AN EXPERIMENTAL EVALUATION OF ANTI-INFLAMMATORY ACTIVITY OF MORINGA OLEIFERA SEEDS

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ABSTRACT

Objective: To evaluate the anti-inflammatory activity of Moringa oleifera seeds in experimentally induced acute and sub acute models of inflammation in rats.

Material and methods: Albino wistar rats of either sex weighing 150-200g were used. Ethanolic and aqueous extracts of Moringa oleifera seeds (EEMO and AEMO) were prepared with the help of Soxhlet’s apparatus. The anti-inflammatory activity was studied using carrageenan induced paw edema method and cotton pellet granuloma method. Histopathological examination of the paw biopsy was done by H&E stain. Statistical analysis was performed using one-way analysis of variance (ANOVA) followed by post hoc dunnett’s test. P < 0.05 was considered statistically significant.

Results: In Carrageenan induced paw edema method, both EEMO and AEMO treated groups demonstrated dose dependent decrease in paw edema compared to control group. In Cotton pellet induced granuloma method all the EEMO and AEMO treated groups demonstrated significant dose dependent decrease in granuloma formation when compared to control.

Conclusion: Thus it can be concluded from our study that the both ethanolic and aqueous extracts of Moringa oleifera seeds possesses anti-inflammatory activity.

Keywords: Moringa oleifera, Carrageenan induced paw edema, Cotton pellet granuloma, Anti-inflammatory.

INTRODUCTION

Moringa oleifera Lam. commonly known as the drumstick tree or the horseradish tree is one of the most widely cultivated and best known of the thirteen species of the family Moringaceae [1-2]. It is grown throughout the tropics and tropics of Africa and Asia [3]. Moringa oleifera is known as a ‘Miracle tree’ as almost every part of it is useful for human. It is often attributed as “natural nutrition for the tropics” for its high nutritional value [4]. The leaves, immature pods and flowers are used as a nutritious vegetable in various Asian countries, particularly in India. The seeds and other parts of Moringa oleifera have long been used in traditional medicine for their medicinal values [5]. The whole plant is reported to have antimicrobial activity and various active principles like pterygospermin and 4-α-L-rhamnosylxy benzyl isothiocyanate have been isolated which have powerful antibacterial and antifungal activity[6-8]. The leaves are reported to have anti-inflammatory, diuretic, antispasmodic and hypotensive activity [5, 9]. The roots are reported to have antispasmodic, hepatoprotective, antiinflammatory and anticonvulsant activity [10-11]. The pods are reported to have hypoglycemic and hypotensive effect [12-13]. The seeds are reported to have antiarthritic, antirheumatic, and antioxidant activities [14-16]. However there are very few studies reporting the anti-inflammatory activity of Moringa oleifera seeds. Hence this study was conducted for the scientific evaluation of anti-inflammatory activity of Moringa oleifera seeds using in vivo acute and subacute models of inflammation.

MATERIAL AND METHODS

Plant Material and Extraction

The pods of Moringa oleifera were collected from University campus and seeds were separated from them and shade dried. The seeds were identified and authenticated by Prof. S. H. Afaq, Department of Pharmacognosy, Imlul Advia, A.K. Tibbyha College, A.M.U. and voucher specimen was submitted (SC-01 30/11). Seeds were powdered with the help of a mechanical grinder and 100 gm of seed powder was extracted separately with 300ml of distilled water and ethanol for aqueous and ethanolic extract respectively with the help of Soxhlet’s apparatus. The extracts were collected in Petri dishes and evaporated till dryness at 40°C in an incubator. Then the extracts were sealed with aluminium foil and stored at 4°C for further experimental work.

Drugs and Chemicals used

Aspirin (Reckitt Benckiser, India). Propylene glycol (BDH, Mumbai) and Carageenan (Sigma Chemicals, USA) were used in the study. The other chemicals used were of analytical grade manufactured by Merck Laboratory (Mumbai, India).

Animals

Albino wistar rats of either sex weighing (150- 200g) were procured from the Central Animal House, JNMC, Aligarh Muslim University. They were housed in polypropylene cages at ambient temperature (25 ± 2 °C), relative humidity (55 ± 5%) and 12-hr light-dark cycle. Animals had free access to standard pellet diet and water ad libitum. The study protocol was approved by the Institutional Animal Ethics Committee (IAEC) and all animal experiments were carried out as per the rules and regulations of IAEC & CPCSEA (Committee for the Purpose of Control and Supervision of Experiments on Animals).

Experimental design

Animals were divided into eight groups of six animals each. Group 1 served as control and was given propylene glycol 2ml/kg, group 2 served as standard and was given aspirin (100mg/kg), groups 3, 4 and 5 were given ethanolic extract of Moringa oleifera (EEMO) seeds at the dose of 50, 100 and 200mg/kg respectively and groups 6, 7 and 8 were given aqueous extract of Moringa oleifera (AEMO) seeds at the dose of 250, 500 and 750mg/kg respectively.

Carrageenan induced paw edema method [17]

This is one of the most commonly employed method for the screening of acute inflammation. All the groups were treated with single dose of respective drug and after 1 hour of the administration of the drugs, acute inflammation was produced by sub plantar injection of 0.1 ml of freshly prepared 1% suspension of carrageenan in normal saline in the right hind paw of the rats. The paw was marked at the level of the lateral malleolus and was immersed every time up to this mark. The paw volumes were measured at 0h, 1h, 2h,
and 3h after the carrageenan injection using digital plethysmometer (Orchid scientific, India).

The percentage inhibition of paw edema at each time interval was calculated by using the following formula:

\[
\text{Percentage inhibition} = \left(\frac{V_U - V_T}{V_C - V_U}\right) \times 100
\]

Where

\[
V_U = \text{Paw volume of test/control group at 0 hr}
\]

\[
V_T = \text{Paw volume of test/control group at that particular time interval}
\]

\[
V_C = \text{Paw volume at different time interval (in ml)}
\]

\[
WT = \text{Weight of the cotton pellets in drug treated animals.}
\]

\[
WC = \text{Weight of the cotton pellets in control animals.}
\]

\[
\text{Statistical analysis}
\]

All the values are expressed as Mean ± SEM (n=6). Statistical significance was calculated by one way ANOVA followed by post hoc Dunnett's multiple comparison test. p < 0.05 was considered to be statistically significant.

\[
\text{RESULTS}
\]

In Carrageenan induced paw edema method, aspirin (100 mg/kg) significantly decreased the paw edema at 1h (p<0.05) and 3h (p<0.001) after carrageenan injection compared to control and percentage inhibition of edema was 55.55% and 81.66% at 1h and 3h respectively. Both EEMO and AEMO treated groups demonstrated dose dependent decrease in paw edema compared to control group. EEMO at the 200mg/kg showed significant reduction of paw edema at 1h (p<0.05) and 3h (p<0.001) after carrageenan injection compared to control and percentage inhibition of edema was 52.77% and 70.65% at 1h and 3h respectively whereas EEMO at 50 mg/kg (p<0.05) and 100 mg/kg (p<0.05) doses showed significant reduction of paw edema only at 3h after carrageenan injection and percentage inhibition of edema was 50% and 51.62% for 50 and 100 mg/kg respectively. AEMO at 250 mg/kg (p<0.05), 500 mg/kg (p<0.05) and 750 mg/kg (p<0.05) doses also showed significant reduction of paw edema only at 3h after carrageenan injection and percentage inhibition of edema was 21.60%, 33.33% and 40% respectively (Table 1 and 2).

\[
\text{Histopathological examination}
\]

Histopathological examination of the paws in control group revealed extensive edema which appears as separation of layers of epidermis, dermis and collagen fibres with extensive infiltration of neutrophils in control group (Figure 1A). Group treated with Aspirin 100mg/kg showed normal epidermal morphology, mild dermal edema with few inflammatory cells (Figure 1B). Whereas groups treated with EEMO and AEMO showed dose dependent decrease in edema and cellular infiltration compared to control group (Figure 1C & D).

Table 1: Effect of Ethanolic extract of *Moringa oleifera* seeds on Carrageenan induced paw edema

<table>
<thead>
<tr>
<th>Groups</th>
<th>Paw volume at different time interval (in ml)</th>
<th>% Inhibition of edema at 3h</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0h</td>
<td>1h</td>
</tr>
<tr>
<td>Control</td>
<td>0.86±0.03</td>
<td>1.22±0.02</td>
</tr>
<tr>
<td>Aspirin (100mg/kg)</td>
<td>0.87±0.04</td>
<td>1.05±0.04*</td>
</tr>
<tr>
<td>EEMO (50mg/kg)</td>
<td>0.78±0.05</td>
<td>1.08±0.05</td>
</tr>
<tr>
<td>EEMO (100mg/kg)</td>
<td>0.88±0.03</td>
<td>1.13±0.03</td>
</tr>
<tr>
<td>EEMO (200mg/kg)</td>
<td>0.83±0.05</td>
<td>1.00±0.05*</td>
</tr>
</tbody>
</table>

EEMO: Ethanolic extract of *Moringa oleifera* seeds, Values are given in mean ± SEM (n=6), *indicates p<0.05, ** indicates p<0.001 when compared to the control group.

Table 2: Effect of Aqueous extract of *Moringa oleifera* seeds on Carrageenan induced paw edema

<table>
<thead>
<tr>
<th>Groups</th>
<th>Paw volume at different time interval (in ml)</th>
<th>% Inhibition of edema at 3h</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0h</td>
<td>1h</td>
</tr>
<tr>
<td>CONTROL</td>
<td>0.96±0.03</td>
<td>1.22±0.02</td>
</tr>
<tr>
<td>ASPIRIN (100mg/kg)</td>
<td>0.87±0.04</td>
<td>1.03±0.04*</td>
</tr>
<tr>
<td>AEMO (250mg/kg)</td>
<td>0.77±0.03</td>
<td>1.09±0.03</td>
</tr>
<tr>
<td>AEMO (500mg/kg)</td>
<td>0.82±0.06</td>
<td>1.11±0.06</td>
</tr>
<tr>
<td>AEMO (750mg/kg)</td>
<td>0.82±0.06</td>
<td>1.11±0.06</td>
</tr>
</tbody>
</table>

AEMO: Aqueous extract of *Moringa oleifera* seeds, Values are given in mean ± SEM (n=6), *indicates p<0.05, ** indicates p<0.001 when compared to the control group.
In Cotton pellet induced granuloma method, Aspirin 100 mg/kg significantly inhibited the granuloma formation by 60.24% (p<0.001) when compared to control. Whereas EEMO significantly inhibited the granuloma formation by 18.22% (p=0.05), 35.86% (p<0.001), and 50.16% (p<0.001) compared to control group, at the doses of 50, 100 and 200mg respectively. AEMO in doses 250, 500 and 750 mg/kg significantly (p<0.001) decreased granuloma formation by 21.37%, 32.03% and 62.33% respectively when compared to control (Figure 2 and 3).

**Fig. 1:** Histological examination of paw sections after 3h of carrageenan injection. (A) Control group (B) Aspirin Group, (C) Ethanolic extract 200mg/kg and (D) Aqueous extract 750mg/kg

**Fig. 2:** Effect of Ethanolic extracts of *Moringa oleifera* seeds on granulation tissue formation in Cotton pellet granuloma method.

EEMO: Ethanolic extract of *Moringa oleifera* seeds, Values are given in mean ± SEM (n=6).

*indicates p<0.05, ** indicates p<0.001 when compared to the control group, using Values given on top of each bar represents percent age inhibition of granuloma formation compared to control group.
DISCUSSION

The most widely used primary test for screening of anti-inflammatory agents is carrageenan induced edema in the rat hind paw. The development of edema in the paw of the rats after injection of carrageenan is a biphasic event [19]. The initial phase of inflammation which is observed during the first hour is attributed to a release of histamine and serotonin and the second phase is due to a release of prostaglandin like substances. In the present study, ethanolic extract Moringa oleifera seeds showed significant reduction of edema in both the phases of inflammation but maximum reduction was observed in the second phase of inflammation (70.65%) which was comparable with aspirin (81.66%). Aqueous extract showed significant (p<0.01) reduction of edema in all the phases of inflammation. The effect of Moringa oleifera seeds lasted for 3 hours parallel to that of aspirin. Histopathological examination of the paws of EEMO and AEMO treated groups further confirmed above results as evidenced by decrease in edema and cellular infiltration compared to control group (Fig. C & D).

The inhibitory effect of Moringa oleifera seeds on first phase inflammation could be due to inhibition of the serotonin and histamine mediated effect and on second phase could be due to the inhibition of the prostaglandin synthesis as suggested by the mechanism of edema formation by carrageenan [20]. This anti-inflammatory activity can be attributed to various phytochemicals like alkaloids, flavonoids, ste rols, glycosides, tannins and terpenoids reported in Moringa oleifera seeds.[12] Sterols like β-sitosterol and flavonoids present in Moringa oleifera seeds are known to target prostaglandin synthesis as involved in acute inflammation [21-22]. Moringa oleifera seeds are also rich in glycosides like benzyl isothiocyanate which have been reported to possess anti-inflammatory activity by decreasing various mediators of inflammation like prostaglandins, NO, IL-6, IL-1β and TNF-α [23]. These inflammatory mediators are responsible for edema formation by increasing vascular permeability and recruitment of various inflammatory cells. The cotton pellet granuloma method has been widely employed to assess the transudative, exudative and proliferative components of sub acute inflammation. The increase in dry weight of the granuloma measures the proliferative phase due to monocyte infiltration and fibroblast proliferation that take place in chronic inflammation [24]. In this study, the ethanolic and aqueous extracts of Moringa oleifera seeds significantly decreased the dry weight of the granuloma when compared to the control group. The percentage inhibition for the ethanolic and aqueous extracts was highest at the doses of 200mg/kg and 750mg/kg i.e. 50.16% and 62.32% respectively (p<0.001) (Figure 2 and 3) which was comparable to that of aspirin (60.24%) and the effect of AEMO 750mg/kg was better than standard drug aspirin.

This anti-inflammatory action may be due to the ability of Moringa oleifera seeds in reducing the number of fibroblasts and synthesis of collagen and mucopolysaccharide, which are natural proliferative agents of granulation tissue formation. The inhibition of production of proinflammatory cytokines, such as IL-1, IL-6 and TNF-α which are powerful chemotactic agents to macrophages and fibroblasts by Moringa oleifera seeds may be responsible for antiinflammatory effect [14]. The anti-inflammatory action may also be due to antioxidants like flavonoids, tocopherols and vitamin C present in Moringa oleifera seeds which decrease oxidative stress generated during inflammation [22].

CONCLUSION

Thus it can be concluded from our study that the both ethanolic and aqueous extracts of Moringa oleifera seeds possesses anti-inflammatory activity. Further more extensive studies are required to elucidate the exact mechanisms and active principle responsible for anti-inflammatory activity of Moringa oleifera seeds so that new potent and safe anti-inflammatory agents can be developed from it.

REFERENCES


