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Investigating the Analgesic and Anti-Inflammatory Potential of 80% Methanolic Extract of Leaves of *Moringa concanensis*

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Abstract

The current study involved the assessment of the analgesic and anti-inflammatory potential of an 80% methanolic extract of leaves of *Moringa concanensis*. The central and peripheral analgesic activity of the extract was evaluated using the tail flick, Eddy's hot plate, and writhing methods, respectively, in mice at 200 and 400 mg/kg doses. While the anti-inflammatory potential was assessed using carrageenan-induced paw edema and cotton pellet-induced granuloma in rats at the same doses. *In vitro* antioxidant and anti-inflammatory activities were also performed. The extract displayed potent antioxidant activity with an IC_{50} of 24.996 µg/mL in hydrogen peroxide and 39.141 µg/mL in superoxide scavenging assays. In the protein denaturation assay, the extract inhibited the denaturation of egg albumin with an IC_{50} of 32.617 µg/ml and 66.42 ± 0.77% inhibition. The extract showed a significant dose-dependent analgesic and anti-inflammatory effect in tested models. At 60 minutes of observation, both 200 and 400 mg/kg doses significantly ($p < 0.001$) increased the latency of paw licking (hot plate) and tail flicking. The extract reduced the number of acetic acid-induced writhes significantly at 200 mg/kg ($p < 0.01$) and 400 mg/kg ($p < 0.001$). Among the tested doses, 400 mg/kg exhibited the highest analgesic effect in hot plate (64.91%), tail flicking (67.74%), and writhing (49.10%) tests. The screening of the extract for anti-inflammatory activity showed that 400 mg/kg has a greater effect than 200 mg/kg. It reduced carrageenan-induced acute inflammation significantly ($p < 0.01$) at the 4th hour with 64.2% inhibition. The similar results were also noted with cotton pellet-induced granuloma ($p < 0.001$) with 51.6% inhibition at the dose of 400 mg/kg.

Major Findings: In the present study, the 80% methanolic extract of the *Moringa concanensis* leaves demonstrated strong dose-dependent analgesic and antiinflammatory effects in mice and rats, with 400 mg/kg showing the highest efficacy. The activity was linked to flavonoids and phenolic compounds supported by *in vitro* antioxidant and protein denaturation inhibition.

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Abbreviations: **MEMC**: Methanolic extract of *Moringa concanensis*; **IAEC:** Institutional Animal Ethics Committee; **GC-MS**: Gas chromatography-mass spectrometry; **H_2O_2**: Hydrogen peroxide; **NBT**:Nitroblue tetrazolium; **PMS:** Phenazine methosulfate; **NADH**: Nicotinamide adenine dihydrogen; **i.p.**: intra peritoneal; **MA**: Maximum analgesia; **IC50:** Half maximal inhibitory concentration; **SD**: Standard deviation; **ANOVA**: One-way analysis of variance; **PGs:** Prostaglandins, **DPPH**: 2,2-Diphenyl-1-picrylhydrazyl; ABTS: 2,2′-azino-bis-(3-ethyl benzothiaz oline -6-sulfonic) acid

1. Introduction

Algesia and inflammation are important aspects of the body's defense mechanism and respond to an external or internal noxious stimulation. These can be either chronic or acute. Algesia is the part of the rapid warning pathway that directs motor neurons to minimize physical damage in the central nervous system. Acute pain occurs suddenly, persists for a short period of time, and may lead to chronic pain, if not treated within 3 months. It is caused by significant bodily tissue damage and the activation of nociceptive transducers at the tissue damage location. Chronic pain which occurs over longer periods of time, may be due to injury or disease, where the body will lose its capacity to return to its normal[1-2].

Inflammation is a protective mechanism to eliminate harmful stimuli (pathogens, irritants, tissue damage) and initiate the tissue damage. Acute inflammation is a short-term response characterized by redness, swelling, heat, and pain. Chronic inflammation is a long-term response that can result from untreated acute inflammation, persistent irritants, or autoimmune diseases and is also associated with cardiovascular diseases, arthritis, and cancers[3,4].

Even though anti-inflammatory and analgesic drugs are available in the large number to treat inflammation and pain, side effects remain a major concern in their long usage. Thus, people rely on herbal medicines, as these account for beneficial effects due to fewer side effects and synergistic effects. The active constituents like flavonoids, alkaloids, phenols present in them play an important role, which leads to their widespread use in modern medicine as well[5-7].

Moringa concanensis Nimmo., belonging to the *Moringaceae* family (Figure 1), is a traditional medicinal plant with significant therapeutic potential, commonly known as the drumstick tree, kadovo saragvo, or Konkan Moringa, and Katumurangai. It has been widely used traditionally among the tribes of the Nilgiris mountain range in Tamil Nadu, India, to treat inflammation, spinal cord pain, and menstrual pain, fertility problems, and various ailments such as skin tumors, fatigue, blood pressure, jaundice, eye problems, and diabetes[8-10]. It has a very strong central trunk, covered with a layer of highly raised bark. The leaves are bipinnate, the bark cracks and has a gray cork, trees show inflorescence, and flowering occurs in the months February to April. Fruits are straight capsules with a brownish tinge and a small gap between the seeds. Seeds are white or light yellow with three angled tiny wings.

The plant is rich in various phytoconstituents, including steroids, triterpenoids, reducing sugar, alkaloids, carbohydrates, amino acids, phenolics, flavonoids, saponins, proanthocyanins,vand tannins. The leaves were reported to contain stigmasterol, α-tocopherol, α-tocopherol acetate, linoleic acid, oleic acid, palmitic acid, methyl palmitate, and isosorbide, as the major compounds[11]. Additionally, cardenolide, digoxin, was isolated from the chloroform extract of leaves[12,13]. The seeds of *Moringa concanensis* contain oleic acid, campesterol, pyranone, palmitic acid, beta sterol, and 1,2-cyclopentanedione, and the bark of *Moringa concanensis* have been reported to contain flavonoids and phenols such as astragalin, quercetin, isoquercetin, and caffeic acid, identified through UPLC-QTOF/MS analysis[14]. The quantitative analysis of the phytoconstituents revealed that the leaves contain the highest amount of flavonoids, phenols, and alkaloids compared to the seeds and flowers[15].

Moringa concanensis has shown a strong *in vitro* antioxidant activity against various free radicals, including hydrogen peroxide, DPPH, hydroxyl radicals, and ABTS, and the literature survey highlighted a wide array of biological activities[16,17]. The ethanolic extract of the leaves showed the potent antiepileptic[10], antidiabetic, immunomodulatory, antibacterial and antifungal activities[18-21]. The methanolic extract of the bark and leaves was studied for its anticancer activity, and scientists attributed the activity to the presence of flavonoids and phenols[22-24]. The fruits and the flowers of *Moringa*

concanensis showed significant anti-inflammatory and analgesic activity, and the alcoholic extract of the roots showed potent anti-arthritic activity[25-27]. While other parts, flower, bark and roots of the plant were studied for analgesic and anti-inflammatory activity, but the leaves, despite being its traditional claim, and rich in phytoconstituents, have not been studied for anti-inflammatory and analgesic activity. Hence, the present study aims to evaluate the analgesic and anti-inflammatory potential of an 80% methanolic extract of *Moringa concanensis* leaves in experimental animals.

2. Materials and Methods

2.1 Collection and Authentication of Plant Material

The twigs of *Moringa concanensis* were collected from Suddapally, Hyderabad, in the month of March 2023, and authentication was done at Government Degree College, Hyderabad. The leaves were picked up from the plant material, shade dried, coarsely powdered, and preserved in an airtight container for later use.

2.2 Preparation of Extract and Phytochemical Testing

Moringa concanensis powder was extracted with petroleum ether until it decolorized in the Soxhlet apparatus. Then the marc was dried and Soxhlet extracted with 80% methanol for 48 hrs. It was filtered, concentrated at 60^0 C under reduced pressure, and evaporated to dryness to get the crude extract. The percentage yield of MEMC was calculated, and it was found to be 15.8%.

The small portion of the methanolic extract of *Moringa concanensis* (MEMC) was subjected to chemical tests for the identification of various active constituents using standard procedures. The tests for the alkaloids, steroids, saponins, tannins, anthraquinones, flavonoids, and phenols, including carbohydrates, fats, and proteins, were performed[28]. The rest of the extract was preserved for further study.



Figure 1. A twig of *Moringa concanenesis*

2.3 Gas Chromatography-Mass Spectrometry (GC-MS) Analysis

The crude extract of MEMC was subjected to a GC-MS analysis using GC-MS instrument, QP2010 SHIMADZU brand, under the conditions of a column (BP5), for sample separation, a helium (He), carrier gas (1 mL/min), and an injection volume of 1 µL. GC-MS NIST107 library was used to identify and analyze the compounds separated in GC-MS[29].

2.4 Animals and Acute Toxicity Study

The male and female Wistar albino mice and rats were used to investigate anti-inflammatory and analgesic activity. The animals were kept in the cages at 22–25^0C., providing 12-hour light and dark cycles, and were permitted to adapt to the laboratory conditions for five days before being used for the activity. Animals were taken care of and used according to the ethical guidelines of laboratory animals. The ethical permission was obtained by the IAEC (Institutional Animal Ethics Committee) GRCP, Hyderabad, to carry out the work[30].

An acute toxicity study was done according to OECD guidelines-425. The plant extract, 2000 mg/kg bd. wt., was given orally to animals following an overnight fast with water and monitored approximately for 24 hours. The animal's neural, behavioral, and autonomic profiles and lethality were observed during the period of 24 and 72 hours and continued for 14 days consecutively. The dose was considered harmful if it caused 2–3 animals to die, and only a single animal died, the same dose was given to confirm the lethality[31].

2.5 *In vitro* Antioxidant Assay

The 80% MEMC was screened for antioxidant activity *in vitro* through H_2O_2 (hydrogen peroxide) and superoxide scavenging methods.

2.5.1. Hydrogen Peroxide Scavenging Assay

Various concentrations of MEMC and standard (ascorbic acid) solutions (10–50 µg/ml) were prepared, and the

reaction mixture was prepared by mixing 1 ml of each concentration of MEMC and standard with 0.6 mL of H_2O_2 (40 mM, in a pH 7.4 buffer solution of phosphate) solution, and solutions were made up to 5 mL with phosphate buffer. The absorption of each concentration mixture was measured using phosphate buffer solution without hydrogen peroxide (blank solution) at 230 nm. The % of free radical scavenged by the test and standard was calculated by using the formula given in equation 1, and IC_{50} value was calculated from the graph, plotted between the percent of scavenged and various concentrations of samples.

$$\%\ of\ Hydrogen\ peroxide\ radicals\ scavenged.$$

$$= \frac{c-a}{c} \times 100 \quad Eq.-1$$

c = Control's absorbance; a = Sample's absorbance

2.5.2 Superoxide Scavenging Activity

To determine superoxide scavenging activity, a nitroblue tetrazolium (NBT) oxidation method was used. The PMS (phenazine methosulfate) and nicotinamide adenine dihydrogen (NADH) systems were used to produce reactive oxygen species that convert the NBT into indigo. Various concentrations (10–50 µg/ml) of MEMC and standard (quercetin) solutions were prepared, and the reaction mixture was prepared by mixing 1 mL of each concentration of MEMC and standard solution with phosphate buffer, 1 mL (pH 7.4), 1mL of NBT (0.156 mM), 1 mL of PMS (0.06 mM), and 1 mL of NADH (0.4 mM) solutions. The absorbance of each solution was taken at 560 nm after incubating each solution for 1 hour at 250^0 C, against the blank. The free radical scavenged by the test and standard was calculated using the formula given in equation 2, and the IC_{50} value was calculated using the graph of % of scavenged plotted against various concentrations of samples[32].

$$\%\ Superoxide\ radicles\ scavenged$$

$$= \frac{Ad-Ad}{Ad} \times 100 \qquad Eq.-2$$

Ad = absorbance of control; Ab = absorbance of test

2.6 Protein Denaturation Assay

In vitro anti-inflammatory activity was evaluated through inhibition of protein denaturation. The sample solutions were prepared by adding buffer solution of phosphate (pH 7.4), 5 ml, egg albumin, 2.8 ml, fresh hen's egg albumin, 0.2 ml and 2 ml of various MEMC and standard concentrations. The control solution was prepared similar way without reference standard/test solution. The samples were heated at 70^0 C for 5 minutes after incubating for 15 minutes at 37^0 C and cooled to room temperature[33]. The absorbance of each concentration was taken at 660 nm, and inhibition of protein denaturation was calculated according to equation 3.

$$\%\ Inhibition\ of\ protein\ denaturation$$

$$1 - \frac{C}{D} \times 100 \quad Eq.-3$$

C - Absorbance of MEMC, D - Absorbance of reference solution

2.7 Analgesic Activity

The tail flick, Eddy's hot plate, and Writhing models were used to evaluate the *in vivo* analgesic potential of 80% MEMC. Twenty-four albino mice (20–25 g) were divided into 4 groups, housing six animals in each group. The group I: normal control, received saline solution; groups II and III: test groups, were given 200 mg/kg and 400 mg/kg bd. wt. of plant extract, orally; and group IV: received diclofenac, 10 mg/kg via the i.p. (intra peritoneal) route. Prior to the administration of treatments, the mice were fasted for 12 hours.

2.7.1 Eddy's Hot Plate Test

The hot plate was maintained at a temperature of 55 ± 1^0 C, steadily and each mouse was placed on it through a restrainer. The reaction time (seconds) of mice to the thermal pain was measured every 15 minutes up to one hour after the treatments were given. Two behavioral traits, such as licking of paws or jumping, were considered as responses of animals to thermal pain. To protect the paw tissues due to heat, a 15-second cut-off time was given to animals. The percentage of maximum analgesia (MA) was calculated using the following equation[34].

$$MA = \frac{A-B}{B} \times 100 \qquad Eq.-4$$

A - Reaction time of treated animals
B - Reaction time of control animals

2.7.2 Tail-Flick Method

The distal end of the tail of each mouse was submerged up-to 2 cm in warm water, maintained with a steady temperature of 55 ± 1^0 C. The response time of each mouse was measured in seconds when it flicked its tail from water due to pain every 15 minutes up to 1 hour after the treatments were given to animals. To avoid harm to the tail, 15-second cut-off time was given to animals. The percentage of maximum analgesia (MA) was calculated using the same formula given under the hot plate method.

2.7.3 Writhing Test

Acetic acid (0.1 ml/10 g bd. Wt., *i.p.*) was given to induce writhing in the animals after 60 min of oral administration of the treatments. The animals were kept in the glass chamber individually, and writhing episodes such as body lengthening, extension of the hind limbs, and back arches were counted for 30 minutes[35]. Percentage inhibition of writhing was taken as a sign of analgesia and evaluated by the formula given in equation 5.

$$\%\ inhibition\ of\ writhes = \frac{C-T}{C}\times 100 \qquad Eq.-5$$

T = Mean number writhes in Test Group; C = Mean number writhes in Control Group

2.8 Anti-Inflammatory Activity

Wistar Albino rats weighing 180–220 gm were used to evaluate the activity.

2.8.1 Carrageenan-Induced Paw Edema

This model evaluates the acute phase of inflammation. The rats were grouped into five, consisting of six animals in each group. The group I received normal saline, normal control; group II received carrageenan, the disease control; groups III and IV were given 200 mg/kg bd. wt. and 400 mg/kg bd. wt. extract, were the test group, and group V was positive control, treated with the diclofenac sodium. The basal paw volume of the left hind paw of each rat was measured after marking in the plethysmograph, which involved the theory of displacement of mercury. The animals were given a subcutaneous injection of carrageenan (0.1 ml of 1% solution) into the sub-plantar region of the left paw after 1 hour of administration of treatments to induce inflammation. After the challenge, paw volume of each rat was determined at intervals of 1, 2, 3, and 4 hrs, and the paw volume was calculated (Equation-6)[36].

$$\%\ of\ Edema\ inhibition = \left[1-\left(\frac{Vt}{Vc}\right)\right]\times 100 \qquad Eq.-6$$

Vt and Vc are the paw edema volume in treatment group and control group

2.8.2 Cotton Pellet-Induced Granuloma

The chronic phase of inflammation is evaluated using this model. The cotton pellets weighing 20±1 were autoclaved at 120^0 C and 15 lbs pressure for 30 minutes. The animals were anesthetized with diethyl ether, and a small incision was made on the shaved flank region of each animal. The cotton pellets were inserted aseptically in the incised region and sutured using catgut. MEMC (200 and 400 mg/kg bd. wt.) and standard (10 mg/kg bd. wt.) solutions were given orally once a day for the next seven days. The cotton pellets were carefully taken out on the 8^{th} day after sacrificing the animals and dried at 60^0 C. Each cotton pellet surrounded by granuloma tissue was weighed, and the percentage inhibition was calculated as given in equation 7 [37].

$$\%\ of\ inhibition = ad - \frac{ad-ac}{ad}\times 100 \qquad Eq.-7$$

ad -pellet weight of test group; ac = pellet weight of control group

2.9 Statistical Analysis

The mean ± SD (standard deviation) was used to represent experimental data, and one-way analysis of variance (ANOVA) was used for statistical analysis. Multiple comparisons between the treatment groups compared to the normal control were made using Dunnett's test. All statistical analyses were carried out using the GraphPad Prism tool (version 9, Inc., San Diego, CA, USA), and Excel 2007 software was used to draw bar and line charts.

3. Results

3.1 Preliminary Phytochemical Screening

Qualitative chemical testing of 80% MEMC confirms the presence of alkaloids, flavonoids, saponin glycosides,

steroids, tannins, antraquinones, and phenols, including carbohydartes, fats, and proteins. The presence of these constituents were further supported by GC-MS analysis and indicates the plant's potential as a source of bioactive compounds with various therapeutic uses.

3.2 GC-MS Analysis

GC-MS technique is the robust approach of phytochemical profiling. In the present study the identification of the compounds were done with the help GC-MS coupled with NIST107 library in the crude methanolic extract of *Moringa concanensis* leaves. It revealed the presence of approximately 25 compounds, (Figure 2) some of which are given in Table 1 with potential pharmacological properties.

3.3 Acute Toxicity Study

An 80% MEMC was found to be safe. It did not show any toxicity or mortality in the tested dose of 2000 mg/kg bd. wt. throughout 14 days of observation. Based on this, two doses, 200 and 400 mg/kg bd. wt. of MEMC were selected for *in vivo* study.

3.4 Antioxidant Assay

An 80% MEMC showed strong antioxidant activity by scavenging H_2O_2 radicals with inhibition of 77.12± 0.90% at 50 µg/mL with IC_{50} of 24.996 µg/mL. The extract displayed comparable results as that of standard which showed 80.42± 0.72% of inhibition with IC_{50} of 22.205 µg/mL at same concentration. The extract also showed efficient superoxide scavenging activity with 63.25±0.38% inhibition at 50 µg/mL, IC_{50} of 39.141 µg/mL. It was found equally potent as that of standard which showed 70.88 ± 0.77% inhibition with IC_{50} 25.448 µg/mL at the same concentration. The data of H_2O_2 and superoxide scavenging are shown in Table 2 and 3.

3.5 *In vitro* Anti-Inflammatory Activity

In vitro anti-inflammatory effect is based on the inhibition of protein denaturation[34]. MEMC displayed an efficient anti-inflammatory activity protecting albumin from denaturing at various concentrations, and results are shown in Table 4. MEMC showed effective inhibition of denaturation of an egg protein compared to the standard

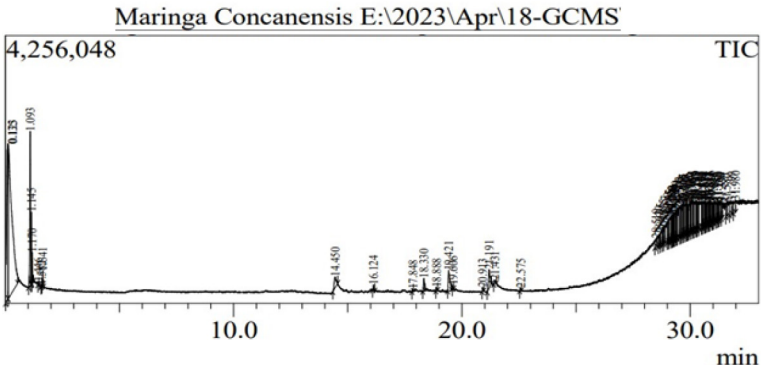


Figure 2: GC-MS chromatogram of methanolic extract of *Moringa concanensis* leaves

Table 1. GC-MS identified compounds of *Moringa concanensis* with their activities

S. No	Phytoconstituents	Nature	Biological activity
1	3 alpha –Phenylthio-3beta-acetoxy-flavanone	Flavonoid	Antioxidant and anti-inflammatory, analgesic[15]
2	Corydaldine	Isoquinoline alkaloid	Analgesic and anti-allergic[38]
3	Phytol,	Diterpene alcohol.	Antioxidant and anti-nociceptive[39]
4	1,2-dihydroanthra[1,2-d] thiazole-2,6,11-trione	Antraquinone derivative	Antifungal, anti-inflammatory and anticancer due to the presence of thiazole[40]
5	D-glucopyranose	Simple sugar	Nutritional
6	Pregnan-20-one,	Steroid	May have anti-inflammatory or hormonal effects[41]
7	1,2-Dihydro-2,4-diphenyl-quinazoline	Quinazoline derivative's	Anti-inflammatory and analgesic, antimicrobial[42]

Table 2. Hydrogen peroxide scavenging activity of MEMC

Concentration (µg/mL)	% inhibition	
	MEMC	Ascorbic acid
10	34.03 ± 0.44	35.77 ± 0.65
20	44.70 ± 0.70	48.62 ± 0.80
30	56.05 ± 0.71	58.51 ± 0.87
40	64.65 ± 0.81	69.70 ± 0.65
50	77.12 ± 0.90	80.42 ± 0.72
IC50 values	24.996 µg/mL	22.205 µg/mL

Table 3. Superoxide radical scavenging activity of MEMC

Concentration (µg/mL)	% inhibition	
	MEMC	Quercetin
10	12.51 ± 0.43	34.86 ± 0.80
20	26.82 ± 0.60	46.61 ± 0.95
30	38.40 ± 0.37	53.71 ± 0.60
40	51.41 ± 0.45	64.38 ± 0.72
50	63.25 ± 0.38	70.88 ± 0.77
IC50 values	39.141 µg/mL	25.448 µg/mL

with IC_{50} of 32.617 µg/ml and 22.387 µg/ml respectively, with inhibitions of 66.42± 0.77% and 78.28 ± 0.22%, respectively. The results indicates that the MEMC has a good anti-inflammatory activity.

3.6 Analgesic Activity of MEMC

3.6.1 Hot Plate Method

The MEMC (200 and 400 mg/kg bd. wt.) significantly prolonged the mice's reaction time to the thermal stimulation, in comparison to the normal control and diclofenac sodium (10 mg/kg bd. wt.). The extract at both 200 ($p < 0.05$) and 400 mg/kg ($p < 0.001$) dosage showed a significant increase in the pain latency period (Figure 3) with a maximum analgesic effect of 57.54% and 64.91%, when compared to the standard with 76.38 % at 60 min time period.

3.6.2 Tail Flick Method

The MEMC at both doses showed significant analgesic effect ($p < 0.001$) in comparison to the control, at 60 min of observation time (Figure 4), with inhibitions, 53.45% and 67.74%, respectively. While the standard diclofenac showed significant ($p < 0.001$) effect with 79.66% inhibition.

Table 4. An anti-inflammatory effect of MEMC *in vitro*

Concentration (µg/mL)	% inhibition	
	MEMC	Diclofenac
10	29.03 ± 0.54	34.96 ± 0.57
20	37.33 ± 0.80	48.44 ± 0.49
30	46.47 ± 0.44	60.37 ± 0.22
40	58.30 ± 0.59	68.60 ± 0.34
50	66.42 ± 0.77	78.28 ± 0.22
IC50 values	32.617 µg/mL	22.387 µg/mL

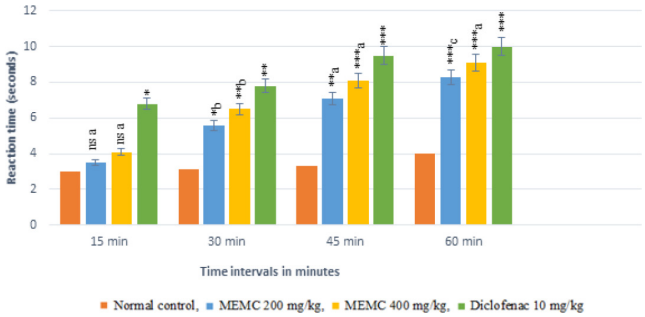


Figure 3. Effect of 80% MEMC on hotplate method
Mean ± SEM (n= 6), comparison of test with control, * - $p < 0.05$, ** - $p < 0.01$, *** - $p < 0.001$, ns - non-significant, and with diclofenac, a - $p< 0.001$, b – $p < 0.01$, c – $p < 0.05$.

3.6.3 Writhing Test

The MEMC decreased the number of writhes significantly at 200 mg/kg ($p < 0.01$) and 400 mg/kg ($p < 0.001$) compared to the negative control. The MEMC decreased the writhes significantly with 37.28 (200 mg/kg) and 49.10 % (400 mg/kg) inhibition dose-dependently, compared to the negative control and displayed the comparable effect with diclofenac, with 65.59 % inhibition. (Table 5).

3.7 Anti-Inflammatory Activity

3.7.1 Carrageenan-Induced Paw Edema

The MEMC significantly inhibited ($p < 0.01$) paw edema induced by carrageenan in the 4th hour (Figure 5), with 47.4% (200 mg/kg), 64.2% (400 mg/kg), and it was similar to the reference standard diclofenac sodium (10 mg/kg) with 75.6% inhibition. The extract at 400 mg/kg dose showed more effectiveness and activity was found to be similar to diclofenac sodium (10 mg/kg).

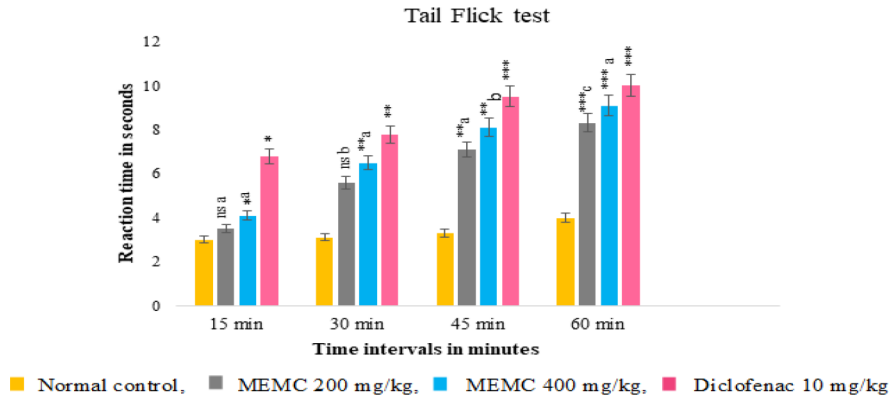


Figure 4. Effect of 80% MEMC on Tail Flick method.
Mean ± SEM (n= 6), comparison of test against control: * = p < 0.05, ** = p < 0.01, *** = p < 0.001), ns - non- significant, against diclofenac: a - p < 0.001, b - p < 0.01.

Table 5. Analgesic activity of MEMC in writhing method

Treatment	No. of writhes in 30 min (Mean ± SEM)	% Inhibition
Normal control	56.15 ± 1.17	-
MEMC (200 mg/kg)	46.65 ± 0.79^{*a}	37.28
MEMC (400 mg/kg)	33.91 ± 0.73^{**a}	49.10
Diclofenac sodium (10 mg/kg)	30.82 ± 0.67**	65.59

Values, mean ± SEM (n= 6), comparison of test against control: * = p < 0.01, ** = p < 0.001 and against diclofenac: a - p < 0.01

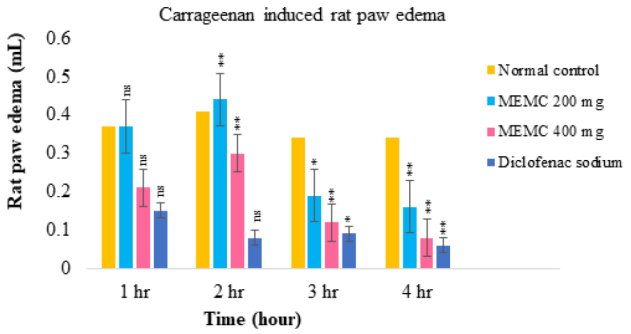


Figure 5. Effect of 80% MEMC on rat paw edema
mean ± SEM, (n= 6), comparison of test with control (* - p < 0.05, ** - p < 0.01, ns - non-significant)

3.7.2 Granuloma Induced with Cotton Pellets

This method is used to measure chronic phase of inflammation[37]. MEMC significantly (p < 0.001) inhibited the granuloma formation at 400 mg/kg dose compared to the control with a % inhibition of 51.6, and decreased the chronic phase of inflammation which is similar to that of diclofenac sodium with 57.90%. Even MEMC at 200 mg/kg was found to have significant effect (Table 6).

Table 6. Effect of MEMC on cotton-pellet induced granuloma

Treatment	Cotton pellets dry weight (mg) Mean±SEM	% Inhibition
Normal control	57.82 ± 1.52	-
200 mg/kg MEMC	48.67 ± 1.29*	41.65
400 mg/kg MEMC	39.51 ± 1.08**	51.6
Diclofenac sodium (10 mg/kg)	33.52 ± 0.86**	57.90

Values, mean ± SEM (n= 6), comparison of test against control: * = p < 0.01, ** = p < 0.001

4. Discussion

The search for the potential analgesic and anti-inflammatory drug from the traditional medicinal plant with minimal side effects is a socially and scientifically justified approach. Chronic pain and inflammation are the major health concerns that are often treated with synthetic drugs that can have significant side effects[43]. Use of the traditional medicinal plants is one of the best approaches, as these have exceptionally fewer side effects and have been used for many centuries. *Moringa concanensis*, one of the important traditional plants, rich in bioactive compounds, has been used by the Ayurveda, Unani, and many local communities to treat many illnesses, including pain and inflammation[8]. However, there has been no scientific data regarding the analgesic and anti-inflammatory activity of the leaves of *Moringa*

concanensis, which are the potent part of the plant. Thus, the present study investigates the analgesic and anti-inflammatory potential of this plant.

The phytochemical screening is one of the proximate analyses to identify the active constituents in the extracts. An alcoholic extract of the leaves of *Moringa concanensis* was reported to contain various active constituents, the presence of which was further supported in the present study. An 80% MEMC showed the presence of alkaloids, flavonoids, steroids, tannins, anthraquinones, and phenols[15-16]. Furthermore, GC-MS analysis of the MEMC showed the presence of some specific compounds belong to alkaloids (Corydaldine), flavonoids (3 alpha –Phenylthio-3beta-acetoxy-flavanone), terpenoids (Phytol), steroids (Pregnan-20-one), antraquinone (1,2-dihydroanthra[1,2-d] thiazole-2,6,11-trione) and quinazoline derivatives, which have been reported for their antioxidant, analgesic, and anti-inflammatory activities. These were the novel compounds that have not yet been reported in the leaves[44].

The antioxidant activity of 80% MEMC was determined using hydrogen peroxide and superoxide radicals scavenging activity. H_2O_2 is one of the reactive oxygen species (ROS) and is present in appreciable amounts in our body. However, its abnormal accumulation may lead to oxidative stress, lipid peroxidation, and DNA damage. MEMC showed strong antioxidant activity by scavenging H_2O_2 radicals, and it was found to be equally potent to the ascorbic acid. The MEMC also displayed significant superoxide scavenging activity. The superoxide radicals are produced by NAD(P)H oxidases present in various cells like endothelial, leukocytes, and other vascular cells, which modulate the expression of mediators and contribute to pain and inflammation[32]. Based on the results, it was inferred that MEMC showed significant concentration-dependent antioxidant activity against both hydrogen peroxide and superoxide radicals, showing potency comparable to ascorbic acid, a well-known antioxidant.

The antioxidant activity of the MEMC is attributed to the presence of phenolic compounds and flavonoids, which are known for their capacity to neutralize the free radicals and block their formation; thus, the presence of these constituents in the methanolic extract of the leaves of *Moringa concanensis* contributes to its potent antioxidant activity[13] and makes them a promising candidate for investigation in the management of pain and inflammation.

In the present study, the hot plate, tail flick, and acetic acid-induced writhing tests[34-35] were employed to evaluate analgesic potential of 80% MEMC, and anti-inflammatory activity was evaluated with carrageenan induced paw edema and cotton pellet induced granuloma inflammation models[36-37]. The doses of 200 mg/kg and 400 mg/kg of the extract were used to evaluate both the activities, and diclofenac sodium was used as reference standard.

The hot plate and tails flick tests were used to evaluate the central analgesic activity of the extract[35]. Mice claws and tail are very sensitive to the heat, and by subjecting the claws and tail to the constant heated plate and continuously heated water, the analgesic activity can be evaluated. In the study, a 15 sec cut-off time was given, and jumping, licking the claws, and flicking the tail were considered to evaluate the reaction time. The mice treated with 200 and 400 mg/kg prolonged the reaction times, with pain threshold of 57.54% and 64.91%, respectively, at 60 minutes, post administration, compared to the standard drug with 76.38 %. Similarly, in the tail immersion test, MEMC demonstrated dose-dependent inhibition with 53.45% and 67.74% for 200 and 400 mg/kg, respectively, nearing the efficacy of the standard, which showed 79.66% inhibition. The results suggest MEMC modulates nociceptive signaling in the central nervous system, via activation of PAG (periaqueductal gray matter), triggering release of endogenous peptides like enkelphalins or endorphins and to suppress pain impulse transmission at spinal synapse.

Peripheral analgesic effect was evaluated by acetic acid-induced writhing test[35]. The intraperitoneal injection of acetic acid irritates and stimulates the abdomen region and encourages the release of many endogenous inflammatory chemicals, such as histamine, serotonin, and PGs and induces the writhes. The extension of the lower limbs, lengthening of the body, and tightening of the abdominal muscles are writhes induced by acetic acid and are indicators of chemically induced peripheral pain. The MEMC significantly reduced writhes, with 37.28% and 49.10%, inhibition at 200 and 400 mg/kg respectively, comparable to diclofenac (65.59%). The activity is likely due to the inhibition of the release of the endogenous substances like prostaglandins, leukotrienes, and other inflammatory mediators released by acetic acid[45].

The previous report identified the presence of high concentrations of flavonoids and phenols in *Moringa concanensis*, known for their antioxidant and anti-

inflammatory activity[15,34]. The present study confirmed these constituents using phytochemical testing and GC-MS analysis which provide identification of specific compounds in the methanolic extract. Thus, the observed analgesic effects of MEMC are likely to the presence of flavonoids and phenols in the MEMC[34]. The study demonstrated the dose-dependent activity. A dose of 400 mg/kg of MEMC was more effective compared to the 200 mg/kg, indicating the presence of higher concentrations of phytoconstituents is required to achieve the significant effect of the extract.

The carrageenan-induced paw edema model is a commonly utilized method for assessing acute inflammatory responses, as carrageenan has non-antigenic and localized proinflammatory properties. In this study, acute inflammation was induced via sub-plantar injection of 1% carrageenan into rats' left hind paw, eliciting a biphasic succession. The first phase involves the release of endogenous mediators such as serotonin (5-HT), bradykinin, and histamine, followed by the overproduction of COX-2 and its pro-inflammatory prostaglandins in the later phase, which amplify vascular permeability and edema formation. The extract at both tested doses of 200 and 400 mg/kg significantly reduced the rat paw edema from the first to the fourth hour following carrageenan induction. Therefore, 80% MEMC's anti-inflammatory activity might be due to the result of interferences in these phases, especially in the later stages[36,37,45].

The cotton-pellet induced granuloma model, a well-established method for studying chronic inflammation, was employed to evaluate the anti-inflammatory effect of MEMC in the chronic phase of inflammation. This model induces granuloma formation at a site of implantation in rats, characterized by initial protein-rich exudate accumulation, fluid infiltration, and retaining macrophages, fibroblasts, and neutrophils. Over time, this progresses into highly vascularized granulomatous tissue. The cotton absorbs the granuloma and increases its wet weight (fluid and protein influx) and dry weight (indicative of granulomatous tissue mass).

In the present study, MEMC administration at doses of 200 and 400 mg/kg significantly decreases granuloma tissue formation, evidenced by reduced dry weight of cotton pellets with inhibition of 41.65% ($p < 0.01$), and 51.6% ($p < 0.001$), respectively. MEMC showed similar efficacy to that of the reference drug diclofenac sodium ($p < 0.001$) which showed 57.90% inhibition. Based on these results, it is clear that MEMC suppresses the chronic inflammation by inhibiting the infiltration of macrophages, fibroblasts, and neutrophils, as well as impeding the migration of phagocytes and leukocytes to the inflamed area. The observed anti-inflammatory activity in both acute and chronic phases may be attributed to bioactive constituents in MEMC, including flavonoids, phenols, steroids, and terpenoids, which are known for their anti-inflammatory activity[37].

5. Conclusion

The present study demonstrates that the 80% methanolic extract of *Moringa concanensis* leaves exhibited significant analgesic and anti-inflammatory effects in both acute and chronic models. The extract showed central and peripheral analgesic activities along with potent antiinflammatory action, likely due to the synergistic effects of flavonoids, phenolic compounds, and other bioactive compounds modulating nociception and inflammation pathways. These findings uphold the traditional use of leaves of *Moringa concanensis* in alleviating pain and inflammation. Further research is recommended to fractionate the crude extract, isolate bioactive compounds, and study their specific mechanism of action, thereby advancing therapeutic potential.

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7. Conflict of interest

The authors declare that there is no conflict of interest.

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9. Authors' Contributions

Conceptualization, S.M. and S.K.; methodology, S.M. and S.K.; software, S. M., S.K. and N.B.; formal analysis, M.G.R and C.M.; investigation, S.M. and S.K.; resources, S.M. and S.K..; data curation, S.M. S.K. A.K. and V.B.D; writing—

original draft preparation, S.M. and S.K.; writing—review and editing, S.M. and S.K.; visualization, S.M. S.K. V.B.D and N.B.; supervision, M.G.R. and S.M. All authors have read and agreed to the published version of the manuscript.

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