

ANTI-INFLAMMATORY EVALUATION OF LEAF EXTRACT OF *MORINGA OLEIFERA*Gurvinder Pal Singh^{1*}, Rakesh Garg², Sudeep bhardwaj³, Sandeep Kumar Sharma⁴¹*Senior Lecturer, Department of Pharmacognosy, Adesh Polytechnic college, Muktsar, Punjab, India.

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ABSTRACT

Moringa oleifera Lam. Is a small or medium-sized tree, about 10m high, found wild in the sub-Himalayan tract. The leaves are rich in vitamin A and C and are considered useful in scurvy and catarrhal affections. The leaves are rich in ascorbic acids, amino acids, sterols, isoquercetin glucoside, carotenes, rhamnetin, kaempferol and kaempferitrin. Flowers are traditionally used as tonic, diuretic and abortifacient considered as anthelmintic and also used to cure inflammation, muscle disease, tumors and enlargement of the spleen. All part of this plant is used for the treatment of ascites, rheumatism. Venomous bites and for enhancing cardiac function. In present study, the anti-inflammatory activity was investigated by employing main model Carrageenan induced paw edema (Winter et al., 1962). The results showed a dose dependent decrease in size of odema when observed at 0hr, 1hr, 2hr, 3hr, and 4hr. This effect corresponded with the maximum effect of test dose at 2 hr (Carrageenan-induced paw). The p value<0.0001 was considered to be statistically significant.

Keywords: CMC Carboxy Methyl Cellulose, Per Oral, National Botanical Research Institute.

INTRODUCTION

The Plants have provided mankind a botanical basis of medicinal agents, with natural products once serving as the source of all drugs¹. Reliance on plants as the source of medicine is prevalent in developing countries where traditional medicine plays major role in health care². The rural population of a country is more rely to customary ways of treatment because of its easy availability and cheaper cost³. Herbal therapy, although still an unwritten science, is well established in some cultures and traditions, and has become a way of life in almost 80% of the people in rural areas, in the World *Moringa oleifera* Lam. is a small or medium-sized tree, about 10m high, found wild in the sub-Himalayan tract, from Chenab east wards to Sarda and cultivated all over the plains of the India.⁴

Geographical source

Moringa oleifera Lam is a small genus of quick growing trees distributed in India, Arabica, Asia Minor and Africa. This tree is indigenous to northwest India. It is widely cultivated and naturalized in tropical Africa, tropical America, Sri Lanka, Mexico, Malabar, Malaysia and the Philippine islands.

Chemical constituents

The leaves are rich in vitamin A and C and are considered useful in scurvy and catarrhal affections. The leaves are rich in ascorbic acids, amino acids, sterols, isoquercetin glucoside, carotenes, rhamnetin, kaempferol and kaempferitrin⁵. Analysis gave the following values: moisture, 75.0; protein, 6.7; fat (ether extr.), 1.7; carbohydrates, 13.4; fibre, 0.9; and mineral matter, 2.3%: calcium, 440; phosphorus, 70; and iron, 7.0mg/100g; copper (1.1 are present. Leaves contain carotene (precursor of vitamin A), 11,300 I.U., vitamin B1, 2010 vitamin C, 220mg; and tocopherol, 7.4mg/100g. Estrogenic substances and a pectin esterase are reported to be present.⁶ The essential amino acids present in the leaf proteins are (g/16g): arginine, 6.0; histidine, 2.1; lysine, 4.3; tryptophan, 1.9; phenylalanine, 6.4; methionine, 2.0; threonine, 4.9; leucine, 9.3; isoleucine, 6.3; and valine, 7.1. The biological value and digestibility co-efficient of leaf proteins (at 5% level of protein uptake) are respectively 41% and 77%. Non-protein nitrogen accounts for 16% of the total nitrogen of tender leaves. The nitrogen distribution in the non-protein fraction is as follows: soluble humin N, 5.25; insoluble humin N, 5.25; amide N, 7.89; basic N, 34.21; and non-basic N, 47.37%. Feeding trials rats have shown that drumstick leaf powder has a high supplementary value to rice diet⁷

Table no 1: Phytochemical constituents isolated from *Moringa oleifera* Lam.

Parts	Phytochemical constituents
Roots	4-(α -L-rhamnopyranosyloxy)-benzylglucosinolate and benzylglucosinolate 10
Stem	4-hydroxymellein, vanillin, β -sitosterone, octacosanic acid and β -sitosterol 11
Bark	4-(α -L-rhamnopyranosyloxy)-benzylglucosinolate 10
Whole gum exudates	L-arabinose, D-galactose, D-glucuronic acid, L-rhamnose, D-mannose, D-xylose and leucoanthocyanin 12-13
Leaves	Glycoside niazirin, niazirin and three mustard oil glycosides, 4-[4'-O-acetyl- α -L-rhamnosyloxy) benzyl] isothiocyanate, niaziminin A and B 14-15
Mature flowers	D-mannose, D-glucose, protein, ascorbic acid, polysaccharide 16
Whole pods	Nitriles, isothiocyanate, thiocarbonates, 0-[2'-hydroxy-3'-(2''-heptenyloxy)]-propylundecanoate, 0-ethyl-4-[(α -1-rhamnosyloxy)-benzyl] carbamate, methyl-p-hydroxybenzoate and β -sitosterol 14-15
Mature seeds	Crude protein, Crude fat, carbohydrate, methionine, cysteine, 4-(α -L-rhamnopyranosyloxy)-benzylglucosinolate, benzylglucosinolate, moringyne, mono-palmitic and di-oleic triglyceride 10
Seed oil	Vitamin A, beta carotene, precursor of Vitamin A 17-18

Traditional Uses

Flowers are traditionally used as tonic, diuretic and abortifacient considered as anthelmintic and also used to cure inflammation, muscle disease, tumors and enlargement of the spleen. All part of this plant is used for the treatment of ascites, rheumatism. Venomous bites and for enhancing cardiac function. The tri pinnate leaves are found useful in scurvy and catarrhal affections. *Moringa oleifera* is already esteemed by people in the tropics and sub-tropics for the many ways it is used medicinally by local herbalists. In India juice from leaves is believed to have a stabilizing effect on blood pressure and is to treat anxiety, to control glucose levels in case of diabetes, used as the remedy for diarrhea, dysentery and colitis (inflammation of the colon). Sometime leaf juice with carrot juice, used as a diuretic (to increase urine flow).

Collection of the Plant Materials

The Leaves of plant of *Moringa oleifera* Lam. (Family - Moringaceae) was collected from Botanical Garden of N.B.R.I. (National Botanical Research Institute), Lucknow, India in month of Oct 2010. The plant materials were authenticated by Dr. Tariq Husain chemo taxonomist at National Botanical Research Institute, Lucknow and the voucher specimens (97816) were deposited in the departmental herbarium of National Botanical Research Institute, Lucknow, India for future reference

Animal Protocol

Wistar Albino Rat weighing, 150-200g, maintained on standard laboratory diet and had free access to tap water were employed in the present study. They were housed in the departmental Animal House and were exposed to normal light / dark cycle. Present study was approved from CPCSEA (Regd no 222/2000/cpcsea).

Carrageenan induced Paw-edema

In this experiment, carrageenan-induced rat hind paw edema was used as the animal model of acute inflammation⁸. Briefly, twenty four wistar Albino rat divided as Group-1 (Control group), Group-2 (Standard group), Group-3 (Test Group1: 200mg/kg) and Group-4 (TestGroup2: 500mg/kg), consisting of 6 rat in each group. The inhibition of carrageenan-induced oedema on the sub-plantar region of the paw of the rat was used to measure that anti-inflammatory activity of the extract. The extract in 1%w/v CMC was administrated orally to each test group of six wistar rat according to their body weight, by the means of a cannula, Indomethacin (20mg/kg) body weight suspended in 0.1 ml of 1% w/v CMC. The extract, standard and control were given to the rat an hour before injecting the sub-plantar region of the left hind paw of each rat with 0.1ml of 1% w/v carrageenan solution in normal saline. Increase in linear paw circumference, a measured by plethysmometer at 0hr, 1hr, 2hr, 3hr, 4hr, and 8hr after the carrageenan injection. The mean percent change in paw volume in control, drug treated and test group was taken as an index of increase in paw volume which is a measure of the odema.

RESULT AND DISCUSSION

Anti-inflammatory Activity by Carrageenan Induced Paw Edema

Anti-inflammatory activity of ethanolic extract was determined by carrageenan induced paw edema method. The measurement of paw size was carried out by mercuric displacement method at the time intervals of 0, 1, 2, 3, 4, 8 hr after the administration of the test drug. Results are shown in Table. 2

Table no 2 : Anti inflammatory activity of 50% ethanolic Extract of *Moringa oleifera* on carrageenan induced paw edema in rat.

Treatment	Dose (mg/kg)	Increase in paw volume (ml)				Mean % of paw volume			
		Mean ± S.E.M				1 h	2 h	3 h	4 h
		1 h	2 h	3 h	4 h				
Control Group (CMC)	1ml/100g	1.748 ±0.019	1.815 ±0.020	1.525 ±0.026	1.373 ±0.031	-	-	-	-
Standard Group (Indomethacin)	20mg/kg	0.525 ±0.007	0.858 ±0.014	0.901 ±0.010	1.163 ±0.057	29.88	46.96	59.2	84.6
Test Group-1 Extract of M.O. (200ml/kg)	200mg/kg	1.262 ±0.014	1.468 ±0.011	1.35 ±0.015	1.262 ±0.013	72.4	80.66	88.8	91.97
Test Group-2 Extract of M.O. (500mg/kg)	500mg/kg	0.547 ±0.008	1.083 ±0.010	1.15 ±0.012	1.183 ±0.039	31.03	59.66	75.6	86.13

The results given are mean S.E.M.; number of animal used (n=6) (p<0.001) experimental groups are compared with control.

Table no 3 ; percentage of inhibition of paw volume of experimental groups

Treatment	Dose (mg/kg)	Mean % of inhibition of paw volume			
		1 h	2 h	3 h	4 h
Control Group (CMC)	1ml/100g	-	-	-	-
Standard Group (Indomethacin)	20mg/kg	70.12	53.1	40.8	15.4
Test Group-1 Extract of M.O. (200ml/kg)	200mg/kg	27.6	19.34	11.4	8.1
Test Group-2 Extract of M.O. (500mg/kg)	500mg/kg	68.97	40.4	24.04	13.8

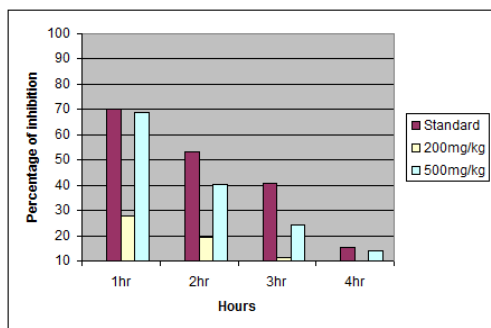


Fig no1 Percentage of inhibition of paw volume through graphical representation

DISCUSSION

Inflammation is considered as primary physiologic defense mechanism that helps the body to protect itself against infection, burn, toxic chemicals, allergens or other noxious stimuli. An uncontrolled and persistent inflammation may act as an etiologic factor for many of these chronic illnesses. The anti-inflammatory drugs with modern system of medicine have been reported for many severe side effects. The plant part of *Moringa oleifera* Lam. have been successfully used in many diseases of liver, spleen, kidney, stomach etc. by the traditional communities. Since, little work has been performed regarding the anti-inflammatory activity. So, an attempt was made to explore the anti-inflammatory potential on *Moringa oleifera* Lam. leaf.

The anti-inflammatory activity was investigated by employing main model Carrageenan induced paw edema for *Moringa oleifera* Lam. leaf ethanolic extract. Twenty four wistar rats were distributed in four groups as Group-1 (Control group), Group-2 (Standard group; Indomethacin), Group-3 (Test group 1: 200mg/kg, p.o.), and Group 4 (Test group 500mg/kg, p.o.). The results showed a dose dependent decrease in size of edema when observed at 0hr, 1hr, 2hr, 3hr, and 4hr. This effect corresponded with the maximum effect

of test dose at 3 hr (Carrageenan-induced paw). The p value < 0.001 was considered to be statistically significant. The Carrageenan-induced Rat paw edema is a biphasic process. The release of histamine or serotonin occurs in the first phase and the second phase is associated with the production of bradykinin, protease, prostaglandin and lysosome. Therefore, the inhibition of carrageenan-induced inflammation by the extract of *Moringa oleifera* Lam. could be due to the inhibition of the enzyme cyclooxygenase and subsequent inhibition of prostaglandin synthesis. The response is believed to be mediated by the prostaglandin pathway. This is the first such kind of study of anti-inflammatory activity on *Moringa oleifera* Lam. leaf.

CONCLUSION

In conclusion, the actions of extract upon the inflammation models tested justified its usefulness in herbal formulation and could be useful in primary health care. Further, isolation and identification of the compound responsible for biological activity need to be explored.

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