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Original Research Article

An experimental study to evaluate the anti-inflammatory effect of moringa oleifera leaves in animal models

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ABSTRACT

Background: Inflammatory diseases are a major cause of morbidity and disability of work force throughout the world. The treatment of inflammation with standard steroidal and non-steroidal anti-inflammatory drugs shares the risk of toxicity on various organ systems. Moringa oleifera, an herbal plant has been claimed to be effective in the treatment of various types of inflammatory conditions. However, there is lack of scientific studies to ratify these claims. Therefore, the present study was undertaken to explore the anti-inflammatory activity of aqueous extract of leaves of Moringa oleifera (AEMO) in experimentally induced inflammation in albino rats.

Methods: The study was commenced after obtaining approval from Institutional Animal Ethical Committee using AEMO leaves in Albino wistar rats (150-200 gm) of either sex. The anti-inflammatory activity was evaluated using carrageenan induced paw edema model, cotton pellet induced granuloma method and formaldehyde induced paw edema method. For each set of experiment, animals were divided in three groups of six animals each. In each experiment, 1st group was given normal saline (5 ml/kg/day), 2nd group was given standard anti-inflammatory drug dexamethasone (0.5 mg/kg/day) and 3rd group was given Moringa oleifera (200 mg/kg/day).

Results: Aqueous extract of Moringa oleifera leaves at dose of 200 mg/kg, p.o. exhibited the significant anti-inflammatory effect in all the models used in this study.

Conclusions: It can be concluded from our study that aqueous extracts of Moringa oleifera leaves possess anti-inflammatory activity.

Keywords: Anti-inflammatory, Albino rats, Moringa oleifera, Paw edema

INTRODUCTION

Inflammation is the body‘s attempt for self-protection, with the aim of removing harmful stimuli, including damaged cells, irritants or pathogens and to begin the healing process. The inflammatory process protects our body from diseases by releasing cells and mediators that combats foreign substances and prevent infection.[1] However, sustained, excessive or inappropriate inflammation is the cause of numerous diseases including rheumatoid arthritis, psoriasis and inflammatory bowel disease.[2]

Most of the clinically important medicines for treatment of inflammatory conditions are mainly steroidal or non-steroidal anti-inflammatory chemical therapeutic agents. Though these agents have potent anti-inflammatory activity, their long term administration is required for management of chronic inflammatory conditions. Furthermore, these drugs incorporate risk of various and severe adverse effects. Therefore, naturally originated agents with very little side-effects are needed to substitute chemical therapeutic. The ideal drug would be one that enhances the salutary effects of inflammation, yet controls it's destructive and harmful complications.[3] Therefore, agents obtained from natural sources which have fewer side-effects are desired to substitute currently available anti-inflammatory agents in allopathic medicine. These therapeutic agents need to be scientifically ratified in order to validate their present day status as anti-inflammatory agents. Ayurvedic stream offers some drugs that have a significant potential as anti-inflammatory agents. Among them *Moringa oleifera*, which is easily available throughout the country, have

demonstrated anti-inflammatory potential. However, the studies assessing the anti-inflammatory activity of this drug are inconclusive; hence the present study was desired to evaluate the anti-inflammatory action of aqueous extracts of leaves of *Moringa oleifera*.

The plant /tree *M. Oliefera* is known as sigru in Sanskrit and as Soanjan in Hindi.[4] *Moringa oleifera* 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.[5] It is grown throughout the subtropics and tropics of Africa and Asia.[6] *Moringa oleifera* is known as a 'Miracle tree' as almost every part of it is useful for humans. It is often attributed as "natural nutrition for the tropics" for its high nutritional value.[7] The leaves, immature pods and flowers are used as a nutritive 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.[8] The leaves are reported to have anti-inflammatory, diuretic, antispasmodic and hypotensive activity.[9] The roots are reported to have antispasmodic, hepatoprotective, anthelmintic and anticonvulsant activity.[10] The pods are reported to have hypolipidemic and hypotensive effect.[11] The seeds are reported to have antiarthritic, antitumour, and antioxidant activities.[12,13] Aqueous extract of leaves show the presence of amino acids, alpha and beta - carotene, sterols, terpenes, saponins, tannins, carbohydrates, glycosides, alkalois and flavonoids.[14]

METHODS

The study was commenced after obtaining approval from Institutional Animal Ethical Committee (Approval letter No. IAEC/2015/02, dated- 14/05/2015) of Lala Lajpat Rai Memorial Medical college, Meerut, India, registered under CPCSEA India (Registration No. 819/04/ac/CPCSEA).

Animals

Adult wistar albino rats of either sex weighing 150-200 gm were obtained from rat rearing unit of central animal house of the institute. The selected rats were housed in standard polypropylene cages under controlled conditions of temperature (24±2°C) and 12 hour light/dark cycle. The animals had free access to standard rat pellet diet (Vet care India Ltd.) and tap water ad libitum. After one week of acclimatization, the animals were considered suitable for study.

Preparation of plant extract

Fresh leaves of *Moringa oleifera* were shade dried and powdered with the help of a mechanical grinder and 100gm of leaves powder was extracted separately with 300ml of distilled water to obtain aqueous extract with the help of Soxhlet's apparatus. The extract was collected in Petri dishes and evaporated till dry at 40ºC in an incubator. Then the extract was sealed with aluminium foil and stored at 4ºC for further experimental work.[15] The extract was used in aqueous form in the concentration of 125mg/ml.

Drugs and chemicals

Carrageenan (Grace lifetech pvt. ltd.), Dexamethasone (Cadila healthcare limited), Formalin (Balaji formalin pvt. ltd.), Ampicillin (Cadila healthcare limited) were used in this study.

Experimental design

For evaluation of anti-inflammatory activity, total 3 methods of inflammation were used. Animal were divided into three groups of six animals each. Group 1 served as control and was given normal saline (5 ml/kg), group 2 served as standard and was given dexamethasone (0.5 mg/kg), group 3 was given aqueous extract of Moringa oleifera leaves (200 mg/kg). Total 54 animals were utilised for this study.

Carrageenan induced paw edema model

This is one of the most commonly employed methods for the screening of acute inflammation. A 1% w/v suspension of carrageenan was prepared freshly in normal saline and injected intramuscular into sub plantar region of left hind paw (0.1 ml) of adult wistar rats. All the groups were treated with single dose of respective drugs. Animals, in the control group received only normal saline. Test drugs and selected standard drug were administered orally as per body weight, one hour before carrageenan challenge. A mark was made at the ankle joint of each animal. Paw volume upto ankle joint was measured in drug treated and untreated groups just before and 3 hours after carrageenan challenge using a plethysmograph.[16,17]

Edema was measured and percentage reduction in edema was calculated using the following formula:[18]

$$\% \text{ reduction in edema} = \frac{\text{Mean edema (control)} - \text{Mean edema (drug treated)}}{\text{Mean edema (control)}} \times 100$$

Cotton pellet induced granuloma method

This method was used for the evaluation of sub acute inflammation. Sterile cotton pellets, each of 10 mg, impregnated with 0.4 ml of aqueous solution of ampicillin were used. Under anesthesia, one pellet was inserted subcutaneously through a skin incision in the nape of neck of each animal. Anesthesia was induced using ketamine in the dose of 60 mg/kg, i.p. and Xylazine in the dose of 8 mg/kg, intraperitoneally.[19] The incision were sutured by silk 2.0 sutures and wound was sealed with betadine solution to prevent contamination and animals were rehabilitated. Drug treatment was started 2

hours after cotton pellet implantation and continued for 5 consecutive days. On day 6, the pellets were dissected out, dried for 24 hours at 60°C and dry weight was determined. The weight of granulomas formed was calculated by subtracting initial weight from the final dry weight of cotton pellets and % protection by the drug was calculated.[20]

Formaldehyde induced Paw edema method

Formaldehyde induced paw edema is the model used for chronic inflammation. In this, 0.l ml of 2% solution of formaldehyde was injected intramuscularly into the subplantar tissue of the right hind paw of adult wistar rats on day 1 and day 3 to induce paw oedema and the paw volume was measured daily by plethysmometric method for 10 days in the respective groups as follows:[21]

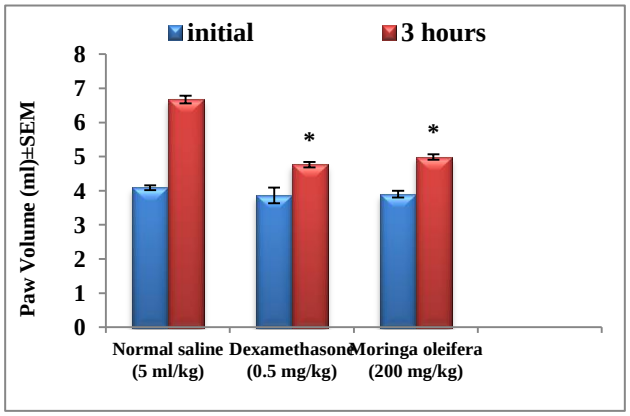
(1). Group 1- Control group was given 0.9% NaCl solution in a single oral dose of 5 ml/kg b.w. for 10 consecutive days.
(2). Group II - This group was treated with Dexamethasone in a single oral dose of 0.5 mg/kg b.w. for 10 consecutive days.
(3). Group III- This group was administered with aqueous extract of *Moringa oleifera* leaves in a single oral dose of 200 mg/kg b.w. for 10 consecutive days.

Statistical analysis

The data obtained was expressed as mean ± SEM. Statistically significant differences between groups were calculated by the application of one way analysis of variance (ANOVA) followed by LSD test. p values were calculated referring to appropriate tables. p values less than 0.05 ($p<0.05$) were considered as statistically significant.

RESULTS

Carrageenan induced rat paw edema



*p<0.05 significant in comparison to control (LSD test)

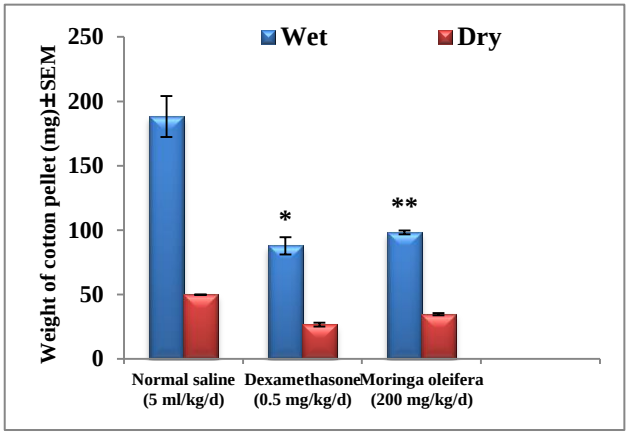
Figure 1: Effect of aqueous extract of *Moringa oleifera* leaves on carrageenan induced hind paw edema (n=6).

Animals pre-treated with dexamethasone exhibited significant reduction in rat paw edema after 3 hour of dexamethasone administration, with the percentage of inhibition being 28.64 compared to the control group.

Aqueous extract of leaves of *Moringa oleifera* showed significant anti-inflammatory effect by reducing rat paw edema by 25.19% at 3 hour after test drug administration when compared to control group (Figure 1).

Cotton pellet induced granuloma method

The standard drug dexamethasone exhibited maximum anti-inflammatory activity by reducing the wet weight of cotton pellets from 188.25±16mg to 87.25±6.75mg (54.45 %) and dry weight from 49.87±0.13 mg to 26.75±1.50mg (46.36%) when compared with control group (Normal saline treated). *Moringa oleifera* showed significant reduction in wet weight from 188.25±16 mg to 98.25±1.50 and dry weight of cotton pellets from 49.87±0.13 mg to 34.87±0.88 mg, the percentage of inhibition being 47.81% and 30.74% respectively (Figure 2).



*p <0.05 and **p<0.01 (significant in comparison to control).

Figure 2: Effect of Aqueous extract of *Moringa oleifera* leaves on granulation tissue formation in cotton pellet implantation method (n=6).

Formaldehyde induced paw edema method

There was significant inhibition of inflammation by dexamethasone throughout the duration of experiment. Dexamethasone produced maximum inhibition 58.71% on 10th day and this was significantly greater than the inhibition caused by *Moringa oleifera*.

Aqueous extract of leaves of *Moringa oleifera* significantly inhibited ($p<0.001$) paw edema, starting from the 5th day, compared to control. Maximum inhibition (41.48%) was observed on 9th day following treatment (Figure 3).

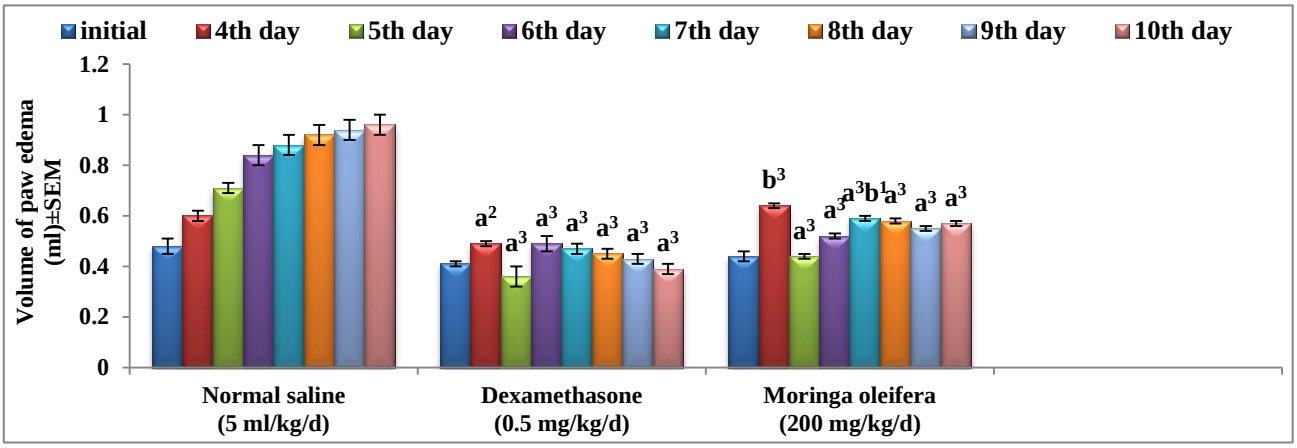


Figure 3: Effect of Aqueous extract of *Moringa oleifera* leaves on formaldehyde induced paw edema (n=6).

DISCUSSION

Carrageenan induced rat hind paw edema is an acute inflammatory model which involves sequential release of several mediators. In the initial phase (first 1.5 h) there is release of histamine and serotonin, the second phase is mediated by bradykinin lasting from 1.5 to 2.5 h and the third phase, mediated by prostaglandin, occurs from 2.5 to 6 h after carrageenan injection.[22] In the present study, the aqueous extract of *Moringa oleifera* leaves at a dose of 200 mg/kg significantly decreased the rat paw edema induced by carrageenan in all phases, suggesting that the possible mechanism of the anti-inflammatory action of *Moringa oleifera* could be due to inhibition of release of mediators in all phases.

Further verification of the anti-inflammatory activity and effect on the transudative and proliferative components of sub acute inflammation was done by using the cotton pellet induced granuloma model. The inflammatory responses have been divided into 3 phases: transudative, exudative and proliferative. The fluid absorbed by the pellet greatly influences the wet weight of the granuloma, and the dry weight correlates with the amount of granulomatous tissue formed.[23] In the present study, the aqueous extract of *Moringa oleifera* leaves at a dose of 200 mg/kg each significantly reduced cotton pellet induced granuloma formation in rats.The possible mechanism could be due to inhibiton of monocyte infiltration and fibroblast proliferation. Activated monocytes release a series of pro-inflammatory cytokines, inducing tumor necrosis factor-α (TNF-α) which facilitates inflammatory cell infiltration by promoting the adhesion of neutrophils and lymphocytes to the endothelial cell.[24] So, it can be construed that the anti-inflammatory effect might be due to active constituents (flavonoids, tannins, rhamnose, xylose, galactose, arabinose, galacturonic acid) that are present in the aqueous extract of *Moringa oleifera* leaves.[25] The anti-inflammatory effect of these active constituents have been reported.[26]

Formaldehyde induced paw edema is the model used for chronic inflammation. It closely resembles arthritis and is used to screen anti-arthritic and anti-inflammatory agents as injection of formaldehyde subcutaneously into hind paw produces localized inflammation and pain. The nociceptive effect of formalin is biphasic, an early neurogenic component followed by a later tissue mediated response.[27] Thus formalin-induced arthritis is a model used for the evaluation of an agent with probable anti-proliferative activity. In addition, it has been reported that formaldehyde injection produces edema and an increase in vascular permeability.[28] The aqueous extract of *Moringa oleifera* leaves showed statistically significant inhibition of paw edema in formaldehyde induced paw edema model in the dose (200 mg/kg, po) employed in the present study. The results obtained from this model suggest the usefulness of aqueous extract of *Moringa oleifera* leaves in the treatment of inflammation associated with chronic diseases like arthritis.

Although the results of present study have demonstrated the anti-inflammatory activity of *Moringa oleifera* yet, the exact mechanism of action of this plant needs further exploration. However, if the pure active principal of this plant could be isolated and evaluated, constituents of this plant could be used more rationally for treatment of different types of inflammations.

CONCLUSION

In the present investigation it could be concluded that Aqueous extract of *Moringa oleifera* leaves (200 mg/kg) have exhibited anti-inflammatory activity in all models for inflammation.

The data obtained from the present study have demonstrated that the aqueous extract of *Moringa oleifera* leaves might be helpful in preventing inflammatory conditions and is expected to serve as good bio-resource for generating a readily available herbal formulation that might be equally potent and cost effective than the conventional synthetic drugs. However,

the modes of anti-inflammatory actions of the studied extract are still obscure. For that, further study for detailed investigation of the mechanism of action of these extract is needed using various experimental animal models.

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