Anti-Inflammatory Activity of *Moringa Oleifera* Leaf and Pod Extracts Against Carrageenen Induced Paw Edema In Albino Mice.

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Summary

The ethanolic and aqueous extracts of the leaf and pod of Moringa oleifera were tested to study the effects on the inflammatory reaction, using the technique of carageenan induced paw edema in albino mice. Both the ethanolic and alcoholic extract showed significant anti-inflammatory activity comparable to the reference standard Diclofinac sodium.

Key words: Anti-inflammatory activity, *Moringa oleifera*, Carrageenen.

Introduction

The plant Moringa *oleifera* Lam (Moringaceae) is the most widely cultivated variety of the genus Moringa and is distributed in the sub-Himalayan ranges of India, Sri-Lanka, Mexico, Arabia and South Western Africa. The leaves and pods of Moringa oleifera remove all kinds of pain, good vesicent, expectorant, stimulant and abortifacient. The decoction of the leaf is used as a stimulant, analgesic and diuretic. The pods are edible, seeds are useful as purgative, antipyretic, cures eye diseases, head complaints and are used in venereal affections. Leaf and pod of Moringa oleifera contains 4-Hydroxymellein, β-sitosterol and vanillin. The present study was undertaken to screen the anti-inflammatory activity of the leaf and pod of *Moringa oleifera*. [1] [2]

Experimental Section

The leaf and pod of *Moringa oleifera* were collected from the local areas of Udaipur district, Rajasthan, India during February 2010 and were authenticated by Prof.R Katewa, Department of Botany, M.B.College, Udaipur Rajasthan.

Preparation of Extracts

Leaves and fruits (pods) of *M. oleifera* will be ground separately in a mortar. Each of the plant tissues will be soaked in approximately 400ml of 95% ethanol and water on an electrical shaker for three hours at room temperature and then left to stand overnight. The mixtures were filtered into conical flasks using Whitman filter paper No. 1. The filtrate was then concentrated on a rotary evaporator at 50^0C to yield semi-solid masses whose weights were determined. The extracts were then stored in a refrigerator at 4^0C. The prepared extract was weighed and mixed with known concentration of ethanol. The extract will be subjected to photochemical screening and administered to the animals in the course of this study.

Animals

Healthy albino mice of either sex and of approximately the same age, weighing about 20-30 gm were used for the study. They were fed with standard chow diet and *ad libitium*.They were housed in polypropylene cages maintained under standard condition (12 hour light, 12 hour dark cycle; 25 ± 3oC, 35-60 % humidity). The experimental protocol was subjected to the scrutiny of the Institutional Animal Ethical Committee and was cleared by the same before starting. The acute toxicity studies of ethanol and aqueous extracts were carried out according to OECD guidelines. 1000 mg/kg dose of both the extracts was found non-toxic in mice and was taken for the further study.

Anti-Inflammatory Activity

Animals were divided into eight groups (n=6) starved overnight with water ad libitum prior to the day of experiment. The control group receives vehicle orally, while other group receives test drug and standard drug respectively. Left paw is marked with ink at the level of lateral malleolus; basal paw volume is measured plethysmographically by volume displacement method using Plethysmometer by immersing the paw till the level of lateral malleolus. The animals are given drug treatment. One hour after dosing, the mice are challenged by a subcutaneous injection of 0.05ml of 1% solution of carrageenan into the sub-plantar side of the left hind paw. The paw volume is measured again at 1, 3, & 5 hours after challenge. The increase in paw volume is calculated as percentage compared with the basal volume. The difference of average values between treated animals and control group is calculated for each time interval and evaluated statistically. The percent Inhibition is calculated using the formula as follows. [3] [4] [5]

Statistical analysis

Results expressed as mean ± S.E., were evaluated by unpaired student T test. Values of $p < 0.05$ were considered statistically significant.

Results and Discussion

The rat-paw volume was measured 1, 3 And 5 hours after injection of carrageenan. At a dose of 1000 mg/kg the Moringa oleifera Treatment significantly inhibited the development of oedema at 1, 3 and 5 hours (Reduction by 55.69, 64.77 and 77% respectively). Treatment with Dicofenac Sod significantly ($p<0.05$ and $p<0.01$) inhibited the development of edema 1, 3 and 5 hours (64.97, 69.94 and 81.629% respectively). These findings indicate that aqueous pods extract of Moringa oleifera at 1000 mg/kg reduces the carrageenan induced edema to similar extent as the potent anti-inflammatory drug Diclofenac Sod. Moreover, these results provide further evidence that the pod of Moringa oleifera contain anti-inflammatory principle that may be useful in the treatment of the acute inflammatory conditions. The aqueous and ethanolic extract of the pod and leaf of *Moringa oliefera* showed significant reduction ($p<0.05$ and $p<0.01$) in the edema volume at a dose of 1000 mg/kg body weight, which is comparable to standard drug Diclofenac sodium. Indigenous drug systems can be source of variety of new drugs which can provide relief in inflammation, but their claimed reputation has to be verified on a scientific basis. Alcoholic extract showed maximum anti-inflammatory activity. However, the activity produced by both the extracts was found to be less effective than

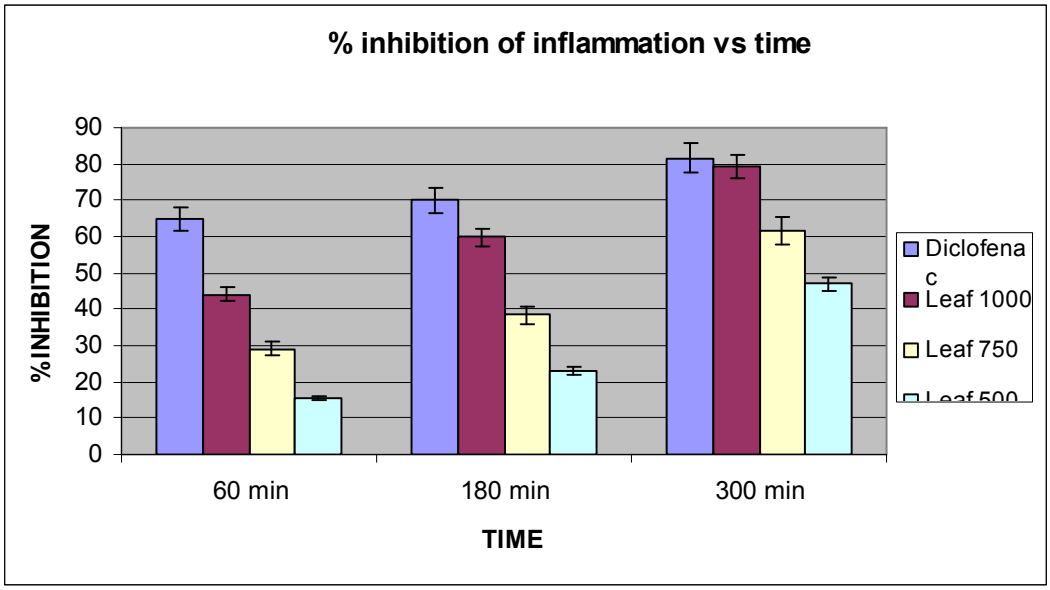
standard Diclofenac sodium. Edema represents the early phase of inflammation in carageenan induced paw edema and is the simplest and most widely used model for studying anti-inflammatory activity. The paw edema induced by the subplantar injection of carageenan in rats is biphasic, the first phase 1 hr. involves the release of serotonin and histamine while the second phase (over 1 hr.) is mediated by prostaglandins, the cyclooxygenase products and the continuity between two phases is provided by kinins. Both extract showed significant anti-inflammatory activity at 5 hr. against carageenan injection suggesting that the extracts predominantly inhibit the release of prostaglandins like substance. The percentage of paw edema was found to be better with the ethanolic extract than the aqueous extract. The activity may be attributed due to the presence of 4-Hydroxymellein, β-sitosterol and vanillin. The aqueous and ethanolic extract of the leaf of *Moringa oliefera* showed significant reduction in the edema volume at a dose of 1000 mg/kg body weight, which is comparable to standard drug Diclofenac sodium. Indigenous drug systems can be source of variety of new drugs which can provide relief in inflammation, but their claimed reputation has to be verified on a scientific basis. Alcoholic and aqueous extract of M.oleifera leaf and pod showed anti-inflammatory activity. [8] [9]

Table-1: Anti-inflammatory activity of Moringa oleifera extracts on Carageenan induced paw edema in mice

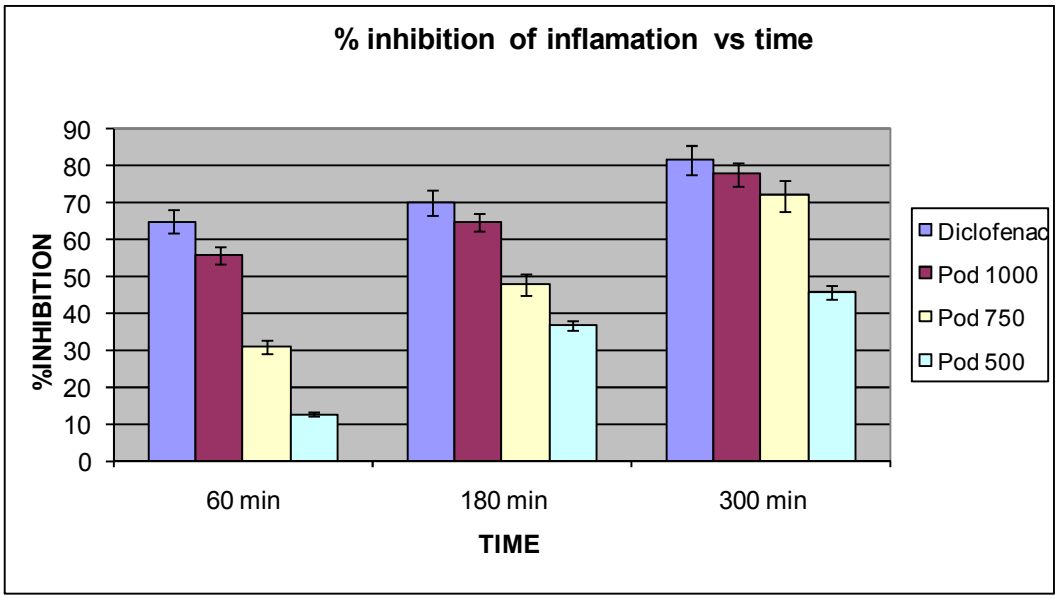
Treatment	Dose mg/ kg	Volume displaced in ml			% Percentage inhibition		
		1 hr	3 hr	5 hr	1hr	3 hr	5 hr
Control	------	2.00±0.066	2.25±0.020	3.40±0.03	------	------	------
Diclofenac Sodium	25	0.70±0.033*	0.67±0.028*	0.62±0.041*	64.9	69.9	81.6
M.oleifera leaf	1000	1.10±0.078*	0.90±0.052*	0.70±0.053*	44.0	59.9	79.1
M.oleifera leaf	750	1.40±0.050*	1.38±0.045*	1.30±0.050*	29.1	38.4	61.6
M.oleifera leaf	500	1.80±0.041*	1.70±0.052*	1.65±0.038*	15.4	23.1	46.9
M.oleifera pod	1000	0.87±0.038*	0.79±0.027*	0.75±0.021*	55.6	64.7	77.6
M.oleifera pod	750	1.37±0.028*	1.07±0.72*	0.95±0.039*	30.9	47.8	71.8
M.oleifera pod	500	1.95±0.040*	1.90±0.051*	1.85±0.039*	12.5	36.7	45.5

Results expressed as Mean± S.E.M (n=6), p<0.05*

Graph No.13: % inhibition of Inflammation by different groups of mice:



Graph No.14:-% inhibition of Inflammation by different groups of mice:



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