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Anti-Inflammatory Effect of Ethanolic Extract of *Moringa oleifera Leaves* on Albino Rats.

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ABSTRACT

To study the anti-inflammatory effect of ethanolic extract of *Moringa oleifera* leaves using the technique of Carrageenan induced hind paw edema in albino rats. It is a randomized control study. Albino rats of the Wistar strain between weight 100-150 g were used. The animals were starved overnight with water being provided *ad libitum.* The Ethanolic extract of *Moringa Oleifera* (EMO), and the standard drug were administered by oral route. One hour later the rats were challenged with the subcutaneous injection of 0.1 ml of 1% solution of carrageenan on the plantar surface of the left hind paw. The paw was marked with ink at the level of lateral malleolus and immersed in the water chamber of the digital plethysmometer for measuring paw volume. The paw volume was measured before and after carrageenan injection at 1,2,3,4,6 &24 hrs. The efficacy of the drug was tested on its ability to inhibit paw edema. The ethanolic leaf extract of *Moringa oleifera* showed significant ($p<0.05$) reduction of paw volume edema at 100 ,200 ,400 mg/kg compared with the control group. The percentage inhibition of paw edema was maximum at 4hrs with EMO 400 mg/kg of 63% whereas Aspirin showed 70% inhibition of paw edema. The ethanolic leaf extract of *Moringa oleifera* exhibited anti-inflammatory activity in a dose dependent manner.

Keywords : EMO, anti-inflammatory, carrageenan

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INTRODUCTION

Moringa oleifera Lam syn *M. pterygosperma* (Family-Moringaceae) is known as shigru (Sanskrit); Sahijan(Hindi) ; Drumstick tree (English) is naturalized in the tropical and subtropical regions worldwide [1,2]. The plant is referred to many names like horseradish tree, ben oil tree, miracle tree, and "Mother's best friend" [3]. *Moringa* tree can grow well in hot dry lands to an average height of 5-10 m. *Moringa oleifera* has been stated 5000 years ago in the Charaka Samhita for its use in quick healing of abscesses both on oral intake and local application as paste. It is well known in folk medicine of African bushmen in the treatment of rheumatic swellings [4] and ancient Egyptians for wound healing properties [5]. Leaves are tripinnately compound bearing several small leaf legs, rachis slender, thickened and articulated at base [6]. Leaves are of high nutritive value and ingredients in the leaf like ascorbic acid, carotenes, flavonoids, isoquercetin, glycosides like niazirin, 4-hydroxymellein, β sitosterol and vanillin is claimed to possess anti-inflammatory property [7,8]. Inspite of the bountiful knowledge on inflammation, the modern day anti-inflammatory medicines are always a subject to their side effects, economy factor and potency. The present study is undertaken to screen the anti-inflammatory activity of leaf extract of *Moringa oleifera.*

MATERIALS AND METHODS

Materials

Collection of plant material

The leaves were collected for the local areas of Syampur, Bhubaneswar,Odisha 751003 and its identity was confirmed by taxonomist of Regional Plant Research Centre (RPRC), Bhubaneswar.

Preparation of extract

Fresh leaves were collected dried in shade and powdered. The powder was extracted with 90% ethanol using hot continuous percolation method in a Soxhlet apparatus for 18 hrs. Extract filtered using Whitman filter paper no 1 and concentrated in rotary evaporator to yield a semi solid mass of 42 g (yield 8.4 % w/w). Extract stored in refrigerator at 4^0 C and used for oral administration.

Chemicals

Aspirin (Burgoyne Burbidges and Co,India), Carrageenan (Sd fine-Chem Ltd,India) and solvent, chemicals used were of analytical grade.

Animals

Adult Wistar Albino rats of either sex (100g –200g) were randomly selected from the central animal facility. The animals kept at ambient temperature of 22 ± 1^oC, 12hr light and dark cycle allowed. Food, water given *ad libitum* . Animals were acclimatized to laboratory conditions for 7 days prior to taking them for experimentation. The study was approved by the Institutional Animal Ethical Committee (IAEC) of Siksha O Anusandhan University, Bhubaneswar.

Methods

The animals were randomly divided into 6 groups with 6 rats each; Group I: Control (normal saline given orally at 2ml/kg body weight); Group II: Standard (Aspirin at 200 mg/kg); Group III,IV,V,VI (EMO 50, 100, 200, 400 mg/kg respectively). The Ethanolic extract of *Moringa Oleifera* (EMO), and the standard drug were administered by oral route after making suspension with tween 80. One hour later the rats were challenged with subcutaneous injection of 0.1 ml of 1% solution of carrageenan on the plantar surface of the left hind paw and the right hind paw served as control [9,10]. The paw was marked with ink at the level of lateral malleolus and immersed in the water chamber of the digital plethysmometer for measuring paw volume. The paw volume was measured before and after carrageenan injection at 1, 2, 3 ,4, 6 and 24 hrs. The efficacy of the drug was tested on its ability to inhibit paw edema. The increase in paw edema is calculated as percentage compared with the basal volume. The difference of average values between the treated animals and the

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control group is calculated for each time interval and evaluated statistically. The percentage inhibition of paw edema is calculated using the formula [11] as follows:

% inhibition = (Vc – Vt/Vc) × 100.

Vc = Paw edema in control group animals
Vt = Paw edema in test group animals

Statistical Analysis

Results were expressed as mean ± SD. Statistical analysis done by using one way ANOVA followed by Post-hoc Dunnett's Test. In all tests a p-value of less than 0.01 and 0.05 was taken as significant.

RESULTS

Table 1 shows the effect of EMO and Aspirin on paw volume edema at different hours. Figure 2 shows the percentage inhibition of paw edema by Aspirin and EMO at different hours compared with the control. The maximum percentage of paw edema inhibition is seen at 3rd and 4th hour, where Aspirin shows 64% and70%, and EMO 400 mg/kg shows 56% and 63% respectively comparing with the control (Figure 2).

Table 1: Effect of ethanolic extract of *M.oleifera* and Aspirin on carrageenan induced paw volume edema.

DRUGS	0 Hour	1 Hour	2 hour	3 Hour	4 Hour	6 Hour	24 Hour
Control Normal saline 2ml/kg	0.11 ±0.01	0.33±0.03	0.63±0.03	0.88±0.04	0.97±0.05	0.72±0.05	0.28±0.05
Standard Aspirin 200mg/kg	0.1±0.01	0.18±0.01**	0.27±0.04**	0.31±0.03**	0.29±0.04**	0.29±0.04**	0.24±0.04
EMO 50 mg/kg	0.1±0.01	0.32±0.02	0.62±0.02	0.9±0.08	0.96±0.1	0.72±0.06	0.33±0.03
EMO 100 mg/kg	0.1±0.01	0.30±0.03	0.55±0.04*	0.68±0.06**	0.72±0.07**	0.63±0.06*	0.26±0.04
EMO 200 mg/kg	0.1±0.01	0.29±0.03*	0.47±0.06**	0.58±0.08**	0.55±0.08**	0.54±0.1**	0.25±0.03
EMO 400 mg/kg	0.11±0.01	0.27±0.04*	0.37±0.04**	0.38±0.04**	0.35±0.03**	0.43±0.02**	0.24±0.03

Results are Mean±SD (n=6).*p<0.05when compared to control. **p<0.01when compared to control.

Figure 1: % Paw edema inhibition at different hours by EMO & Aspirin compared with control

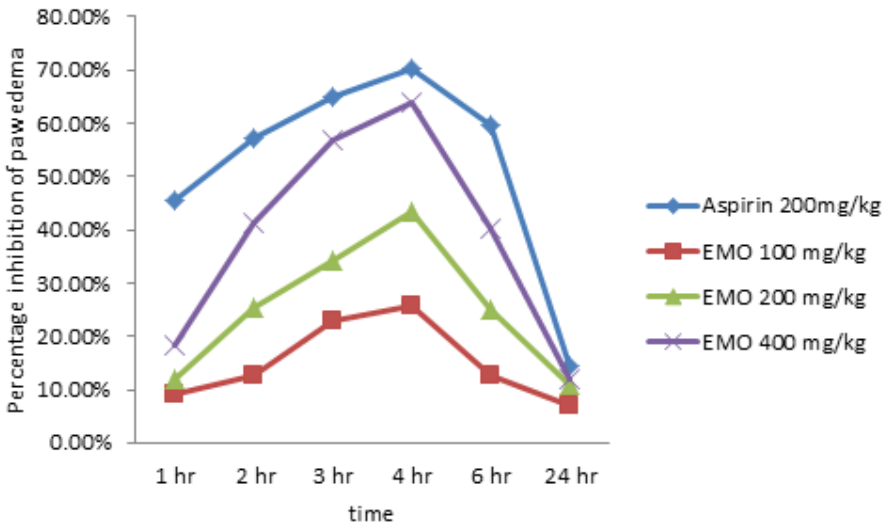

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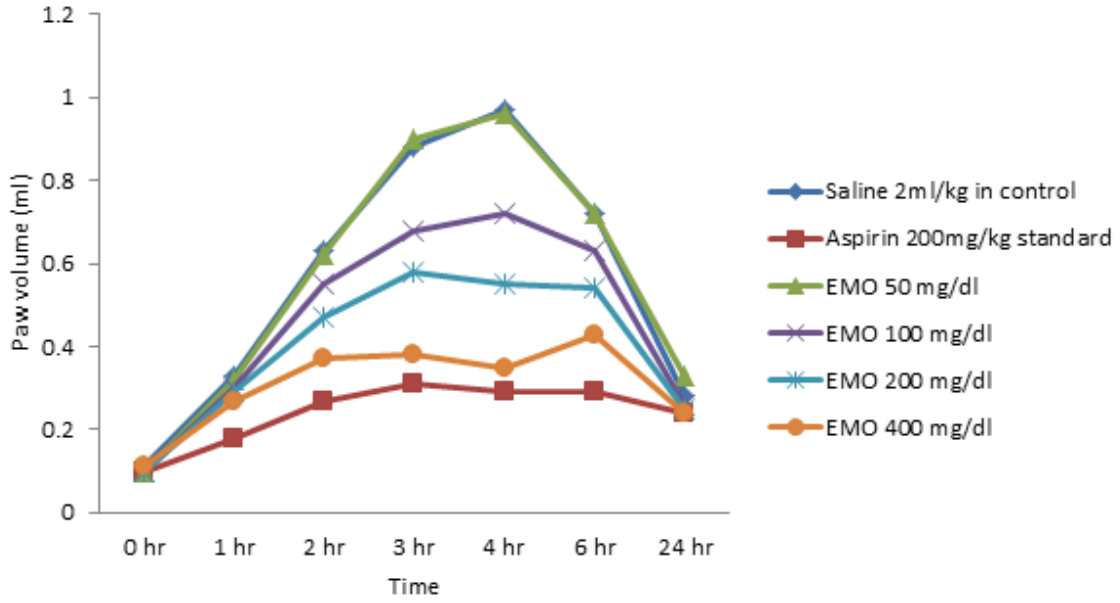
Figure 2: Effect of ethanolic extract of *M.olifera* on carrageenan induced paw volume edema.
(Mean paw volume edema versus time for Aspirin and EMO)



DISCUSSION

Inflammation is characterized in phases, first phase by vascular permeability, exudation of plasma, release of mediators, the second phase is due to migration of leucocytes and the final phase is by granuloma formation [12]. This study is a subacute study using carrageenan as a phlogistic agent .The paw edema induced by carrageenan in rats is biphasic,the first phase (0 -2 hrs) due to release of 5-HT, histamine, bradykinin from mast cells, plateau phase(3hrs) maintained by kinins [13], second phase (4hrs) produced by prostaglandins ,protease and lysosomes [14].

In this study ethanolic leaf extract of *Moringa Oleifera* showed significant($p<0.01$) reduction of paw edema at 100,200,400 mg/kg in the 3rd and 4th hour compared with control in a dose dependent manner suggesting that the extract predominantly inhibited the release of prostaglandin like substances. Aspirin is a non-selective COX inhibitor used here as standard also showed significant ($p<0.01$) reduction of paw edema volume compared with control.

The anti-inflammatory activity of leaf extract of EMO may be postulated to its COX inhibitory (Prostaglandin synthesis inhibition) activity in a dose dependent manner. As inflammation is also mediated by the lipoxygenase pathway [15], hence we could also suspect a possible action of the test drug on the lipoxygenase pathway.

The anti-inflammatory activity of ethanolic leaf extract of *Moringa Oleifera* may be attributed due to the phytochemical ingredients in it like flavonoids, isoquercetin, glycosides like niazirin ,4-hydroxymellein, β sitosterol and vanillin [3].

CONCLUSION

This study demonstrates the efficacy of EMO as an anti-inflammatory agent and scientifically validates the folklore claims about this plant. Further studies are required for the isolation of chemical constituents involved in the anti-inflammatory action of this plant.

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