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Evaluation Of the Anti-Inflammatory Effect of *Moringa Oleifera* Leaves in Animal Models.

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ABSTRACT

Inflammatory diseases are a major cause of morbidity and disability throughout the world. Inflammation is a part of the complex biological response of body tissues to harmful stimuli. Cytokines and other growth factors activate the Mitogen Activated Protein Kinase (MAP) pathway ultimately resulting in inflammation. *Moringa oleifera*, an herbal plant has been claimed to be have significant anti-inflammatory property with less adverse effects. The study was commenced after obtaining approval from Institutional Animal Ethical Committee in Wistar rats (200-250 gm) of either sex. 18 adult albino rats were divided into 3 equal groups. 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). After 1 hour of drug administration, 0.1ml of 1% carrageenan was injected into the left hind paw of each animal and paw edema was measured after 1 & 3 hours. Paw edema was noticed more in control group and significantly reduced in test group comparable to standard group. The results were analysed statistically and p value was found to be statistically significant. Hence this study shows that *Moringa oleifera* has got significant anti-inflammatory activity in animals.

Keywords: Cytokines, *Moringa oleifera*, Inflammation, Edema.

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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].

Inflammation is a part of the complex biological response of body tissues to harmful stimuli, such as pathogens, damaged cells or irritants [3]. The cardinal sign of inflammation warmth, pain, swelling, and redness and hyperemia was described by Celsus 2,000 years ago [4]. Cytokines and chemokines promote the migration of neutrophils and macrophages to the site of inflammation [5, 6]. Cytokines and other growth factors activate the Mitogen Activated Protein Kinase (MAP) pathway ultimately resulting in inflammation [7].

Inflammation could be acute, sub-acute or chronic in nature. When the inflammation gets exaggerated and sustained without apparent benefit it produces severe adverse consequences, it should be intervened with proper anti-inflammatory drugs to prevent the complications. Currently many anti-inflammatory drugs are available of which commonly used ones include NSAID's & Corticosteroids. Chronic use of these drugs have their own gastrointestinal, renal and neurological adverse effects which limits the chronic usage [8]. Hence there is always a surge for a drug with minimal side effects and additional therapeutic advantages.

Therefore, agents obtained from natural sources which have fewer side-effects are desired to substitute currently available anti-inflammatory agents in allopathic medicine. Among them *Moringa oleifera*, which is easily available throughout the country, have demonstrated anti-inflammatory potential. *Moringa oleifera* is known as *Sigru* in Sanskrit and as *Soanjan* in Hindi [9]. *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 [10]. It is grown throughout the subtropics and tropics of Africa and Asia [11]. Aqueous extract of leaves shows the presence of amino acids, alpha and beta - carotene, sterols, terpenes, saponins, tannins, carbohydrates, glycosides, alkaloids and flavonoids. The leaves are reported to have anti-inflammatory, diuretic, antispasmodic and hypotensive activity [12]. Hence with this background this study was done to confirm the anti-inflammatory property of *Moringa oleifera* leaves in adult Wistar rats.

MATERIALS AND METHODS

This study was conducted at the Central animal house, Government Vellore Medical College, Vellore after obtaining Clearance from Institutional Animal Ethical Committee. 18 adult Wistar rats of weight 200 -250 gms of either sex was obtained from Central animal house, Government Vellore Medical College. All the animals were maintained under 12:12 hour light: dark cycle and were fed with standard laboratory chow and water ad libitum.

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 40°C in an incubator. Then the extract was sealed with aluminium foil and stored at 4°C for further experimental work. 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) were used in 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 subcutaneously into sub plantar region of left hind paw (0.1 ml) of adult Wistar rats. For evaluation of anti-inflammatory activity, Animals 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(test group) was given aqueous extract of *Moringa oleifera* leaves (200 mg/kg) one hour before carrageenan challenge. A mark was made at the ankle joint of each animal. Paw volume up to ankle joint was measured in drug treated and untreated groups 1 hour and 3 hours after carrageenan challenge using a plethysmograph(13, 14) Paw Edema was measured and percentage reduction in edema was calculated using the following formula(15)

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

RESULTS

Animals pre-treated with standard (Dexamethasone) exhibited significant reduction in rat paw edema after 3 hour of drug administration, with the percentage of inhibition being 42.6% compared to the control group.

Aqueous extract of leaves of *Moringa oleifera* (Test) showed significant anti-inflammatory effect by reducing rat paw edema by 39.3% at 3 hours after test drug administration when compared to control group (Table 1 & Figure 1).

Results were analysed statistically and showed significant paw volume reduction with p-value of <0.005 in test group when compared to control group which is statistically significant.

Table 1: Measurement Of Paw Edema (in ml)

Animals	Control		Test		Standard	
	1 HR	3 HR	1 HR	3 HR	1 HR	3 HR
1	3.0	6.1	3.0	3.7	3.0	3.5
2	3.2	6.0	3.4	3.6	3.1	3.4
3	3.0	6.3	3.0	3.8	3.2	3.6
4	3.4	6.2	3.3	3.6	3.4	3.5
5	3.2	6.1	3.2	3.7	3.1	3.6
6	3.0	6.0	3.0	3.8	3.0	3.5
Mean	3.1	6.1	3.1	3.7	3.1	3.5

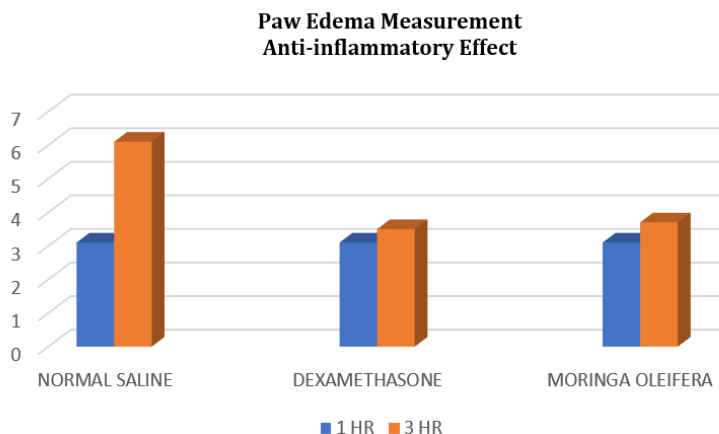


Figure 1: Measurement Of Paw Edema (in ml)

DISCUSSION

Carrageenan-induced rat paw edema test is the most sensitive and reproducible test for anti-inflammatory effects. The Carrageenan injection into rat paw produces an acute and local inflammatory response. It is associated with three distinct phases. The first phase is early mediated by mast cell degranulation and histamine and serotonin release (1 hr), the second phase (60 to 150 min) is characterized by bradykinin release and pain, and further eicosanoid production in the late phase (3-4hr) [16].

In this study, after 1hr of carrageen injection all the animal developed edema in paw and the mean paw volume is measured. Carrageen is a substance with polysaccharide it produces acute inflammatory edema after subcutaneous injection by releasing chemical mediators. Aqueous extract of leaves of *Moringa oleifera* (Test) showed significant anti-inflammatory effect by reducing rat paw edema by 39.3% at 3 hours after test drug administration when compared to control group with significant p value < 0.005. This proves that *Moringa oleifera* has anti-inflammatory property. This effect is comparable to Standard (Dexamethasone) which showed 42.6% reduction in rat paw edema

The possible mechanism could be due to inhibition of monocyte infiltration and fibroblast proliferation by *Moringa oleifera*. 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. So, it can be construed that the anti-inflammatory effect might be due to active constituents (flavonoids, tannins, rhamnase, xylose, galactose, arabinose, galacturonic acid) that are present in the aqueous extract of *Moringa oleifera* leaves [17]. The anti-inflammatory effect of these active constituents have been reported similarly in other studies [18].

CONCLUSION

We conclude that aqueous extract of *Moringa oleifera* leaves have exhibited significant anti-inflammatory activity by reducing paw edema in Wistar Rat Animal model. The data obtained from the present study might be helpful in treating 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.

The limitation of this study is being done on a small number of rats and needs to be done on more numbers for greater validity.

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