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

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ABSTRACT: The present study evaluates the Anti-inflammatory and analgesic activities of various extracts of Leaves of Moringa oleifera using various experimental models. The analgesic activity of Leaves of Moringa oleifera carried out using acetic acid-induced writhing in mice and tail flick test in rats. The anti-inflammatory activity was evaluated using carrageenan-induced rat paw edema and cotton pellet-granuloma formation in rats. The effects of the administration of reference standard (diclofenac) were also evaluated. Treatment with Methanol extract showed significant inhibition of carrageenan induced rat paw edema. however Methanolic and Aqueous extracts were found to be more effective in increasing latency period in tail flick method. The results obtained indicate that Moringa oleifera has analgesic and anti-inflammatory activities that supports the folk medicinal use of the plant.

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

I. INTRODUCTION

On earth, plants occupy a unique position since they are the foundation to life. They are the primary producers in all food chains. Plants directly supply 90% of human calorific intake and 80% of proteins intake. Plants are being used as a potential source of medicine for time immemorial. More than 70% of India's 1.1 billion populations still use the traditional herbal medicine[1]. India has an ancient heritage of traditional medicine.[2-3] The Materia Medica of India provides a great deal of information on the folklore practices and traditional aspects of therapeutically important natural products. In the last few decades there has been an exponential growth in the field of herbal medicine. It is getting popularized in developing and developed countries owing to its natural origin and lesser side effects. More than 700 mono and polyherbal preparations in the form of decoction, tincture, tablets and capsules from more than 100 plants are in clinical use.[4] Herbal preparations called "phytopharmaceuticals" or "phytomedicine" are preparations made from different parts of plants. They come in different formulations and dosage forms including tablet, capsule, elixir, powder, extract, tincture, cream and parenteral preparations. Herbal products in the crude state are also used. The pharmacist among all health care practitioners is in the best position to provide information about drug safety and effectiveness. If any herb is used as therapeutic agent it should be considered as a drug.[5-6]

Inflammation is one of the most important and very complex experiences. The international association for the study of pain has defined pain as; "An unpleasant sensory and emotional experience associated with actual or potential damage, or described in terms of such damage".[7] "Nociception can be defined as response specific to potentially tissue damage stimulation". It is mechanism where by noxious peripheral stimuli are transmitted to the central nervous system. Pain is a subjective experience, not always associated with 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, Sigru
Gujarati - Midho Saragvo, Segto, Seyla
Telgu - Sajana, Munaga
Tamil - Murungai

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M. oleifera is the utmost commonly available species in the Pakistan, Afghanistan, Bangladesh and sub-Himalayan tracts of India. This quickly developing tree utilized by old Egyptians. It is an enduring softwood tree with timber of short quality. It is as of now a paramount product in India. All parts of this tree are palatable devoured by people. Parts used:It consists of fresh or dried leaves, bark, pods, roots, flowers and seeds.[8] Leaves are interchange, strangely pinnate layout and 22– 72 cm. Every pinnae three to nine sets of 1 to 2 cm long. The ovate leaflets with whitish belowand delicate dull green above. The white hued fragrant blooms that will be at a slant monosymmetric. The soil grown foods units, entitled "drumsticks" are 17 – 47 cm, 9 - ribbed containers maiden by 3 valves to discharge seeds. Each one tree create 16,000 - 24,000 in a year. All parts fit for human consumption but the roots, which are utilized as sauce, contain spirochin. a conceivably deadly incapacitating operator.[9]

Phytochemicals constituents are the compounds originated by plants. In particular, moringaceae composites comprising sugar, rhamnose. Glucosinolates, isothiocyanates, an impartially compound are also present. Leaves contain glycosides known as niazirin and other known as niazirinin. Leave also contains three mustard oil glycosides.[10] Nitrile glycosides: niazirin, niazirinin mustard oil glycosides: niaziminin A& B. Phenolic flavonoids- Quercetin & its glucosides, kaempferol & its glucosides, 5-caffeoylquinic acid,3- caffeoylquinic. Vitamins: Carotene (Vit. A), Ascorbic acid (Vit. C), Riboflavin (Vit. B2), Tocopherols (Vit. K), Nicotinic acid (Vit. B3). Essential Amino Acids, Proteins Calcium, Phosphorus, iron, copper & Iodine.[11] The plant exhibited various biological activities. The leaves have anti-inflammatory and anthelmintic properties and vitamin rich. They used in wound, tumor, helminthiasis.

The present work relates usually to a composition and method for managing pain and inflammation. More principally, the present development relates to the pain and inflammation managing composition, which contains extracts from plant species.

II. EXPERIMENTAL WORK

In the present study, crisp leaves of Moringa oleifera were gathered from local area of Sehore district and was authenticated. After authentication, leaves were properly washed and cleaned with water and dehydrated beneath shadow at room environment. After completely drying, the material was subjected to evaluation using different parameters. Then the leaves were taken for the size reduction using cutter mill in order to get coarser to a fine powder. After size reduction, the powder passed through 40# sieve to get uniformly sized powder. The powdered material was subjected to various standardization parameters like anatomical & histological studies, microscopy and determination of various phytochemical parameters as per pharmacopoeias / literatures.[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 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 $^{\circ}$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

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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 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 ± 5ºC 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 and180 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 x (1-vt/vc), where "vc" speaks to 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.

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

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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 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 μ1, μ2 and κ1 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 ± SEM					
		0 min.	30 min.	60 min.	90 min.	120 min.	180 min.
1	Control	1.39 ± 0.012	1.37 ± 0.022	1.53 ± 0.015	1.58 ± 0.020	1.85 ± 0.019	2.68 ± 0.031
2	Standard	1.81 ± 0.030	1.93 ± 0.020	2.21 ± 0.017	2.49 ± 0.026	3.46 ± 0.006	5.72 ± 0.071
3	MEMO	1.51 ± 0.011	1.72 ± 0.009	1.94 ± 0.018	2.20 ± 0.016	3.26 ± 0.030	5.40* ± 0.014
4	AEMO	1.30 ± 0.026	1.55 ± 0.007	2.00 ± 0.039	2.44 ± 0.039	3.31 ± 0.009	5.91** ± 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 oleiferaaqueous extractdisplayed highly significant

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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 Choncjrous 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.

Table 4: Anti-inflammatory outcome of different extracts of Moringa oleifera on edema prompted by carrageenan in rat paw

Sr. No.	Group	Mean paw edema volume in ml ± SEM					Difference	% inhibition in paw edema after 4 h.
		0 h.	1 h.	2 h.	3 h.	4 h.		
1	Control	1.01 ± 0.02	1.10 ± 0.09	1.26 ± 0.01	1.15 ± 0.07	1.09 ± 0.05	0.08	0.00
2	Standard	1.03 ± 0.09	1.31 ± 0.11	1.20 ± 0.02	1.15 ± 0.04	1.05 ± 0.09	0.02	75.00
3	AEMO	1.07 ± 0.05	1.42 ± 0.09	1.15 ± 0.13	1.14 ± 0.10	1.10 ± 0.21	0.03	60.00*
4	MEMO	1.09 ± 0.25	1.21 ± 0.14	1.07 ± 0.17	1.02 ± 0.08	1.14 ± 0.16	0.05	33.75

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MEMO – Moringa oleifera methanolic extract
AEMO – Moringa oleifera aqueous extract
* = Significant, ** =($p<0.05$), highly significant n = 6, quantity of rats used in every group

V. CONCLUSION

The present work relates usually to a method for managing pain and inflammation. More principally, the present development relates to the pain and inflammation managing composition, which contains extracts from plant. The plant material was used for estimation of tannins and total phenolic in which the Moringa oleifera found with the amount of total tannins (22.30 %). The phenolic content was found to be 60.21 % w/w. The % yield of aqueous and methanolic extract of was 7.55 % w/w and 9.1 % w/w respectively. Methanolic extract and aqueous concentrate of Moringa oleifera indicated the existence of flavonoids, tannins, glycoside, alkaloids, saponins, steroids, carbohydrates, proteins, amino acid, vitamins and phenolic compounds. Both aqueous extract (5.91 ± 0.004seconds) and methanolic extract (5.40 ± 0.014seconds) showed significant analgesic activity when compared to standard drug (5.72 ± 0.071seconds), ($p<0.05$). Moringa oleifera methanolic and aqueous extracts lessened the carrageenan prompted edema by 60.00 % and 33.75 % correspondingly on oral dosing of 500mg, as equated to the untouched control set. Findings of these studies conclusively demonstrate that the novel drug delivery technology for the herbal extracts can knock the market because of the reasons of many pharmaceutical problems occurred in conventional delivery systems.

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