory pain.

Key words: Antidiarrheal, Castor oil, Paw volume, *Aegle marmelos*, Antiinflammatory

INTRODUCTION

Plants have been used in treating human diseases for thousands of years. Some 60,000 years ago, it appears that ancient people valued herbs as medicinal agents; this conclusion is based on a grave in Iran in which pollen grains of eight medicinal plants were found [1], [2]. One of these allegedly ancient medicinal herbs, yarrow was discussed as a modern medicinal plant [3], [4].

Since prehistoric times, shamans or medicine men and women of Eurasia and the America acquired a tremendous knowledge of medicinal plants. All of the native plant species discussed in details in this work was used by native people in traditional medicine. The fact that hundreds of additional species were also used by First Nations
Canadians suggests that many of these also have important pharmacological constituents that could be valuable in modern medicine [5], [2].

Up until the 18th century, the professions of doctor and botanist were closely linked. Indeed, the first modern botanic gardens, which were founded in 16th century in Italy. Padova and Florence, were medicinal plant gardens attached to medical faculties or schools [6], [7].

The use of medicinal plants is not just a custom of the distant past. Perhaps 90% of the world's population still relies completely on raw herbs and unrefined extracts as medicines. A 1997 survey showed that 23% of Canadians have used herbal medicines. In addition, as much as 25% of modern pharmaceutical drugs contain plant ingredients [8], [9].

India has a rich heritage of usage of medicinal plants in the Ayurvedic, Siddha and Unani system. Many Indian plants have been investigated for their beneficial use in different diseases and reports occur in numerous scientific journals [10]. The country has about 15000 medicinal plants that include 7000 plants used in Ayurveda, 700 in Unani, 600 in siddha, 450 in Homeopathy and 30 in modern medicines [11], [12]. The plant extracts and its product play an important role in treating many symptoms [13], [14]. Medicinal plants have been tested for biological, antimicrobial and hypoglycemic activity [15]. They have been also tested for antiulcerogenic, antihelminthic, hepatoprotective, analgesic, antipyretic, antileishmanial and insecticidal activities [16], [12].

**MATERIALS AND METHODS**

**Carrageenan-Induced Rat Paw Edema:**
Rats were divided into four groups (n=6). Acute inflammation was produced by sub planter administration of 0.1 ml of 1% w/v carrageenan in normal saline in the right hand paw of the rats. The paw volume was measured at 0-h and 3-h after carrageenan injection by using plethysmometer. Animals of group I received normal saline (3 ml/kg b.w., intraperitoneal) and served as saline control. The group II received reference drug Diclofenac sodium (10 mg/kg b. w., i.p). The groups III and IV received ethanolic extract of *Aegle marmelos* (200 and 400 mg/kg b.w., i.p, respectively) and Animals of all groups were treated with the extract and reference drug 1 hour before the administration of carrageenan [17], [18].

The difference between the initial and subsequent readings gave the actual edema volume. Edema was expressed as the mean increase in paw volume relative to control animals [19], [20]. The percentage inhibition of edema was calculated by the following equation:

\[
\text{% inhibition of edema} = 100 \left(1 - \frac{V_t}{V_c}\right)
\]

Where \(V_c\) is the edema volume in the control group and \(V_t\) is the edema volume in test group.

**Castor oil induced diarrhoea**
Rats were allowed to fast for 18 h and divided into 4 groups of 6 animals each. One group received 10 ml/kg 0.5% v/v aqueous Tween 80 orally and served as a negative control. Another group received the standard drug loperamide (3 mg/kg, p.o.) as positive control, third and fourth groups received extract at a dose of 100 and 200 mg/kg body weight, respectively. After 1 h of treatment, all the animal groups were challenged with 1 ml of castor oil orally and observed for consistency of faecal materials [21], [22]. After this administration, the animals were placed separately in metabolic cages with filter paper, which was changed every hour. The severity of diarrhoea was assessed each hour for 6 hours. The total number of diarrhoeal droppings excreted and the total weight of faeces were recorded within a period of 24 h and compared with the control group [23], [18]. The total number of diarrhoeal droppings of the control group was considered 100%. The results were expressed as a percentage of inhibition of diarrhoea [24], [25].

**RESULTS AND DISCUSSION**
In carrageenan induced paw oedema activity, the paw volumes and percentage of inhibition of the control, standard and test compounds are shown in Table No: 6.3. The tests compounds are compared with diclofenac as a standard at a dose of 10mg/kg body weight for anti-inflammatory activity. Presently diclofenac showed 20% inhibition of inflammation at 3 hours when compared to control.
Alcoholic extracts of *Aegle marmelos* leaves (200 mg/kg and 400 mg/kg) shown significant inhibition of inflammation with 10% and 30% respectively at 4 hours when compared with control. The results of test compounds were found to be statistically significant at value $P<0.001$.

**Table-1: Anti-inflammatory activity *A. marmelos***

<table>
<thead>
<tr>
<th>Group</th>
<th>Dose (mg/kg)</th>
<th>Change in paw volume (ml) Mean±SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>0min</td>
</tr>
<tr>
<td>Control</td>
<td>--</td>
<td>0.2±0.01</td>
</tr>
<tr>
<td>Std (Diclofenac Sodium)</td>
<td>10</td>
<td>0.2±0.02</td>
</tr>
<tr>
<td>Ethanol extract of <em>Aegle marmelos</em> (test I)</td>
<td>200</td>
<td>0.3±0.02</td>
</tr>
<tr>
<td>Ethanol extract of <em>Aegle marmelos</em> (test II)</td>
<td>400</td>
<td>0.2±0.01</td>
</tr>
</tbody>
</table>

The results are expressed as means ± S.E.M. Differences in mean values between groups were analyzed by a one-way analysis of variance (ANOVA). Statistical significance was assessed as $p < 0.001$.

Here presence of alkaloids, tannins, flavonoids, steroids, anthrecene glycosides and saponins has been associated with various degrees of anti-inflammatory activities. Therefore the anti-inflammatory effects observed in this study may be due to the activity of one or a combination of some of the identified constituents. It may suggest that the inhibitory effect of the constituents in the extract on edema formation is probably due to inhibition of the synthesis and/or release of the inflammatory mediators, especially the cyclooxygenase products. The carrageenan induced paw edema test is effectively controlled with the arachidonate COX inhibitors due to its COX-dependent mechanism [17], [19].

**Table-2: Anti-diarrhoeal activity of *A. marmelos***

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dose mg/kg</th>
<th>Mean frequency of diarrhea ± SEM</th>
<th>Mean wt of fecal drops ± SEM</th>
<th>Mean wt of faeces ± SEM after 4hr</th>
<th>% protection</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>-</td>
<td>3.6±0.021</td>
<td>10.6±0.24</td>
<td>1.16±0.031</td>
<td>0.00</td>
</tr>
<tr>
<td>Loperamide</td>
<td>3</td>
<td>0.4±0.02</td>
<td>1.8±0.37</td>
<td>0.15±0.033</td>
<td>86.72</td>
</tr>
<tr>
<td><em>Aegle marmelos</em></td>
<td>400</td>
<td>2.2±0.03</td>
<td>4.6±0.05</td>
<td>0.6±0.07</td>
<td>48.27</td>
</tr>
</tbody>
</table>

The results are expressed as means ±S.E.M. Differences in mean values between groups were analyzed by a one-way analysis of variance (ANOVA). Statistical significance was assessed as $p < 0.001$. 

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Evaluation of Anti-diarrhoeal activity by castor oil induced method:
The Anti-diarrhoeal activity of *Aegle marmelos* was assessed by castor oil induced diarrhoea in rats was illustrated in table below showed significant anti-diarrhoeal activity at 400 mg/kg orally. Anti-diarrhoeal activity was comparable with the standard drug loperamide [23].

**CONCLUSION**

Through these studies it can be concluded that leaves of *Aegle marmelos* have shown great potential of anti-inflammatory and anti-diarrhoeal activity. Based on phytochemical screening, we have concluded that the both test I and test II have anti-inflammatory activity in carrageenan-induced paw edema in rats. This extracts has showed significant decrease in paw edema volume when compared to control and standard drugs. The Based on phytochemical screening, we have concluded that the both test I and test II have anti-diarrhoeal activity in castor oil method in rats.

**REFERENCES**