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(RESEARCH ARTICLE)

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Evaluation of anti-inflammatory activity in Wistar rats using ethanolic extract of moringa leaves by Carrageenan induced inflammation

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Abstract

Around the world, inflammatory disorders are a leading source of morbidity and disability among workers. Standard steroidal and non-steroidal anti-inflammatory medication treatments for inflammation carry the same risk of harming different organ systems. It has been suggested that the herbal plant Moringa oleifera can effectively treat a variety of inflammatory diseases. However, there aren't enough scientific studies to support these assertions.

From the beginning of human civilization, medicinal plants have been used to treat a variety of illnesses. According to the World Health Organization, almost 80% of people in underdeveloped nations struggle to pay for synthetic medications and must rely on traditional medicines, mostly made from plants, to meet their basic medical needs. In numerous tropical and subtropical nations, Moringa oleifera LAM (Moringaceae) has been utilized as a traditional medicine. It is a very nutrient-dense vegetable that may be used to cure microbiological infections, poisonous bites, and rheumatism. Researchers looked into the Methanol extract of *Moringa oleiferous* (MEMO) anti-inflammatory properties. Using a plethysmometer, In order to investigate the anti-inflammatory properties of ethanolic extract of Moringa oleifera leaves (EEMO) in experimentally induced inflammation in albino rats, the current study was conducted. The results of the present study revealed that oral administration of EEMO exhibited anti-inflammatory effect in the models studied

Keywords: Anti-inflammatory; Albino rats; *Moringa oleifera*; Inflammation; Paw oedema; Plethysmometer

1. Introduction

1.1. Inflammation

Inflammation is a complex biological response of the body's immune system to injury, infection or damage.[1][2]

It's a natural defense mechanism that aims to protect the body.

A dynamic system, inflammation is triggered in response to mechanical trauma, burns, microbial infections, and other harmful stimuli that can endanger the host's health. This process involves changes in blood flow, increased vascular permeability, tissue destruction through leucocyte activation and migration with the production of reactive oxygen derivatives (oxidative burst), and the production of local inflammatory mediators, including PGs, leukotrienes, and platelet-activating factors caused by phospholipase A2, COXs, and lipoxygenase. Proinflammatory cytokines, such as TNF-α, IL-1β, and vascular endothelial growth factor (VEGF), are key players in inflammation.[3]

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1.2. Anti-inflammation

The process of preventing or lessening inflammation, which is the body's normal reaction to damage, infection, or negative stimuli, is known as anti-inflammation. It entails the application of drugs or therapies that lessen the redness, swelling, discomfort, and heat that are indicative of inflammation.[4]

1.3. *Moringa oleiferous*

The tree or plant *Moringa oleiferous* is referred to as Soanjan in Hindi and Sigru in Sanskrit. Often referred to as the horseradish or drumstick tree, Moringa oleifera is one of the thirteen species in the *Moringaceae* family, which is also the most well-known and extensively grown. It is cultivated throughout Africa and Asia's tropics and subtropics. Because practically every aspect of Moringa oleifera is beneficial to humans, it is referred to as a "Miracle tree."[5] Because of its high nutritional content, it is frequently referred to as "natural nutrition for the tropics. “Many Asian nations, especially India, eat the leaves, young pods, and blooms as a nutritious vegetable.

Traditional medicine has long utilized *Moringa oleiferous* seeds and other parts for their therapeutic properties. The foliage is claimed to possess hypotensive, diuretic, antispasmodic, and anti-inflammatory properties. The roots are said to possess hepatoprotective, anticonvulsant, anthelmintic, and antispasmodic properties. The pods are said to have hypotensive and hypolipidemic effects.[1][6]

The seeds are said to possess antioxidant, antitumor, and antiarthritic properties. Amino acids, alpha and beta-carotene, sterols, terpenes, saponins, tannins, sugars, glycosides, alkaloids, and flavonoids are all present in an aqueous extract of leaves.

Figure 1 Plant *Moringa Olifera*

1.4. Plethysmometer

In preclinical research, a paw plethysmometer is a tool used to quantify changes in mouse paw volume, mainly for evaluating medication efficacy, inflammation, and oedema. It is frequently employed in pharmacological research to assess analgesic and anti-inflammatory medications.[8]

Working Principle:

- The rodent's paw is positioned inside a digital or fluid-filled chamber.
- The gadget logs variations in volume brought on by oedema or inflammation.
- Measurements taken before and after inflammation are compared.
- The reduction of swelling is the basis for evaluating the efficacy of anti-inflammatory medications.

Figure 2 Plethysmometer

Applications:

- Research on inflammation and arthritis.
- Research on pain and analgesic medications.
- Evaluation of vascular permeability.

This apparatus is a useful tool for testing novel therapies for inflammatory diseases in pharmaceutical and biological research.[7]

2. Method

The study was commenced after obtaining approval from Institutional Animal Ethical Committee of Malla Reddy institute of pharmaceutical sciences, Hyderabad, India, registered under CPCSEA India.

2.1. Animals

Wister rats of both sexes (200–250gm) are employed. Following their arrival, the animals were divided into treatment groups and randomly assigned to polypropylene cages with bedding made of paddy husk. In the polypropylene cages, the animals were kept in a well-ventilated environment with a light-dark cycle of 12:12. Libitum, standard pellet diet, and drinking water were supplied during the experiment. Following CPCSEA norms, the rats were acquired from Prasad Vyas Lab in Uppal, Hyderabad. Prior to the commencement stage, the animals were given a week to get used to the laboratory environment. Every experimental technique was carried out in compliance with IAEC criteria.[11][12]

2.2. Plant collection

Moringa oleiferous plant leaves were collected in and erodes in the month February from the locality near to bollarum, Secunderabad, Telangana.

Figure 3 Leaves of Moringa

2.3. Drying and pulverization of the plant material

After collection, the leaves were washed to remove the dust particles and allowed to dry in a shade for complete drying.

Then the dried leaves were powdered into coarse powder in a mixer grinder. [15][16]

Figure 4 Dried leaves of moringa

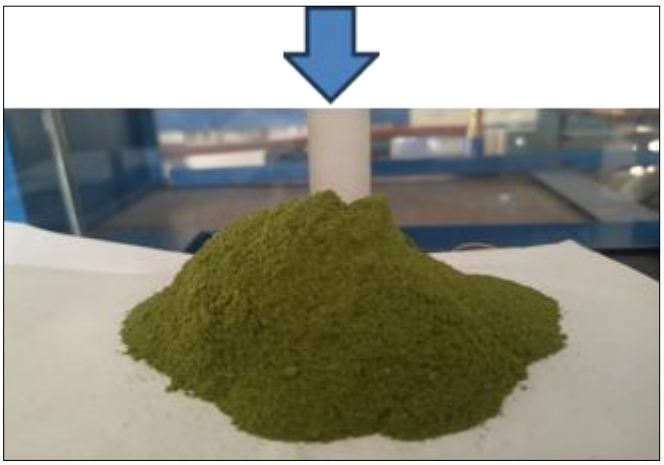

Figure 5 Powder of Moringa

2.4. Preparation of plant extract (Soxhlet apparatus)

The Soxhlet extraction method is a widely used technique for extracting bioactive compounds from plant materials, including moringa powder, by utilizing a solvent to continuously extract desired compounds. To begin, a suitable amount of moringa powder (40g) is weighed and placed in a thimble made of cotton. The thimble is then inserted into the Soxhlet extractor, which is connected to a round-bottom flask containing the chosen solvent. The solvent, which is ethanol, depending on the target compounds, is heated to its boiling point in the flask. As the solvent evaporates, it moves up into the Soxhlet chamber, where it condenses and drips over the moringa powder. This cycle repeats, with the solvent repeatedly passing over the moringa powder and extracting its bioactive constituents. The process is typically run for several hours (usually 4-6 hours) to ensure complete extraction. Throughout the procedure, the temperature is carefully controlled to maintain the solvent at its boiling point while cooling water is circulated through the condenser to facilitate efficient condensation.[17]

Figure 6 Beginning of the extraction

Figure 7 During of the extraction

Figure 8 Last cycle of extraction (colour less siphon tube)

Once the extraction is complete, the thimble containing the moringa powder is removed, and the solvent in the round-bottom flask is evaporated using a rotary evaporator or gentle heating. This step ensures that the extracted compounds are isolated from the solvent. The resulting extract, which may be oily, resinous, or powdery, is then dried to remove any remaining solvent traces. This can be done by placing the extract in a drying oven or desiccator. After drying, the final extract is collected and stored in a clean, labelled container in a cool, dark place to maintain the integrity of the bioactive compounds. Soxhlet extraction is highly effective for obtaining high yields of bioactive substances from moringa powder, making it ideal for research or commercial purposes where concentrated extracts are needed.[17][21]

2.5. Drugs and chemicals

- Carrageenan (Grace lifetech pvt. ltd.),
- Diclofenac (Cipla healthcare limited),
- Saline 0.9% and
- Distilled water was used in this study.

2.6. Experimental design

For evaluation of anti-inflammatory activity, the carrageenan induced inflammation method was used. Animal were divided into five groups of six animals each. Group 1 served as control and was given normal saline (5 ml/kg), group 2 served as standard and was given Diclofenac-standard anti-inflammatory drug (0.5 mg/kg), group 3,4 and 5 was given Ethanolic extract of Moringa oleifera leaves 100 mg/kg, 250mg/kg and 500mg/kg respectively. Total 30 animals were utilized for this study.[21][23]

2.7. Carrageenan induced paw oedema model

This is among the most widely used techniques for acute inflammatory screening. Adult Wistar rats were given 0.1 ml of a newly/Freshly made 1% w/v carrageenan suspension in normal saline intramuscularly into the sub plantar region of their right hind paw. Each group received a single dosage of the appropriate medication which cause acute inflammation. The control group's animals were given merely regular saline. An hour before to the carrageenan challenge, test medications and a chosen standard medication were given orally based on body weight. Every animal had a mark made at the ankle joint. [24]

Using a plethysmoFigure, paw volume up to the ankle joint was assessed in drug-treated and untreated groups immediately prior to and three hours following the carrageenan challenge.

Following each hour of the carrageenan injection for up to four hours, the percentage inhibition of right paw oedema was determined.

- Paw oedema =(V_t-V_o)
- V_o= Paw oedema at the time zero
- V_t= Paw oedema at the time (1,2,3, and 4)

$$\text{Percentage inhibition of oedema} = \frac{(\text{Vt}-\text{Vo})\ \text{control} - (\text{Vt}-\text{Vo})\ \text{treated groups}}{(\ \text{Vt}-\text{Vo})\text{control}} \times \frac{100}{1}$$

- Inducing carrageenan to the animal
- Inducing diclofenac to the animal
- Inducing moringa extract to the animal

2.8. Acute Toxicity studies

- Oral acute
- Intraperitoneal

2.9. Sub chronic toxicity studies

Figure 9 Measuring paw volume of Moringa treated animals' Statistical analysis

Figure 10 Measuring paw volume of Diclofenac treated animal

Table 1 Observation

Animals	**1**	**2**	**3**	**4**	**5**	**6**
Normal Paw volume Albino Wistar rats	0.6 mL	0.7 mL	0.8 mL	0.9 mL	0.8 mL	0.6 mL
CONTROL GROUP: Carrageenan 0.1mL +Saline Solution paw oedema volumes	1.8 mL	1.6 mL	1.6 mL	2.1 mL	1.9 mL	1.7 mL
STANDARD GROUP: Carrageenan 0.1mL +Diclofenac 10mg/kg paw oedema volumes	0.8 mL	0.9 mL	0.7 mL	0.8 mL	0.7 mL	0.7 mL
TEST GROUP-1: Carrageenan 0.1mL + Moringa Extract 100mg/kg paw oedema volumes	1.6 mL	1.5 mL	1.4 mL	1.8 mL	1.7 mL	1.5 mL
TEST GROUP-2 Carrageenan 0.1mL + Moringa Extract 250mg/kg paw oedema volumes	1.3 mL	1.2 mL	1.0 mL	1.2 mL	1.4 mL	1.0 mL
TEST GROUP-3: Carrageenan 0.1mL + Moringa Extract 500mg/kg paw oedema volumes	0.7 mL	0.7 mL	0.8 mL	0.7 mL	0.9 mL	0.8 mL

The acquired data was presented as mean ± SEM. Differences between groups that were statistically significant were determined by applying the LSD test after one-way analysis of variance (ANOVA).

3. Results

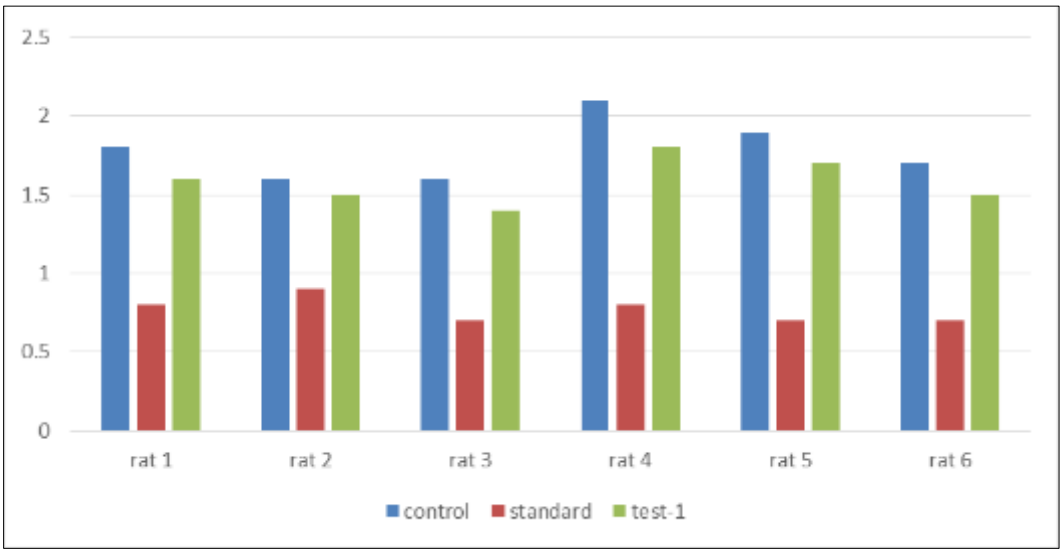

Figure 11 *Reduction of inflammation with 100mg moringa extract*

Animals pre-treated with Diclofenac shows significant reduction in inflammation after 5 hrs of Diclofenac administration. Percentage of inhibition being 64.32% compared to the control group

Ethanolic extract of leaves of moringa oleifera(100mg/kg) exhibited anti-inflammatory activity by reducing rat paw oedema by 42.35% after 5 hours test drug administration when compared to standard

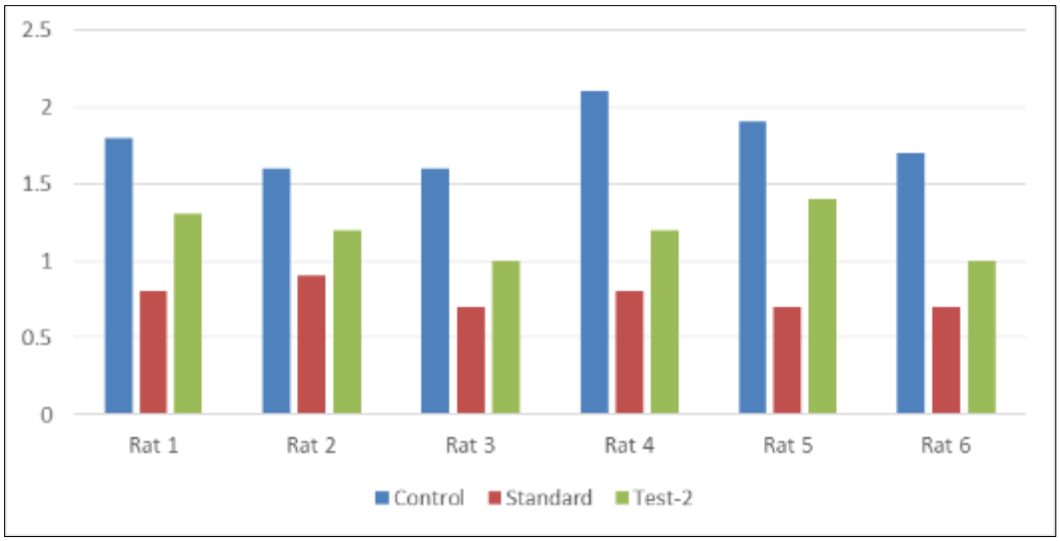

Figure 12 Reduction of inflammation with 250mg moringa extract

Animals pre-treated with Diclofenac shows significant reduction in inflammation after 5 hrs of Diclofenac administration. Percentage of inhibition being 64.52% compared to the control group

Ethanolic extract of leaves of moringa oleifera(250mg/kg) exhibited anti-inflammatory activity by reducing rat paw oedema by 52.47% after 5 hours test drug administration when compared to standard

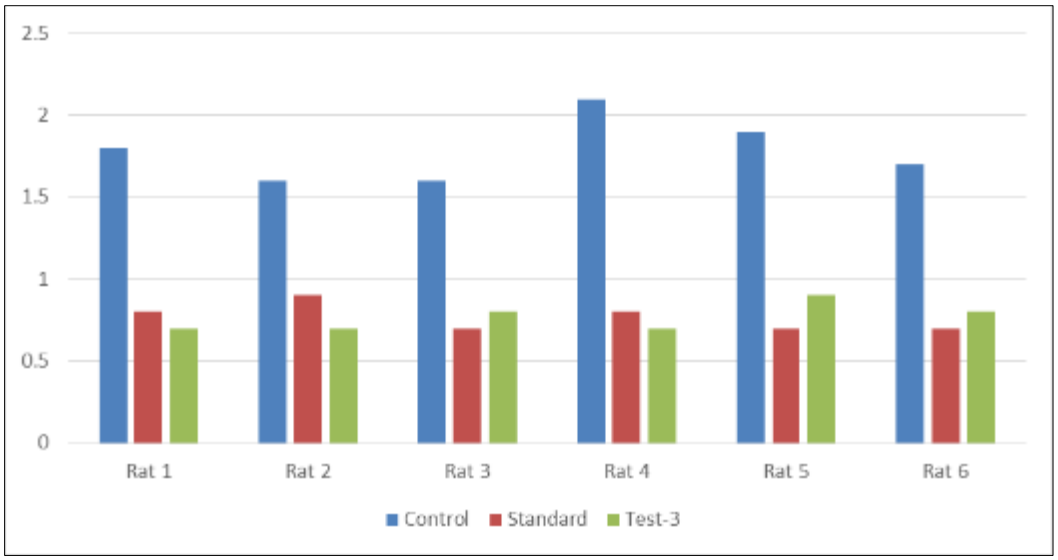

Figure 13 Reduction of inflammation with 500mg moringa extract

Animals pre-treated with Diclofenac shows significant reduction in inflammation after 5 hrs of Diclofenac administration. Percentage of inhibition being 64.54% compared to the control group

Ethanolic extract of leaves of moringa oleifera(500mg/kg) exhibited anti-inflammatory activity by reducing rat paw oedema by 62.82% after 5 hours test drug administration when compared to standard

4. Conclusion

As far as we know, the anti-inflammatory properties of *Moringa oleiferous* leaf ethanolic extract have been assessed. Because *Moringa oleiferous* leaves contain flavonoids and alkaloids, their ethanolic extract has strong anti-inflammatory properties. The use of extract in traditional system medicine to treat anti-inflammatory disorders is supported by these findings. Our interest in more molecular research on the plant of other extracts is justified by these facts.

Compliance with ethical standards

Disclosure of conflict of interest

If two or more authors have contributed in the manuscript, the conflict of interest statement must be inserted here.

Statement of ethical approval

The study was commenced after obtaining approval from Institutional Animal Ethical Committee of Malla Reddy institute of pharmaceutical sciences, Hyderabad, India, registered under CPCSEA India.

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