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TO EVALUATE THE ANTI-INFLAMMATORY ACTIVITY OF ETHANOLIC LEAF EXTRACT OF MORINGA OLEIFERA PLANT IN ALBINO WISTAR RATS

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ABSTRACT

Introduction: Moringa Oleifera is widely found in Asian subcontinent and it has been used as an analgesic and anti-inflammatory in Indian folk medicine. In this study we compared the antiinflammatory effects of Moringa Oleifera Ethanolic extracts in experimentally Carrageenan induced model of inflammation in rats. Material and methods: A total of 30 Male Albino Wistar sex weighing 150-200g (n= 30) were used. Ethanolic extracts of Moringa Oliefera Leaf [eemo] were prepared with the help of soxhlet's apparatus. The anti-inflammatory activity was studied using carrageenan induced paw edema method . 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, moringa olefera treated groups demonstrated dose dependent decrease in paw edema compared to control group. Conclusion: We can be conclude that the Ethanolic Extracts of Moringa Oleifera Leaf possesses anti-inflammatory activity.

KEYWORDS: Moringa Oleifera, Carrageenan, Anti-Inflammatory.

INTRODUCTION

Moringa olefera belongs to Moringaceae family there are 13 species out of which , *Moringa oleifera* indigenous to sub-Himalayan tracts of Northern India ^[1], among which *Moringa oleifera* has so far become the most used and studied.

Ayurvedic traditional medicine mentions that *Moringa* oleifera can prevent 300 diseases and its leaves have been exploited both for preventive and curative purposes. [2]. Moringa is among the species utilized by traditional Siddha healers. [3] Ancient Egyptians used *Moringa oleifera* oil for its cosmetic value and skin preparation. [4]; even the species became popular among Greeks and Romans, [5]. *Moringa oleifera* has been dubbed "miracle tree", or "natural gift", or "mother's best friend". In India, herbal drugs are an integral part of The Indian System of Medicine (Ayurveda) which is an ancient and mainstream system. [6]

Moringa is one such genus whose various species have not been explored fully despite the enormous reports having potentials such as: cardiac and circulatory stimulants; antitumor; antipyretic; antiepileptic; anti-inflammatory, diuretic antispasmodic; antiulcer; diuretic antihypertensive; cholesterol lowering; antioxidant; antidiabetic; antitumour, hepato-protective; antibacterial and antifungal activities. [7,8,9,10-11] These are also being used for treatment of different ailments in the indigenous

system of medicine. [12] . Some previous reports indicate that ethanolic extract of the leaves possesses significant antiinflammatory activities.

Therefore the present study is aimed to evaluate the antiinflammatory activity of ethanolic extract of moringa olefera plant in animal models using Albino Wistar Rats.

MATERIALS AND METHODS Plant Material and Extraction

Fresh leaves of *Moringa oleifera* of were collected from periphery of Aurangabad city and its identity was confirmed by Dept. of Botany, Maulana Azad College Aurangabad. leaves were dried in the shade inside the room for two days and later made into powder. 90% ethanol was used to extract the powder using the method of soxhlation for 18 hrs. Whitman filter paper No. 1 was used to filter the extract and concentrated to yield a semi solid mass of 48 gm (yield 9.2% w/w). and was refrigerated at 4°C and for lateruse.

Chemicals: Indomethacin (indocap, Jagsonpal Pharmaceuticals), carrageenan [sigma chemical co. usa] and other solvent chemicals used were of analytical grade.

Animals Male Wistar Albino Rats (150-200 g) were procured from central animal house of M.G.M. Medical College and Hospital aurangabad. were used. Food,

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water was given *ad libitum and* were acclimatized for laboratory conditions 7 days before the experiments. The experimental study protocol was approved by the Institutional Animal Ethics Committee (IAEC) of MGM MEDICAL COLLEGE constituted as per the guidelines laid by the committee for the purpose of control and supervision of experiments on Animals (CPCSEA).

Paw edema was induced by Carrageenan as described winter et al ^[13] by injecting 0.1 ml of 1% w/v carrageenan suspended in 1% Carboxy Methyl Cellulose into sub-plantar tissues of the left hind paw of each rat. Rats were divided into five groups; each group consisting of six animals.

- Group 1 -- CONTROL [Normal Saline 0.2ml,oral]
- Group 2 [STD] -- Indomethacin[20 mg/kg, intraperitoneal]
- Group 3 -- EEMO [100 mg ,oral]
- Group 4 -- EEMO [200 mg, oral]
- Group 5 -- EEMO [400 mg, oral]

The paw thickness was measured before injecting the carrageenan and after ,30,60,120, 180, min. using mercury plethysmometer by immersing the paw up to the marked point. The anti-inflammatory activity was calculated as percentage inhibition of oedema in the animals treated with extract under test in comparison to the carrageenan control group.

The percentage (%) inhibition of edema is calculated using the formula

% inhibition = $T_0 - T_t/T_0 \times 100$

Where T_t is the thickness of paw of rats given test extract at corresponding time and T_o is the paw thickness of rats of control group at the same time.

STATISTICAL ANALYSIS

All the values are expressed as Mean \pm standard error of mean (SEM). 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.

RESULTS AND DISCUSSION

Table 1: Comparison of Anti-Inflammatory activitie and paw edema (ml) at different time

Groups	0min	30 min	60min	120min	180min
Group1	0.79 ± 0.01	1.70±0.29	1.29 ±0.04	1.69 ± 0.09	1.67 ± 0.04
mean \pm S.E.M					
Group 2 [std]	0.89 ± 0.02	1.19 ± 0.07 *	1.27 ± 0.09 *	1.53 ± 0.16 *	0.52 ± 0.03* *
Group3 [mo100] mean ± S.E.M	0.92 ± 0.01	1.22 ± 0.07**	1.37 ± 0.04*	1.34 ± 0.08*	0.87 ±0.04**
Group4[mo200] mean ± S.E.M	0.90 ± 0.03	1.21 ± 0.11**	1.19 ± 0.07*	1.16 ± 0.01**	0.82 ± 05**
Group5mo[400] mean ± S.E.M	0.90 ±0. 02	1.18 ± 0.08 **	$1.3 \pm 0.08^{\text{ns}}$	1.36 ± 0.02**	0.70 ± 0.05**

Values are *p<0.01 – significant, **P<0.001 -- Highly Significant

In group 2 (Indomethacin) there was significant decrease in the paw edema at 30 min (p<0.05) and 3hrs (p<0.001) as compared to control and results were highly significant attend of 3hrs. In group 3(100 mg) there was dose dependent decrease in paw edema compared to control group which was significant at the end of 3hrs . In Group 4 (200 mg) there was significant reduction of

paw edema at 30 min (p<0.05) and 3hrs (p<0.001) at 30 min and 3hr respectively which was highly significant at the end of 3 hrs. In group 5(400 mg)there was significant reduction of paw edema throughout the period from 30 min. to 3hrs and highly significant results were obtained at end of 3 hrs comparable with the standard drug .

Table 2 percentage of inhibition of paw volume in different groups at different times

Groups	30 min	60min	120min	180min
Group 2[std]	30 %	5 %	10 %	68.86 %
Group3[mo100]	28 %	10 %	20 %	47.90 %
Group4[mo200]	28 %	6 %	31 %	50.89 %
Group5mo[400]	30 %	1 %	19 %	58.08 %

In group 2 (Indomethacin) the percentage of inhibition of paw volume was 30% and 68.86% at 30min and 3hr respectively. In group 2 (100mg) the percentage of inhibition of paw volume was 28 % and 47.90 % at 30min and 3hrs respectively. In group 3 (200mg) the percentage inhibition of edema was 28 % and 50.89 % at 30 min and 3hrs respectively.in group 5(400 mg) the

percentage inhibition of edema was 30 % and 58.08 % at 30 min. and 3hrs respectively.

DISCUSSION

The most widely used primary test for screening of antiinflammatory agents is carrageenan induced edema. The development of edema in the paw of the rats after injection of carrageenan is a biphasic event. [14] The initial

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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 leaf extracts at 400 mg dose showed significant reduction of edema in both the phases of inflammation but maximum reduction was observed in the second phase of inflammation (68.86%) which was comparable with indomethacin (58.08%).

The inhibitory effect of Moringa oleifera leaf extract 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 [15]. Many bioactive compounds naturally present in *Moringa oleifera* leaves, such as flavonoids and phenolic acids, may be involved in to anti-inflammatory process. Further studies should be done to investigate the potential anti-inflammatory action and the mechanism of action of other bioactive compound naturally present in *Moringa oleifera* leaves. [16]

SUMMARY AND CONCLUSION

Thus it can be concluded from our study that the Ethanolic leaf extracts of Moringa Oleifera at 400 mg shows significant anti-inflammatory activity. Further more extensive studies are required to elucidate the exact mechanism responsible for anti-inflammatory activity of Moringa oleifera leaf in larger animal group so that new potent, safe, and economical anti-inflammatory drugs can be developed from it. Finally, human studies are needed to evaluate the anti-inflammatory of *Moringa oleifera* leaves also in human beings.

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