PROTECTIVE EFFECT OF METHANOLIC EXTRACT OF PSIDIIUM guajava LEAVES ON ACUTE AND CHRONIC INFLAMMATION IN RATS

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ABSTRACT

Objective: The aim of the present study was to evaluate the therapeutic efficacy of methanolic extract of P. guajava (MEPG) leaves on acute and chronic inflammation in rats using carrageenan-induced paw edema and cotton pellet induced granuloma models respectively. Methods: a) Acute inflammation (Carrageenan induced rat paw oedema): Adult male and female Albino rats were divided into four groups where Group-I, II,III and IV served as control, test groups (MEPG at a dose of 100 and 300 mg/kg p.o) and standard (diclofenac (10 mg/kg, i.p)) respectively. All the rats were treated with 1 % (w/v) of carrageenan in the right hind paw. The paw volume was measured at frequent intervals and the percentage of inhibition was calculated. b) Chronic inflammation (Cotton pellet implantation): Sterile cotton pellets (10 mg) were implanted s.c. in rats under light ether anaesthesia. Adult male and female Albino rats were divided into four groups where Group-I, II, III and IV served as control, test groups (MEPG at a dose of 100 and 300 mg/kg p.o) and standard (phenylbutazone (100 mg/kg)) respectively. At the end of study cotton pellets along with the granulomatous tissue were weighed and percentage increase in weight of cotton pellet was calculated. Results: In this study, treatment with MEPG significantly decreased the paw edema and weight of the cotton pellet with granuloma in rats compared to control group. Conclusion: MEPG was found to exhibit protective effect in both the acute and chronic models of inflammation.

KEYWORDS: Inflammation, Psidium guajava leaves, Anti inflammatory agents, carrageenan-induced paw edema, cotton pellet induced granuloma, MEPG.
INTRODUCTION

Inflammation is the reaction of living tissues to injury, infection or irritation. It is the response to injury of cells and body tissues through different factors such as infections, chemicals, thermal, and mechanical injuries. Anti-inflammatory refers to the property of a substance or treatment that reduces inflammation or swelling.\[1\] The drugs which are used presently for the management of pain and inflammatory conditions are either narcotics or non-narcotics (NSAIDs), and have known toxic and lethal effects. About 34-46% of the users of NSAIDs usually sustain some gastrointestinal damage due to the inhibition of the protective cyclo-oxygenase enzyme in gastric mucosa. It is therefore, essential that efforts be made to introduce new medicinal plants, to develop cheaper, effective and safe analgesic and anti-inflammatory drugs.\[2\]

Psidium guajava L. is a small medicinal tree that is native to South America. It is popularly known as guava (family Myrtaceae) and has been used traditionally as a medicinal plant throughout the world for a number of ailments. In traditional medicine, this plant had been found to exhibit hepatoprotective,\[3\] antioxidant,\[4\] anti-inflammatory,\[5\] antinociceptive,\[5,6\] antimicrobial,\[7,8,9\] antigenotoxic, anti-diarrhoeal,\[10\] cytotoxic,\[11\] antidiabetic,\[12\] anti-anaphylactic,\[13\] antitussive\[14\] activities.

The budding leaves of \textit{P. guajava} contained huge amounts of soluble polyphenolics, gallic acid, catechin, epicatechin, quercetin, and rutin. The leaf oil of Psidium guajava contained a mixture of sesquiterpene -caryophyllene (18.3\%) as the \(\beta\)-hydrocarbons (54.9\%) and oxygenated sesquiterpenes (20.9\%) with -cadinol (3.6\%), (E)-nerolidol\(\alpha\)-ol (6.9\%), \(\alpha\)principal sesquiterpene hydrocarbon and selin-11-en-4 (3.2\%) as the main oxygenated sesquiterpenes.\[6\] Hence the present study was undertaken to evaluate the therapeutic efficacy of methanolic extract of \textit{P. guajava} leaves as anti-inflammatory agent.

MATERIALS AND METHODS

The mature leaves of \textit{P. guajava} were collected from local area and was authenticated by Dr L. Rasingam, Scientist In-charge, Botanical Survey of India, Deccan Regional Centre, Attapur, Hyderabad, Telangana. The leaves obtained from \textit{P. guajava} plant were shade dried in order to prevent the loss of active constituents for about a week. Drying is done with occasional shifting in order to keep the moisture content as low as possible which helps to
reduce the microbial infestation. The dried leaves were finely pulverized by mechanical grinder and stored in air tight containers in order to prevent the moisture.

**Preparation of plant extract**
The dried powdered material (350 g) obtained was further extracted with methanol in soxhlet apparatus in sufficient volume for 18 hrs. The solvent was distilled off in reduced pressure and the percentage extractive value was 20.27% w/w and then stored in sealed (air tight) glass bottles at 4°C for further experimental work. The preliminary phytochemical analysis was performed for the extract to identify the phytoconstituents present in it.

**Drugs and chemicals**
Methanol, Diclofenac, Phenyl butazone, Ether and carrageenan were procured from Sai enterprises (Hyderabad, India). All chemicals and reagents were of analytical grade obtained commercially.

**Experimental animals**
Adult Wistar rats of both sex weighing 200-250 g were procured from registered breeders (Mahaveer Enterprises, Hyderabad, India) and maintained under standard laboratory conditions (temperature 25± 2°C with dark and light circle 14/10 h). They were allowed free access to standard dry pellet diet and water ad libitum. The rats were acclimatized to laboratory condition for 10 days before commencement of the experiment. All experimental procedures were reviewed and approved by the Institutional Animal Ethics Committee (Reg. no. 1684/Po/Re/s/13/ CPCSEA).

**Anti-inflammatory evaluation**
Acute inflammation (Carrageen induced rat paw oedema)\(^{15}\)
The overnight fasted rats were divided into four groups (n = 6). The first group (which served as control) received normal saline (5 ml/kg body wt.,p.o.). The second and third groups received the methanol extracts at the doses of 100 and 300 mg/kg b.w., p.o. respectively. The fourth group received diclofenac (10 mg/kg body wt., i.p.). After 30 mins, acute inflammation was produced by the subplantar administration of 0.1 ml of 1 % (w/v) of freshly prepared suspension of carrageenan in the right hind paw of each rat. The paw volume was measured at 0, 15, 30, 60, 120 min after carrageenan challenge by using a
plethysmometer (Ugo Basile, Italy). The difference between the two readings was taken as the volume of edema and the percentage of inhibition was calculated with respect to control and expressed the percentage protection of inflammation by using the following formula:

\[ \% \text{ Protection} = \frac{(\text{Control mean} - \text{Treated mean})}{\text{Control mean}} \times 100\% \]

**Chronic inflammation (Cotton pellet implantation)**[15]

Sterile cotton pellets (10 mg) were implanted s.c. in rats under light ether anesthesia. The animals were treated with two different doses of (100 and 300 mg/kg; s.c.) of methanolic extract of P. guajava for seven days. On the eighth day the animals were sacrificed and the cotton pellets along with the granulomatous tissue were removed, dried at 50°C for 12 h. and weighed. The increase in weight over the initial weight was recorded. A separate group of animals was treated with phenylbutazone (100 mg/kg).

**Statistical analysis**

The data are represented as mean ± standard error of mean (SEM). Degree of significance was assessed by Student’s ‘t’- test. P values less than 0.001 (P<0.001) were considered as statistically significant.

**RESULTS AND DISCUSSION**

Preliminary phytochemical studies showed the presence of steroids in the petroleum ether extract; triterpenoids, alkaloids and phenolic compounds in the ethyl acetate extract; and steroids, alkaloids, phenolic compounds, glycosides and carbohydrates in the methanol extract. Polyphenolic compounds are known to exhibit several important biological activities including anti-inflammatory properties. The polyphenols content may be responsible for its anti-inflammatory action against carrageenan-induced acute inflammation in Wistar rats.

Acute inflammation is produced when water and plasma increases in tissues during arachidonic acid metabolism via cyclooxygenase and lipo-oxygenase enzyme pathways. It has two phases: 1st phase (begins immediately after Carrageenan injection and lasts for 1 hr) is characterized by release of histamine and serotonin; and 2nd phase (begins after 1 hr and lasts for 4 hr) is characterized by bradykinin release by prostaglandin mediator pathways. Irritants or phlogistic agents like carrageenan, formalin, bradykinin, histamine, serotonin etc., when injected into the dorsum of the foot of the rats they produce acute paw edema within a few minutes of injection. Carrageenan induced rat paw oedema has been most commonly used as an ideal experimental animal model for acute inflammation. In this study, treatment
with MEPG significantly decreased the paw edema in rats and the protective effect was found to be more in 300 mg/kg treated group of MEPG. (Table 1).

Cotton pellet induced granuloma has been most commonly used as an ideal experimental animal model for chronic inflammation. A dose dependent reduction in the weight of the cotton pellets was observed after MEPG treatment. The protective effect was found to be more in 300 mg/kg treated group of MEPG, also it was found to be comparable to that of phenylbutazone. (Table 2).

**Table 1: Anti-inflammatory activity of MEPG on Carrageenan-induced paw edema in rats.**

<table>
<thead>
<tr>
<th>Groups</th>
<th>Dose (per kg)</th>
<th>Paw volume after drug/ extract administration (ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>0 min</td>
</tr>
<tr>
<td>Saline</td>
<td>0.01 ml</td>
<td>0.55±0.02</td>
</tr>
<tr>
<td>Diclofenac</td>
<td>10 mg</td>
<td>0.45±0.02</td>
</tr>
<tr>
<td>MEPG</td>
<td>100 mg</td>
<td>0.35±0.028</td>
</tr>
<tr>
<td>MEPG</td>
<td>300 mg</td>
<td>0.375±0.025</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SEM (n=5), *p<0.001 denotes significance with respect to the control group using student t-test.

**Table 2: Anti-inflammatory activity of MEPG on Cotton pellet-induced granuloma in rats.**

<table>
<thead>
<tr>
<th>Groups</th>
<th>Dose (per kg)</th>
<th>Wet weight of cotton pellet (in mg)</th>
<th>Dry weight of cotton pellet (in mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Mean ± SEM</td>
<td>% Inhibition</td>
</tr>
<tr>
<td>Saline</td>
<td>0.01 ml</td>
<td>112.88±2.6</td>
<td>-</td>
</tr>
<tr>
<td>Diclofenac</td>
<td>10 mg</td>
<td>57.18±1.1*</td>
<td>49.34</td>
</tr>
<tr>
<td>MEPG</td>
<td>100 mg</td>
<td>83.42±2.0*</td>
<td>26.098</td>
</tr>
<tr>
<td>MEPG</td>
<td>300 mg</td>
<td>61.91±1.5*</td>
<td>45.15</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SEM (n=5), *p<0.001 denotes significance with respect to the control group using student t-test.

**CONCLUSION**

MEPG was found to exhibit protective effect in both the models at doses 100 and 300 mg/kg which can be assessed by decrease in the paw edema and weight of the cotton pellet with granuloma. Further studies need to be carried out for isolation of bioactive principles and ascertain their efficacy.
Conflicts of interest
The authors declare that there are no conflicts of interest.

REFERENCES


