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Pharma Research

An International Peer Reviewed, Indexed Journal

10.62655/s-epub.2021.v13.i01.pp1-8

Pharmacological Screening of Aquoues and Methanolic Extract of Moringa Oleifera for Anti-Inflammatory and Analgesic Activity

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Article info

Published on:24-05-2021

ISSN: 0975-8216

KEY WORDS:

Herbal Medicines, Pain, Moringa oleifera, Analgesic and Anti-inflammatory Activity.

ABSTRACT:

Using a battery of experimental models, this research compares the anti-inflammatory and analgesic effects of Moringa oleifera leaf extracts. Results from the tail flick test in rats and the acetic acid-induced writhing in mice were used to determine the analgesic efficacy of Moringa oleifera leaves. Rats were tested for their ability to reduce inflammation by inducing paw edoema with carrageenan and by observing the growth of cotton pellet-granulomas in rats. Also assessed were the results of administering diclofenac, a reference standard. The edoema caused by carrageenan in rats' paws was significantly reduced after treatment with the methanol extract. It was discovered that the latency duration could be increased in the tail flick approach more effectively using methanolic and aqueous extracts. The findings corroborate the traditional therapeutic usage of Moringa oleifera, suggesting that it contains analgesic and anti-inflammatory properties.

I. INTRODUCTION

Because they are essential for all forms of life on Earth, plants are in a class of their own. All food chains rely on them as their principal producer. Humans get 80% of their protein and 90% of their calories from plants. For as long as

anybody can remember, plants have been considered as a possible medical resource. Traditional herbal treatment is still practiced by around 70% of India's 1.1 billion people¹. A long legacy of traditional medicine exists in India.²⁻³

There is a wealth of information on the ancient uses and mythology around medicinal plants in the *Materia Medica* of India. The use of herbal remedies has skyrocketed in the past few decades. Due to its natural origins and growing popularity, it is gaining traction in both developing and developed nations.

reduced adverse reactions. There are about seven hundred different herbal remedies available for medicinal use, including decoctions, tinctures, pills, and capsules, derived from over a hundred different plants.⁴ "Phytopharmaceuticals" and "phytomedicine" are terms used to describe herbal remedies derived from various plant components. as addition to tablets and capsules, they are also available as elixirs, powders, extracts, tinctures, creams, and parenteral preparations, among other forms and formulations. Additionally, raw herbal remedies are used. When it comes to questions about the efficacy and safety of medications, no one can answer them better than a chemist. Herbs should be treated as drugs if they have any medicinal value.⁵⁻⁶

Experiencing inflammation is a difficult and crucial event. It is "An unpleasant sensory and emotional experience associated with actual or potential damage, or described in terms of such damage," according to the worldwide organisation for the study of pain.⁷ "Nociception is the body's specialised reaction to stimuli that may cause harm to tissues." This is the process by which harmful external stimuli reach the brain. Because it is a subjective feeling, not everyone who experiences pain also nociception. Inflammation may be acute or chronic type.

Plant material (leaves) of *Moringa oleifera* has been used in present study and the detailed description of the plant is as follows:

Botanical name: *Moringa oleifera*

L.Family: Moringaceae

Vernacular Names: Hindi - Sahijana

Sanskrit - Sobhanjana, Bahola, Salapatra, Sighru

Gujarati - Midho Saragvo, Segto,

SeylaTelgu - Sajana, Munaga

Tamil - Murungai

Humans may savour every bite of this tree. Essential components: Parts of it include bark, pods, roots, flowers, seeds, and leaves, both fresh and dried.⁸ Leaves are alternate, with an unusual pinnate arrangement, and measure 22–72 cm. Three to nine sets of pinnae, each measuring 1 to 2 cm in length. These oval-shaped leaves are delicately dull green on top and pale on the underside. The fragrant, white flowers will be symmetrical and arranged at an angle. The 17–47 cm "drumsticks" used for soil-grown food have nine ribbed containers with three valves for seed dispensing. In a year, only one tree produces 16,000 to 24,000. The roots, while edible in sauce form, contain spirochin, a potentially lethal incapacitating toxin, however all sections are safe for human ingestion. The substances that arise in plants are known as phytochemicals. Composites of moringaceae including sugar and rhamnose are one example. Also included are glucosenolates, isothiocyanates, and an unbiased molecule. The glycosides niazirin and another one with the same name are found in the leaves. Included in the leaf are three glycosides derived from mustard oil. Ten nitrile glycosides, including niazirin and niazirininin niaziminin A and B, glycosides of mustard oil. The phenolic flavonoids quercetin and kaempferol as well as their glucosides 5-caffeoylquinic acid, 3-caffeoylquinic. Carotene (Vit. A), Nicotinic acid (Vit. B3), Riboflavin (Vit. B2), Tocopherols (Vit. K), and Ascorbic acid (Vit. C) are the vitamins. Crucial Elements: Proteins, Amino Acids, Calcium, Phosphorus, Iron, Copper, and Iodine.¹¹ A number of biological processes were shown by the plant. Not only are the leaves nutrient rich, but they also have anti-inflammatory and anthelmintic effects. Used for helminthiasis, wounds, and tumours. Pain and inflammation management are common themes in the current body of study. The formulation that controls inflammation and discomfort, which includes botanical extracts, is the primary focus of the current innovation.

Among the species found most often in the sub-Himalayan regions of India, Pakistan, Afghanistan, and Bangladesh is *M. oleifera*. This rapidly growing tree was used by the ancient Egyptians. This tree is long-lived and produces low-quality softwood. In India, it is now a top-tier product.

II. EXPERIMENTAL WORK

For this research, we used freshly picked, genuine *Moringa oleifera* leaves collected from the Sehore district. The leaves were desiccated in the shade after being correctly washed and rinsed with water after verification.

in a room setting. Various characteristics were used to evaluate the substance once it had dried fully. The leaves were then ground into a fine powder by passing them through a cutter mill, which reduced their size from coarse to fine. The powder was put through a 40# filter to achieve a consistent size after the reduction process. A number of standardisation procedures were applied to the powdered material, including microscopy, anatomical and histological examinations, and the assessment of phytochemical characteristics in accordance with pharmacopoeias and literature.¹²

Preparation of Extracts

The shade dried leaves of *Moringa oleifera* were decreased to a fine triturate (# 40 lattices) and nearby 200gram pulverized powder presented to progressive hot uninterrupted (soxhlet device) extraction using methanol. Ultimately, powder was macerated using water. After powerful extraction prepare, solvents dense off and concentrate was focused on the water bath. Last concentrate accomplished with every

2. Aqueous extract of *Moringa oleifera*

Screening for Analgesic Activity:

Rats of either one gender weighing 130 to 200 grams were picked for the experimentation. They were utilized for evaluating pain relieving potential of the sample. Albino wistar rats were alienated into eight clusters containing six rats. The comforter material of the confines was altered each day. The method of Chandrashekar et al 2004 was utilized to assess pain killing action of the sample concentrate of plants.¹⁴ The animals were allocated into eight clusters (each cluster comprising six rats). The first cluster was worked as control group and taken 5 ml / kg b. wt. (orally) of 5 % acacia solution only, then extcluster of animals was assisted as standard and Pentazocine was administered (5 mg / kg b. wt., I.P.). The rats of remaining clusters were cured with different concentrates of *Moringa oleifera*. The analgesic responses of the extracts of different plant parts

dissolvable was weighed immediately. The colour of the extracts and uniformity will be noted down. The acquired extracts were exposed to chemical exploration, pharmacological screening and for formulation development.

Qualitative Chemical Investigation of Extracts

Different qualitative chemical examinations were conducted for all the extracts of leaves of *Moringa oleifera* to identify their various phytoconstituents. Tests for identification included identification of proteins, amino acids, steroids, flavonoids, glycosides, alkaloids, phenolic compounds and vitamins.¹³

Pharmacological Screening

Albino wistar rats of whichever sex weighing in the middle of 130– 220 g were used in this investigation. They were engaged for assessing acute toxicity study, pain relieving activity study, anti-pyretic activity study and anti-inflammatory activity study. Animals were given marketable laboratory animal feedstuff and water in ample quantity. All the experimental animals were maintained at normal environmental condition of work space. They were housed at 25 °C and 12 h day / night cycle in a group of six rats in hygienic cages. The beds of the cages were changed every day. Six extracts of plant material were utilized for the screening of anti-inflammatory, and analgesic activity. These are listed below.

1. Methanolic extract of *Moringa oleifera* were assessed by means of the method known as tail immersion. The rats were initially weighed and stamped in this procedure. The animals are set into individual controlling pens forgetting the tail hanging uninhibitedly. The creatures are permitted to acquaint to the confines for 30 min. previously the commencement of the test. The bottommost 5 cm tail portion is spotted with marker. This portion of the rat tail is dipped in a mug of newly packed water of accurately $55 \pm 5^{\circ}\text{C}$ temperature. Inside a couple of seconds, the rodent reacts by withdrawing the tail from the container. Now the standard, investigational and control drug or extracts dosages were given to the rats and the response interval was observed at 0, 30, 60, 90, 120 and 180 minute time interval for assessing the activity of the plants extracts.

Screening for Anti-Inflammatory Activity:

Albino wistar rats of whichever gender weighing 150 to 250 g were designated for the testing of the plants extracts. They were employed

for evaluating anti-inflammatory potential of the concentrate of plants material. The testing animals were separated into eight clusters, each cluster having six rats. The eiderdown material of the crates was altered each day. Wister albino rats of whichever gender weighing in the middle of 150 g

- 200 g were contained in regular metal confines. They were delivered with food and liquid in sufficient quantity. The animals were permitted one-week adaptation period earlier the investigational schedule. The animals were distributed into eight clusters each cluster comprising six rats (Shah and Seth, 2010).¹⁵

The first cluster was assisted as a control group and given normal saline (5 ml / kg) only, these cluster of rats was assisted as standard and were given Diclofenac sodium as standard (100 mg

/ kg I.P.). Remaining groups of rats were managed with different extracts through oral route. A spot was made with the marker on the both hind paws of rats just below the tibiotarsal joint so each one time the paw could be dunked in the section of the plethysmograph up to the spot to guarantee the steady paw volume of rats. Following 30 minutes of the above treatment, an incendiary edema was incited in the left rear paw by infusing 0.1 ml of carrageenan 1 % w/v in saline, in the grower tissue of every last one of creatures. The paw volume was checked at the outset hour and emulated by consistently up to the fourth hour after the organization of carrageenan to each one gathering. The contrast between the starting and ensuing perusing gave the genuine edema volume. Swelling was measured as % restraint by utilizing the recipe,

% Inhibition = $100 \times (1 - vt/vc)$, where "vc" speaksto edema volume in control and "vt" edema volume in the gathering treated with test compound.¹⁶⁻¹⁷

III. RESULTS AND DISCUSSION

Percentage Yield of Extraction

The powdered sample of Moringa oleifera were subjected to extraction using continuous hot extraction (soxhlet apparatus). The aqueous and methanolic concentrate of Moringa oleifera was discovered to be 7.55 % and 9.1 % respectively. The different extracts obtained with their percentage yields are recorded in Table 1.

Table 1 : Percentage yield of extracts of *Lagenaria siceraria*, *Ocimum gratissimum*, and *Moringa oleifera* extracts

Sr. no.	Plant name	Extracts	% yield w/w	Physical state of Extract
1	Moringa oleifera	Aqueous (200 g)	25.75 %	Semisolid Viscous
2		Methanol (200 g)	9.1 %	Semisolid Viscous

Qualitative Chemical Investigation of Extracts

The leaves of *Moringa oleifera* were evaluated for the existence of numerous phytoconstituents. (E.g. Glycoside, Terpenoids, Steroids, Tannins, Flavonoids, Carbohydrates, Saponins, Protein, Alkaloids and Amino acid)

Table 2: *Moringa oleifera* extracts qualitative phytochemical study

Sr. No.	Phytoconstituents	M. oleifera	
		Methanolic extract	Aqueous Extract
1	Flavonoids	+	+
2	Alkaloids	+	+
3	Glycoside	+	+
4	Saponins	+	+
5	phenolic compounds and Tannins	+	+
6	Steroids and terpenoids	-	-
7	Carbohydrates	+	+
8	Proteins and Amino acids	+	+
9	Vitamins	+	+

(+) : Present, (-) : Absent

The methanolic concentrate of *Moringa oleifera* indicates the existence of flavonoids, alkaloids, tannins, glycosides, saponins, steroids, carbohydrates, proteins, amino acid, vitamins and phenolic compounds. The aqueous (water)

IV. PHARMACOLOGICAL SCREENING

Screening of Analgesic Activity:

Diverse approaches have remained developed to access the painkilling action of the drugs or plants extracts. These methods are based on the principal of thermal, mechanical and chemical or electrical stimulus. A change in reaction time of the experimental animals which are exposed to a thermal incitement (tail immersion test) was the most widely used method of determining analgesic activity. Thermal injuries precipitate an increase in vascular permeability, proteolysis, systemic inflammatory response and release of chemical mediators who are trailed by

extract of *M.oleifera* indicates the existence of flavonoids, alkaloids, glycosides, tannins, saponins, steroids, carbohydrates, proteins, amino acid, vitamins and phenolic compounds.

persistent pain. It is known that several chemical mediators, i.e., bradykinin and prostaglandin, produces pain in thermal injury and that μ , δ , and κ opioid receptor agonists mediate potent antinociceptive activity in animals subjected to thermal injury. Since pentazocine exhibits high affinity for μ_1 , μ_2 and κ_1 opioid receptor, it is proposed that pentazocine may exhibit antinociception against thermal stimulus via these receptors. The aqueous and methanolic extract of *Moringa oleifera* disclosed significant pain-relieving action over the regular drug pentazocine. The outcomes are presented in table 3.

Table 3: Analgesic activity of extracts of *Moringa oleifera*

Sr. no.	Group	Mean $\pm$ SEM					
		0 min.	30 min.	60 min.	90 min.	120 min.	180 min.
1	Control	1.39	1.37	1.53	1.58	1.85	2.68
		$\pm$	$\pm$	$\pm$	$\pm$	$\pm$	$\pm$
		0.012	0.022	0.015	0.020	0.019	0.031
2	Standard	1.81	1.93	2.21	2.49	3.46	5.72
		$\pm$	$\pm$	$\pm$	$\pm$	$\pm$	$\pm$
		0.030	0.020	0.017	0.026	0.006	0.071
3	MEMO	1.51	1.72	1.94	2.20	3.26	5.40*
		$\pm$	$\pm$	$\pm$	$\pm$	$\pm$	$\pm$
		0.011	0.009	0.018	0.016	0.030	0.014
4	AEMO	1.30	1.55	2.00	2.44	3.31	5.91**
		$\pm$	$\pm$	$\pm$	$\pm$	$\pm$	$\pm$
		0.026	0.007	0.039	0.039	0.009	0.004

MEMO – *Moringa oleifera* methanolic extract

AEMO – *Moringa oleifera* aqueous extract

* = Significant, ** = (p<0.05) highly significant n = 6, quantity of rats used in every cluster

At the initial time points, the methanolic extract and aqueous extracts do not show the analgesic activity as compared with the standard drug pentazocine. At 90 minutes, the aqueous extract and the methanolic *Moringa oleifera* leaves extracts showed modest analgesic action. After 180 minutes of the dosing of the *Moringa oleifera* aqueous extract displayed highly significant

analgesic activity while methanolic extract showed significant to temperate analgesic action when equated to pentazocine regular drug.

Anti-inflammatory Screening:

The current examination was completed to evaluate the legitimacy of the folkloric utilization of this plant in the administration of agony and treatment of incendiary issue. Both in-vitro and in-vivo techniques will be accessible for the assessment of mitigating executors however between the in-vivo systems carrageenan prompted rodent paw oedema examine is accepted to be a standout amongst the most solid furthermore the most broadly utilized. Carrageenan is a mix of polysaccharides made out of sulfated galactose units and is inferred from *Chondrus crispus*, Irish Sea greenery. The oedema, which creates in rodent

paw after carrageenan infusion, is a biphasic occasion. The beginning stage is ascribed to the discharge of histamine and serotonin, the oedema kept up in the middle of first and second stage to arrival of the kinin like substance and the third stage to arrival of prostaglandin like compound at fourth hour.

Oral path of management of the medication is a typical methodology to controlling the medication. For which carrageenan-affected paw irritation has been acknowledged as a valuable phlogistic instrument for exploring systemic calming executor. The concentrate indicated time- subordinate inhibitory movement in carrageenan- instigated paw irritation over a time of 4 h. This shows activity against the arrival of kinins, serotonin and histamine in the early stage, while later stages will be suspected to be arachidonate metabolites creating an edema subordinate on the mobilization of neutrophils. The methanolic extract and aqueous extract of *Moringa oleifera* showed moderately

to noteworthy inflammation suppressing activity on carrageenan prompted edema in rat paw over the standard drug diclofenac sodium.

33,75%, respectively. Due to the many pharmaceutical issues caused by traditional delivery techniques, the results of these investigations show that new drug delivery technologies for herbal extracts have the potential to revolutionise the industry.

V. CONCLUSION

An approach to pain and inflammation management is often at the heart of the current work. The current innovation is mainly concerned with the composition that controls inflammation and discomfort; it includes plant extracts. *Moringa oleifera* was identified as having the highest concentration of total tannins (22.30%), among the plant materials evaluated for total phenolics and tannins. Results showed a phenolic content of 60.21 % w/w. The water-based extract had a yield of 7.55% w/w, whereas the methanolic extract had a yield of 9.1% w/w. There were phenolic substances, carbohydrates, proteins, amino acids, steroids, glycosides, alkaloids, saponins, and flavonoids in the methanolic extract and water concentration of *Moringa oleifera*. When compared to the reference medicine, both the aqueous and methanolic extracts demonstrated substantial analgesic efficacy (5.91 ± 0.004 seconds and 5.40 ± 0.014 seconds, respectively) with a p-value less than 0.05. In comparison to the untreated control group, 500 milligramme oral doses of methanolic and aqueous *Moringa oleifera* extracts reduced carrageenan-induced edema by 60,000 and

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