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Morinaga Oleifera loaded niosomes for healing application: Arthritis

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ABSTRACT
The study aimed to evaluate the potential anti-arthritic properties of *Moringa Oleifera* leaf extract loaded into niosomes. The extract was obtained using the Soxhlet method with various solvents, and the best extract was determined based on its yield. The niosomes were prepared using the Ether Injection Method and incorporated into a gel. The effectiveness of the treatment was tested on rats with induced arthritis through various methods such as paw edema, body weight gain, arthritic index, haematology profile, and Eddy's hot plate thermal analgesia method. The results indicated a positive response to the *Moringa Oleifera* treatment against arthritis. The niosomes were also evaluated for their size and shape and underwent gel permeation testing using a Franz diffusion cell. However, further research is necessary to confirm the effectiveness of the treatment in humans.

Keywords: *Biopesticides, Management, Potato, Viruses,*

1. INTRODUCTION

Arthritis is a broad term used to describe any disorder that affects the joints, which can result in inflammation, pain, and loss of function in one or more joints. Rheumatoid Arthritis (RA) is a common form of arthritis that can cause synovial inflammation, cartilage damage, and bone erosions. [1]

Currently, there is no cure for RA, and patients require lifelong therapy to manage the symptoms of the disease. NSAIDs are commonly used to treat arthritis, but they can cause adverse effects or lack of efficacy in some patients. Herbal preparations, such as *Moringa Oleifera*, have drawn attention due to their potential to provide natural therapy without adverse effects. [2]

Moringa Oleifera is a fast-growing tree that is native to the Indian subcontinent and is widely cultivated for its young seed pods and leaves, which are used as vegetables and in traditional herbal medicine. The leaves of *Moringa Oleifera* have been selected for a detailed pharmacological and pharmacognostical study to identify their distinguishing characters that can be useful in designing a suitable topical dosage form. [3,4]

Niosomes, a multilamellar vesicle prepared from non-ionic surfactants, have been used to increase the bioavailability of drugs through a biological membrane. In this study, *Moringa Oleifera* leaf extract was loaded into niosomes, which act as a

nanocarrier to facilitate the delivery of the active compound. The niosomes were then incorporated into a gel formulation, which showed prolonged action and improved stability due to the formation of a reservoir effect in the skin, increasing the drug retention capacity into the skin. [5]

Overall, the study suggests that *Moringa Oleifera* has potential as a natural agent for treating arthritis, and niosomes loaded with *Moringa Oleifera* leaf extract could be a useful delivery system for improving the effectiveness of the active compound in treating arthritis. [6]

2. MATERIALS AND METHODS

The leaves were obtained locally and washed in running water before being air-dried under shade for 30 days. The leaves were then extracted using a standard Soxhlet method with ethanol as the solvent. A specific amount of dried leaves was weighed and placed into a whatman cellulose thimble, which was then placed in the Soxhlet extractor. The temperature for extraction was set at 80°C, and after 6 hours of extraction, the extracts were concentrated and further dried in an oven to remove the solvent completely. This process would result in a concentrated extract of *Moringa Oleifera* leaves, which could then be used for further analysis or experimentation. [7,8]

Fig: *Moringa Oleifera* leaves

Arthritis Induction: CFA Induced Arthritis:

This passage describes the experimental design for a study investigating the effects of Moringa extract on CFA-induced arthritis in male rats. CFA-induced arthritis is characterized by an acute phase of local inflammatory reactions followed by a chronic inflammatory phase with secondary arthritic lesions. The study used healthy male rats of 8-10 weeks old, kept in standard conditions and allowed to acclimatize for ten days before the experiment. [9]

The hind limbs of all groups except the control group were injected with CFA on day 0. The daily doses of Moringa extract or indomethacin (a nonsteroidal anti-inflammatory drug) were started on day 0 and continued to day 21. The extract was obtained using ethanol as the solvent via a standard Soxhlet extraction method. The extract was concentrated and dried in an oven to remove the solvent completely. [10]

Animals and experimental design:

All the animals were randomly grouped into six animals per group (n = 6):

Group I (Normal control), no CFA injection

Group II (CFA-control), no treatment

Group III given indomethacin 2.5 mg/kg/day as reference standard

Group IV (E500), given 500 mg/kg/day of Moringa extract and

Group V (E250), given 250 mg/kg/day of Moringa extract.

The daily dose for all treatment groups (groups III–V) and CFA-control group was administered at 8–9 AM and the measurements were conducted at 2 PM [11].

Assessment of arthritis:

Effect of Moringa extract on CFA-induced arthritic paw edema

The hind paws edema was observed on days 0, 3, 6, 9, 12, 15, 18, and 21 post CFA injection using digital micrometer gauge. The percentage increase in paw edema was calculated by the following formula:

% increase in paw oedema=To−Tt/To×100

Where, To is the mean of paw thickness at day 0 and Tt is the mean of paw thickness at a particular time. [12]

Effect of Moringa extract on body weight gain

All the animal's body weight was monitored from day 0 and repeated on days 3, 6, 9, 12, 15, 18 and 21 post CFA injection. The percent weight change was calculated using the following formula:

% weight change=Wt−Wo/Wt×100

Where Wt is the weight of animal at time t and Wo is the weight of animal on day 0. [13]

Arthritic index

The severity of induced arthritis were evaluated by a method described by Vijayalaxmi et al., where visual scoring system of the signs and symptoms on scale of 0–4 per limb as where 0: no change, 1: slight swelling and erythema of the limb, 2: mild swelling and erythema of the limb, 3: gross swelling and erythema of the limb, and 4: gross deformity and inability of the limb. A score of the both hind limb was counted and a score more than 1 exhibits the arthritis whereas a maximum score of the arthritis is 8. The measurement was started on day 3 post CFA injections and repeated on days 6, 9, 12, 15, 18, and 21. [14]

Eddy's hot plate thermal analgesia method

In this method heat was the source for pain. A constant heat (55^0C) was given to the animals by hot plate and the response of the animals e.g., jumping, paw licking and withdrawal was observed. First, the animal was placed on the hot plate and allowed to acclimatize for three to five minutes; then, start the instrument with a cut-off period of 18 seconds and 55 ± 0.1°C maximum heat to avoid harming the animals and observe the reaction time (in seconds) and the time taken by the animal to start paw licking or jumping, which is considered as baseline. The procedure was repeated 30, 60, 90, 120 and 180 minutes after administration of calculated dose of Moringa extract & indomethacin. At each measuring time, the test was repeated three times for each animal with 5-minute intervals. [15] The latency time was calculated using the following equation:

Latency time = Tt − To

where Tt is the time taken by the rat to respond at particular measurement time and To is the time taken by the rat to respond at time 0.

Preparation of Niosomes:

Ether injection method

The method described is a niosome preparation method, which involves the use of Pluronic L64 surfactant and cholesterol to form a clear solution in diethyl ether. In a separate vessel, an aqueous solution of the Moringa Oleifera leaf extract is heated to 40oC using a water bath. The mixture of Pluronic L64 surfactant and cholesterol is then added slowly to the preheated aqueous solution of the drug. The solution is kept at 40oC until the diethyl ether is completely evaporated. The resulting solution contains large unilamellar niosomes, which are vesicular structures composed of nonionic surfactants and cholesterol that encapsulate the drug. This method is commonly used for the delivery of drugs and bioactive compounds, as it can protect them from degradation and enhance their bioavailability. [16]

Fig: Preparation of niosomes

Preparation of Gel:

The steps involved in the method are:

1. Accurately weighing 2g of Carbopol 940 and dispersing it in 50 ml of distilled water. The beaker is left aside for half an hour to allow the Carbopol to swell.
2. Stirring the mixture using a mechanical stirrer at 1200 rpm for 30 minutes.
3. Taking 5 ml of propylene glycol and the required amount of niosomal extract in another beaker, and stirring the mixture properly.
4. Adding the niosomal extract solution to the Carbopol dispersion with constant stirring.
5. Making up the volume to 100 ml with distilled water.
6. Adjusting the pH of the gel formulation to the required skin pH (6.8-7) using Triethanolamine. [17]

Fig: Gel Formation

Evaluation of Gel:

Particle size analysis:

A microscopy-based technique to determine the size distribution and mean diameter of the niosomes is very useful. The process involves measuring the size of individual niosomes using an optical microscope with a calibrated eyepiece micrometer. About 200 niosomes are measured, and the average size is calculated to obtain the size distribution range and mean diameter. Additionally, microphotographs of the niosomes are taken using a 9-megapixel digital camera. [18]

***In-vitro* diffusion studies:**

Franz Cell apparatus is a commonly used in vitro skin permeation assay for studying drug delivery. The apparatus consists of two primary chambers separated by a membrane. The test product, in this case, the gel containing niosomal extract, was applied to the membrane through the top chamber. The receiver compartment was filled with 10 ml of distilled water and an egg membrane was placed on it. The gel was covered with the donor compartment and clamped to get undisturbed. The apparatus was placed on a magnetic stirrer with a speed of 300 rpm and temperature was maintained at 32°C. This testing determines the amount of niosomal extract that has permeated the membrane. Sample withdrawn at 1 hour, along with Moringa extract as standards, were subjected to characterization studies by thin layer chromatography (TLC). The samples were injected on a TLC Silica Gel (5 cm × 10 cm) plate and the plate was developed using Toluene: Ethyl acetate (9:1) as the mobile phase. [19]

Fig: *In-vitro* diffusion study

3. RESULTS AND DISCUSSION

PHARMACOLOGICAL STUDY:

Effect of Moringa extract on CFA-induced arthritic paw edema:

The results indicate that both the ethanol extract of Moringa leaves and indomethacin showed significant inhibition of inflammatory paw edema in comparison to the CFA-control group. The Moringa extract at a dose of 250 mg/kg exhibited the highest percentage of inhibition (70.60%) during the chronic phase, which was even more effective than indomethacin (70.28%). These results suggest that the Moringa extract has anti-inflammatory activity and could be a potential alternative to traditional anti-inflammatory drugs.

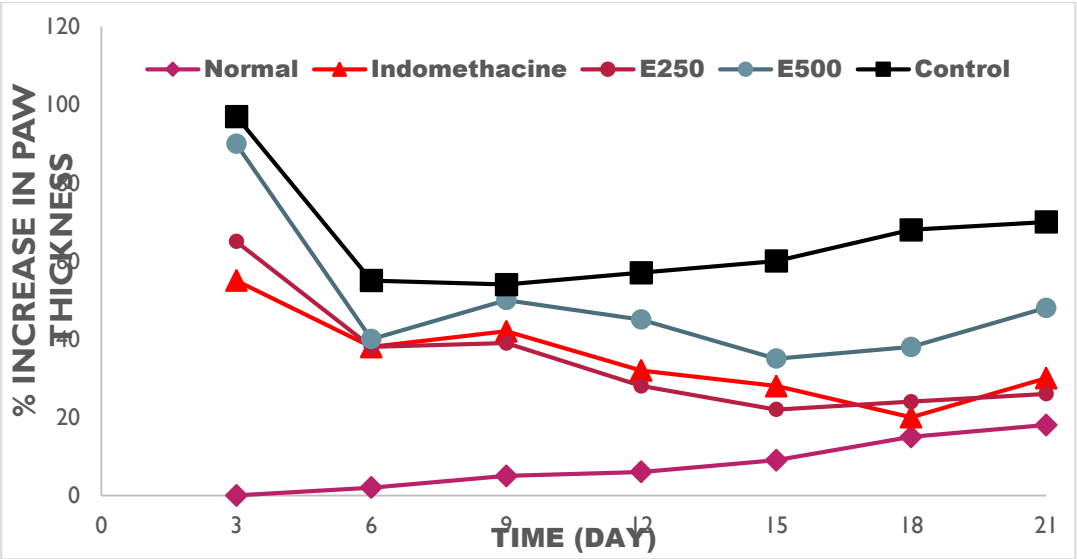

Fig. 1: Percentage increase in paw edema

Percent increase in paw edema in CFA-induced arthritis in rats of CFA-control (no treatment), indomethacin at dose 2.5 mg/kg/day, E500 and E250 groups that were given Moringa leaves extract at dose 500 and 250 mg/kg/day, respectively.

Effect of Moringa extract on animal's body weight:

The study found that the treatment groups given the crude extract at a dose of 250 mg/kg showed a significant increase in body weight at $p < 0.05$. However, at a dose of 500 mg/kg, the increase in body weight was non-significant compared to the CFA-control group. The indomethacin group also showed a non-significant weight gain, despite being almost constant rate increase. The normal group showed a steady increase in body weight. All treatment groups and the CFA-control group

showed a biphasic pattern, where there was a continuous increase in body weight from day 6 until day 18 (established chronic phase), and then the weight started to decrease.

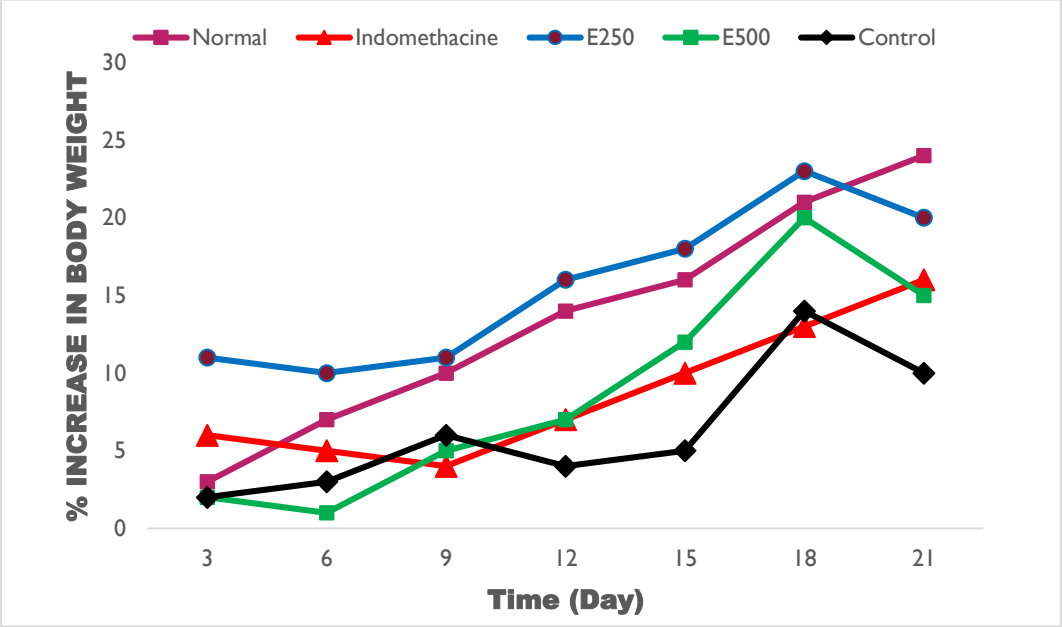

Fig. 2: Percentage increase in body weight

Percent increase in body weight of normal control, disease control, and treatment groups. E500: the animal group that was given Moringa leaf extract at dose 500 mg/kg and E250: the animal group that was given Moringa extract at dose 250 mg/kg.

Arthritic index:

The arthritic score for both hind paws was recorded on day 3 post CFA injection by measuring paw thickness, ankle and knee diameter, swelling and redness of paw's phalanges, and signs of inflammation on the eye, mouth, nose, ear, and tail. The results showed a significant decrease in arthritic index in the indomethacin group and the groups that were given Moringa extract at both doses compared to the CFA-control group. The animal group that was given Moringa extract at a dose of 250 mg/kg body weight showed better arthritic index, indicating less clinical signs of inflammation and arthritis compared to the animal groups that were given either indomethacin or Moringa extract at a dose of 500 mg/kg body weight.

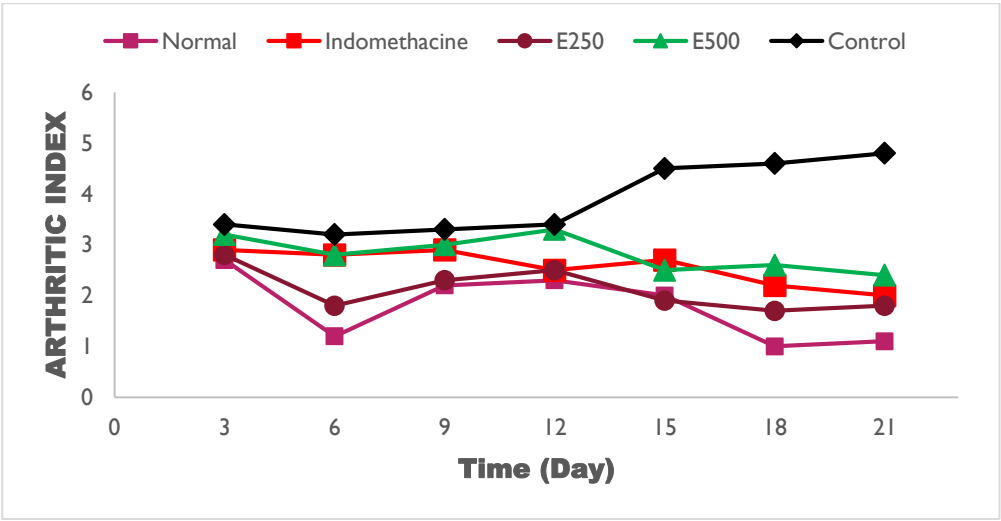

Fig. 3: Arthritic Index

Arthritic index for disease control group, indomethacin group, and two animal groups (E500 and E250) that were given Moringa extract at doses of 500 and 250 mg/kg body weight.

Hematology profile:

Based on Table 1, it can be observed that the CFA-control group showed abnormal results in all the evaluated haematological parameters except WBC. This suggests the development of iron deficiency anemia, which is one of the clinical manifestations of RA. However, all treatment groups including the group that was given indomethacin showed a significant improvement in HGB, RBC, and PCV parameters compared to the CFA-control group. The animal group that was given Moringa extract at a dose of 250 mg/kg showed the highest effect, with a significant difference compared to the CFA-control group.

For WBC, only animal groups that were given either indomethacin or Moringa extract at a dose of 500 mg/kg showed a significant effect compared to the CFA-control group. In the ESR test, all treatment groups showed a significant effect compared to the CFA-control group.

Table 1: Effects of Ethanol Extract of *Moringa oleifera* Leaves on Some Hematologic Parameters in CFA-induced RA Rats.

Normal range	CFA-control (mean ± SEM)	Normal (mean ± SEM)	Indomethacin (mean ± SEM)	E500 (mean ± SEM)	E250 (mean ± SEM)
HGB (g/dL) (13.5–18.4)	11.28 ± 0.757	12.38 ± 0.136	12.99 ± 0.360	13.60 ± 0.712	13.91 ± 0.456
PCV (%) (38.9–54.9)	33.98 ± 0.0.015	39.63 ± 0.007	39.42 ± 0.014	40.58 ± 0.032	41.20 ± 0.005
RBC (10^{12}/L) (7.8–10.2)	6.89 ± 0.278	7.39 ± 0.076	7.78 ± 0.288	7.97 ± 0.610	8.09 ± 0.242
WBC (10^{9}/L) (5.9–19.0)	12.75 ± 0.567	9.59 ± 1.987	7.51 ± 1.342	7.90 ± 2.120	9.76 ± 0.754
ESR (mm/h) (0.5–1.45)	2.09 ± 0.032	1.11 ± 0.128	0.68 ± 0.043	0.73 ± 0.156	0.75 ± 0.217

Eddy's hot plate:

Anti-nociceptive activity refers to the ability to reduce or block pain sensation. Here, the study found that the ethanol extract of Moringa leaves had a significant anti-nociceptive activity in a dose-dependent manner, meaning that the higher the dose of extract given, the stronger the anti-nociceptive effect. The activity was observed 90 minutes after oral administration and reached its peak at 180 minutes, after which the effect started to decline. In comparison, the activity of indomethacin (a commonly used non-steroidal anti-inflammatory drug) started earlier, at 60 minutes, reached its peak at 120 minutes, and then started to decline. This information is shown in Figure 4 of the study.

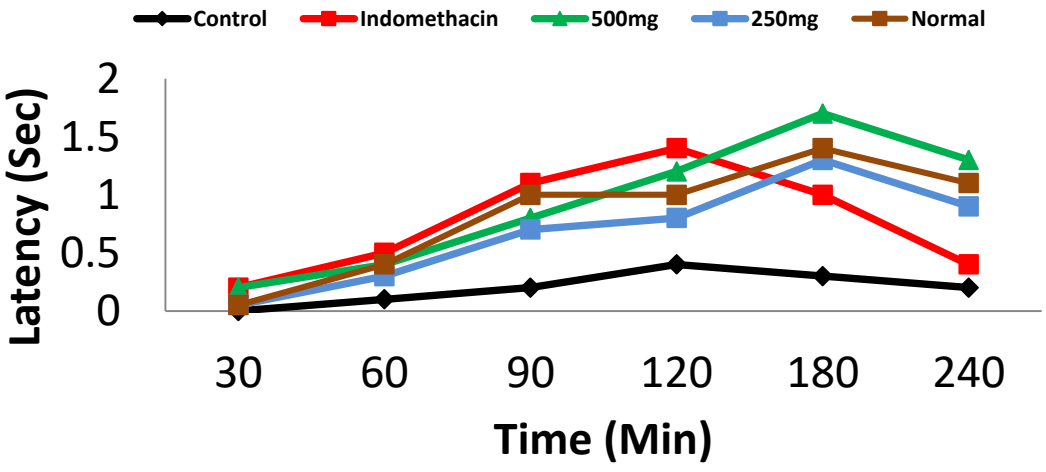

Fig. 4: Thermal analgesia method using Eddy's hot plate

Effect of ethanol extract of Moringa oleifera leaves at different dose levels on latency time of normal rats measured using Eddy's hot plate thermal analgesia method. Moringa extract at doses of 500 and 250 mg/kg body weight were given.

Preparation of Niosomes:

Ether injection method

The ether injection method was chosen for the preparation of niosomes because it is known to produce stable and large unilamellar vesicles compared to other techniques. The method involves dissolving a mixture of Pluronic L64 surfactant and cholesterol in diethyl ether to form a clear solution. In a separate vessel, an aqueous solution of Moringa Oleifera leaf extract is prepared and maintained at 40°C using a water bath. The mixture of Pluronic L64 surfactant and cholesterol is then slowly added to the preheated aqueous solution of the extract. The temperature of the solution is maintained at 40°C until the diethyl ether is completely evaporated. Once the ether is removed, the mixture is transferred and observed to have formed large unilamellar niosomes containing the Moringa Oleifera leaf extract.

Evaluation of Gel:

It is good to know that particle size analysis was carried out to evaluate the size distribution range and mean diameter of the niosomes. An optical microscope with a calibrated Eyepiece micrometer was used to measure around 200 niosomes individually, and the average was taken to determine their size distribution range and mean diameter. Microphotographs of niosomes were taken using a 9-megapixel digital camera. The histogram for particle size distribution and particle size was shown in Figure 5. The results showed that the average vesicular sizes of niosomes ranged from 2.5µm to 7.4µm, indicating that the niosomes were of uniform size and spherical in shape as depicted in the microphotographs in Figure 5.

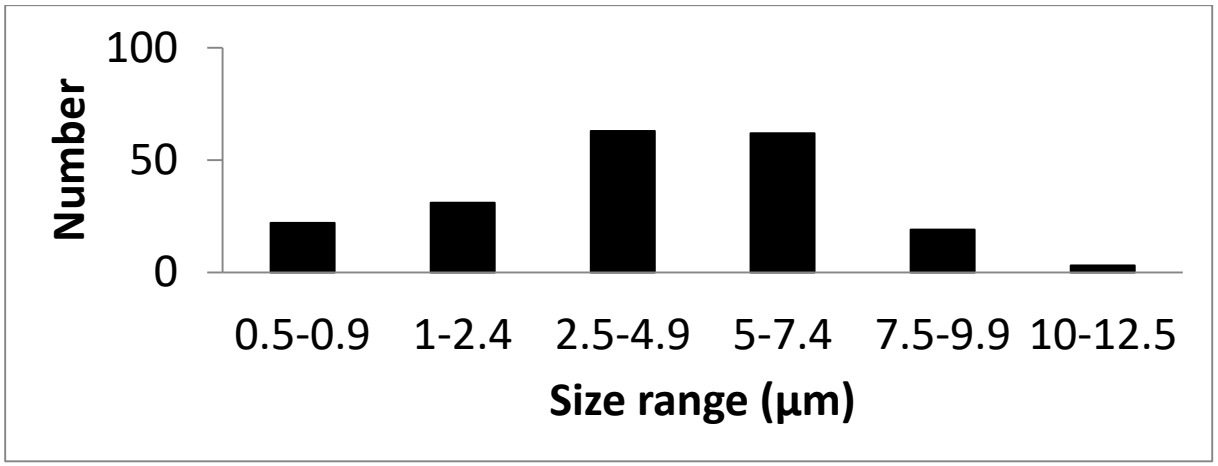

Figure 5: Size distribution of Niosomes

***In-vitro* Diffusion Studies:**

The passage describes the results of a Thin Layer Chromatography (TLC) analysis of a niosomal gel formulation. The major peaks observed in the niosomal gel were at Rf 0.35, 0.46, and 0.56 with peak areas of 35.43%, 27.02%, and 26.08%, respectively. There were also minor peaks at Rf 0.61, 0.79, and 0.95 with peak areas between 1% and 3%. The diffused sample showed three major peaks at Rf 0.45, 0.5, and 0.58 with peak areas of 43.64%, 33.92% and 22.43% respectively. The band at Rf 0.45 and 0.58 were observed in both the pure sample and diffused sample of the formulated gel. The TLC analysis was performed to confirm the penetration of the niosomal gel sample across the semipermeable membrane.

Peak	Maximum Rf	Area (%)
1	0.04	4.43
2	0.08	1.93
3	0.35	35.43
4	0.46	27.02
5	0.56	26.08
6	0.61	1.31

7	0.79	3.15
8	0.95	0.37

Table 2: Analytical data from TLC of Niosomal Gel

Peak	**Maximum Rf**	**Area (%)**
1	0.45	43.64
2	0.50	33.92
3	0.58	22.43

Table 3: Analytical data from TLC of Diffused Sample

4. CONCLUSIONS

Based on the study, it can be concluded that the ethanol extract of *Moringa Oleifera* leaves has significant anti-nociceptive, anti-inflammatory, and anti-arthritic activities. The study also found that a dose of 250 mg/kg of the extract is safe, low-toxic, and effective compared to the group that was given indomethacin or a 500 mg/kg dose. The niosomal formulation of the extract was found to have good particle size distribution and can potentially be a useful medication for the treatment of rheumatoid arthritis. Overall, this study provides scientific evidence that supports the traditional use of *Moringa Oleifera* leaves as a natural remedy for pain and inflammation.

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