



EVALUATION OF THE ANTI-RHEUMATIC AND ANTI-INFLAMMATORY ACTIVITIES OF ETHANOLIC *Moringa Oleifera* BARK EXTRACT USING IN VITRO PROTEIN DENATURATION AND IN VIVO EGG ALBUMIN INDUCED PAW EDEMA MODELS

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ABSTRACT

Moringa oleifera is a medicinal plant traditionally used for the management of various inflammatory conditions. The present study was carried out to evaluate the anti-rheumatic and anti-inflammatory activities of ethanolic *Moringa oleifera* bark extract using **in vitro protein denaturation and in vivo egg albumin-induced paw edema models**. The bark was collected, shade-dried, powdered and extracted with ethanol by maceration. Preliminary phytochemical screening revealed the presence of alkaloids, flavonoids, steroids, tannins, glycosides and volatile oils. In-vitro anti-rheumatic activity was evaluated by the egg albumin denaturation method, where the extract showed concentration-dependent inhibition, with **55% inhibition at 15 mg and an IC₅₀ value of 11.01 mg**. Diclofenac sodium was used as the standard and showed an IC₅₀ value of 11.40 mg. In-vivo anti-inflammatory activity was assessed using egg albumin-induced paw edema in Wistar rats. The extract showed reduction in paw edema at both tested doses, with the high dose producing **36.34% inhibition at 4 h, compared with 37.40% for indomethacin**. The findings suggest that ethanolic *Moringa oleifera* bark extract possesses promising anti-rheumatic and anti-inflammatory activities and may serve as a potential natural source of bioactive anti-inflammatory agents.

KEYWORDS: *Moringa Oleifera*, Bark Extract, Anti-Rheumatic Activity, Anti-Inflammatory Activity, Protein Denaturation, Egg Albumin-Induced Paw Edema, Phytochemical Screening.

INTRODUCTION

Arthritis

Arthritis is a chronic inflammatory disorder affecting one or more joints and is commonly associated with pain, swelling, stiffness, and joint damage. Common forms include rheumatoid arthritis and osteoarthritis. [1,2,3]

Rheumatoid Arthritis

Rheumatoid arthritis (RA) is a systemic autoimmune inflammatory disease that primarily affects the synovial joints. Persistent inflammation may result in cartilage destruction, bone erosion, joint deformity, and disability. [6,7]

Inflammation

Inflammation is a protective physiological response to infection, tissue injury, toxic substances, or other harmful stimuli. Excessive or prolonged inflammation can contribute to tissue damage and chronic inflammatory disorders. [9]

Anti-inflammatory Drugs

Anti-inflammatory drugs are used to reduce excessive inflammation, tissue damage, pain, and other associated symptoms. The major classes include glucocorticoids and non-steroidal anti-inflammatory drugs (NSAIDs), which mainly act by reducing inflammatory mediator production. [10]

Moringa oleifera

Moringa oleifera Lam. is a medicinal plant belonging to the family Moringaceae and is widely used in traditional medicine. Different parts of the plant have been reported to possess anti-inflammatory, antioxidant, analgesic, antimicrobial, hepatoprotective, and anti-arthritis activities. [11]

Phytochemical Constituents

M. oleifera contains several bioactive phytoconstituents, including flavonoids, phenolic compounds, alkaloids, tannins, saponins, steroids, terpenoids, and isothiocyanates. These constituents may contribute to its reported pharmacological activities.^[14]

Rationale of the Study

The presence of various bioactive constituents and the reported traditional use of *M. oleifera* in inflammatory conditions provide a basis for evaluating its anti-rheumatic and anti-inflammatory potential. Therefore, the present study evaluates ethanolic *M. oleifera* bark extract using **in vitro protein denaturation and in vivo egg albumin-induced paw edema models**.

MATERIALS AND METHODS

Collection and Preparation of Plant Material

The bark of *Moringa oleifera* was collected from a local area of Tamil Nadu, India, and authenticated by a qualified botanist. The collected bark was washed with distilled water and air-dried for 5–10 days. The dried bark was powdered and passed through a sieve to obtain a uniform powder.^[15]

Preparation of Ethanolic Extract

About **45 g of powdered bark** was extracted with ethanol by maceration for three consecutive 24-hour periods. The extract was filtered and concentrated using a rotary evaporator to obtain the crude ethanolic extract.^[16]

Preliminary Phytochemical Screening

The ethanolic bark extract was subjected to qualitative phytochemical screening for alkaloids, flavonoids, steroids, tannins, glycosides, volatile oils, reducing sugars and saponins. The tests were performed using standard qualitative procedures based on characteristic colour changes or precipitate formation.^[17]

In vitro Anti-rheumatic Activity:

The anti-rheumatic activity was evaluated using the **egg albumin denaturation method**. Different concentrations of the extract were tested for their ability to inhibit protein denaturation. Diclofenac sodium was used as the standard drug. The absorbance was measured at **660 nm**, and the percentage inhibition of protein denaturation was calculated.^[18]

In vivo Anti-inflammatory Activity:

Wistar albino rats weighing **150–200 g** were used for the study and maintained under standard laboratory conditions. Animals were divided into four groups: control, standard, low-dose extract and high-dose extract, with six animals in each group. The extract was administered at **200 and 400 mg/kg**, while indomethacin (**10 mg/kg**) was used as the standard drug.

Acute inflammation was induced by subplantar injection of **0.1 mL of 1% egg albumin** into the right hind paw. Treatments were administered one hour before induction, and paw thickness was measured at hourly intervals up to 4 hours using a Vernier caliper. The percentage inhibition of paw edema was calculated.^[19]

Statistical Analysis

The results were expressed as **mean $\pm$ SEM**. Statistical analysis was performed using one-way ANOVA followed by Dunnett's multiple comparison test. A value of **P < 0.05** was considered statistically significant.

RESULTS AND DISCUSSION

PRELIMINARY SCREENING

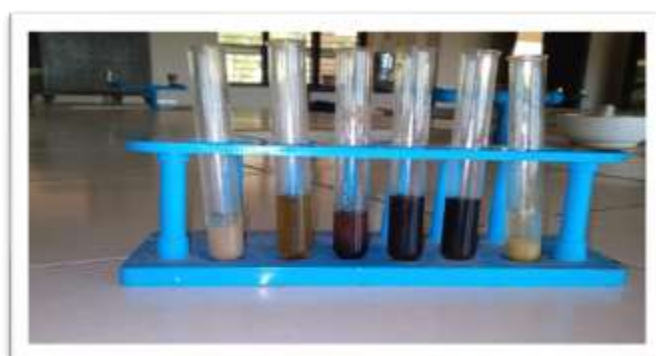


Fig 1: PRELIMINARY SCREENING

**TABLE:1 (PRELIMINARY SCREENING)**

Phytoconstituents	Test	Observations	Results
alkaloids	Mayer's test	Creamy white	+
flavonoids	NaOH test	Yellow color	+
Steroids	Salkowski test	Reddish-brown colour	+
Tannins	Ferric chloride test	Greenish/blue-black colour	+
Glycosides	Fehling's test	Brick-red precipitate	+
Volatile oils	NaOH + HCl test	Turbidity/emulsion	+

Anti-Rheumatic activity

To assess anti-Rheumatic activity in vitro, the albumin denaturation method was utilized. At 15 mg, demonstrated a 55% inhibition and an IC₅₀ value of 11.01 mg, compared to the standard diclofenac sodium, which had an IC₅₀ value of 11.40 mg. During our investigation of various concentrations in a dose-dependent manner, we found that many concentrations exhibited antioxidant and anti-inflammatory properties of MOET. When compared to diclofenac sodium, MOET showed moderate anti-Rheumatic effects.

Table 2: MOET *In vitro* anti-Rheumatic activity

Table 2: NOET <i>In vitro</i> anti-Rheumatic activity						
S.NO	Concentration(mg)	COD	SOD	% inhibition	Average	IC ₅₀ (mg/ml)
1	5 mg	0.29	0.17	41%	43.6 ± 2.58	11.01 mg/ml
2		0.29	0.16	44%		
3		0.29	0.17	41%		
4		0.29	0.16	44%		
5		0.29	0.15	48%		
6		0.29	0.16	44%		
1	10 mg	0.29	0.16	44%	47.6 ± 3.14	
2		0.29	0.15	48%		
3		0.29	0.15	48%		
4		0.29	0.14	51%		
5		0.29	0.14	51%		
6		0.29	0.16	44%		
1	15 mg	0.29	0.13	55%	55.3 ± 2.58	
2		0.29	0.13	55%		
3		0.29	0.12	58%		
4		0.29	0.13	55%		
5		0.29	0.14	51%		
6		0.29	0.12	58%		
In vitro Standard Diclofenac sodium						
1	10 mg	0.29	0.09	68%	71.6 ± 3.51	
2		0.29	0.08	72%		
3		0.29	0.07	75%		

Table 3: MOET Anti- Rheumatic Activity IC₅₀ Value (mg) Compared to Standard Diclofenac Sodium IC₅₀ Value (mg)

GEL And Rheumatic Activity IC ₅₀ Value (mg) Compared to Standard Diclofenac Sodium IC ₅₀ Value			
S.NO	Concentration (mg)	Average (%)	IC ₅₀ (mg)
1	5 mg	44%	11.01 mg/ml
2	10 mg	48%	
3	15 mg	55%	
Standard Diclofenac sodium			
1	5 mg	75%	11.40 mg/ml
2	10 mg	71%	
3	15 mg	65%	

Fig 2: Anti-Rheumatic Activity IC₅₀ Value (mg)

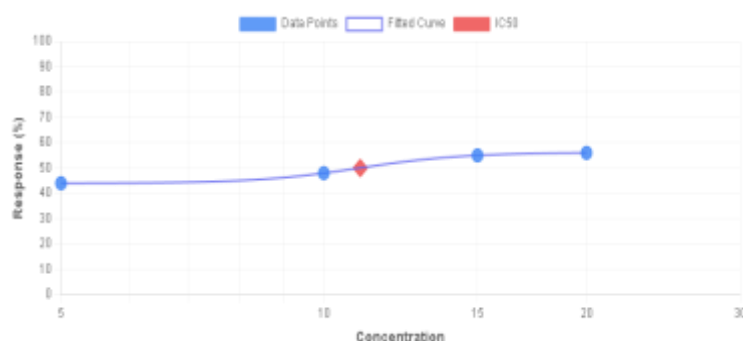


TABLE:4

IN VIVO METHOD:

Effect of *M. Oleifera* on Albumin induced paw edema in rat

GROUPS	1HR	2HR	3HR	4HR	P Value
DISEASE CONTROL	5.90±0.08959	6.15±0.07479	6.40±0.06841	6.55±0.1042	0.6791
STANDARD	5.05±0.09518	4.70±0.1096	4.35±0.04155	4.10±0.04351	0.1558
MOE HD	5.25±0.04091	4.90±0.05416	4.46±0.04683	4.17±0.04583	0.8457
MOE LD	5.60±0.05459	5.30±0.02852	5.05±0.04740	4.75±0.01183	0.0687

All values were expressed as mean ± SEM, n = 6, * P < 0.05, **P < 0.01, ***P < 0.001 as compared to the disease control group. Results were done by one-way ANOVA followed by Dunnett's test

Effect of *E.Moringa Oleifera* on Albumin induced Paw Edema in Rats

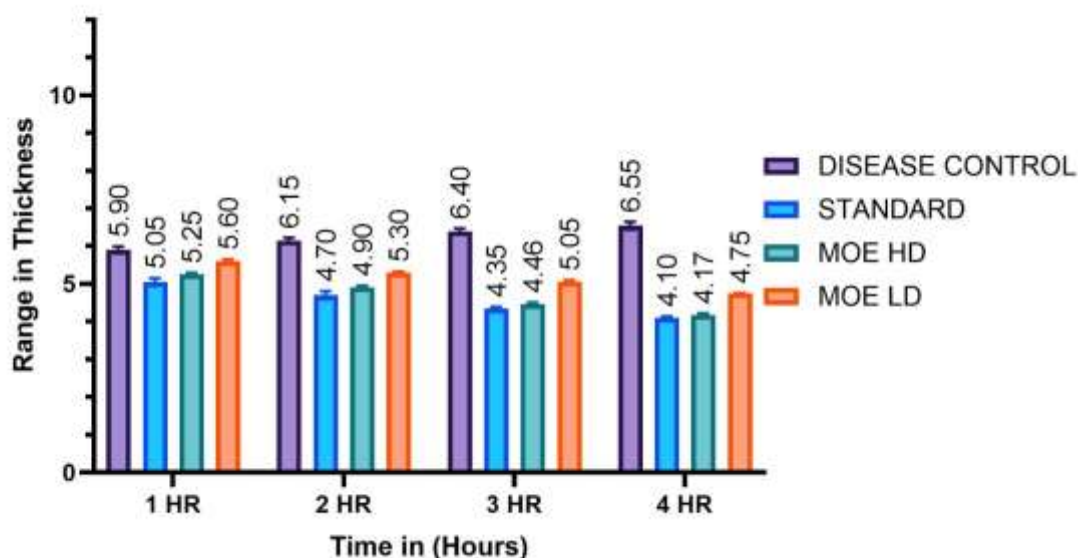


FIG 3: Effect of *E. Moringa Oleifera* on Albumin Induced Paw Edema in Rats

TABLE:5

% Inhibition of paw edema in *M. Oleifera* and Albumin treated rats

Groups	1st Hour	2nd Hour	3rd Hour	4th Hour
Disease control	—	—	—	—
Standard	14.41%	23.58%	32.03%	37.40%
High Dose	11.02%	20.33%	30.31%	36.34%
Low Dose	5.08%	13.82%	21.09%	27.48%

All values were expressed as mean $\pm$ SEM, $n = 6$, * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ as compared to the disease control group. Results were done by one-way ANOVA followed by Dunnett's test

% Inhibition of paw edema in E.M.Oleifera and Albumin treated rats

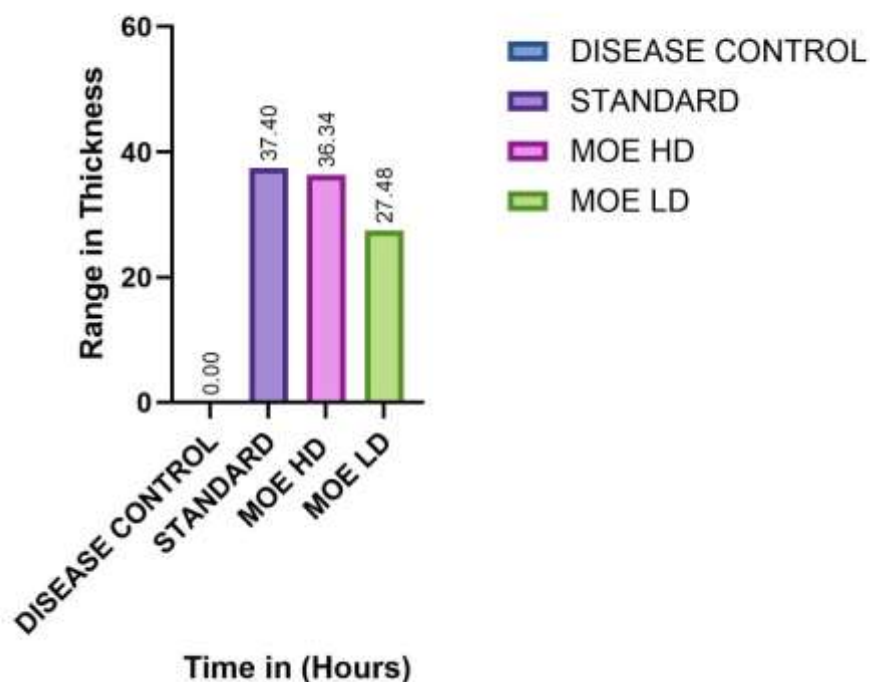


Fig 4 : % inhibition of paw edema in E.M. Oleifera and Albumin treated rats



Fig 5 : IN VIVO Anti-inflammatory activity



Discussion

Phytochemical screening of the ethanolic *Moringa oleifera* bark extract revealed the presence of alkaloids, flavonoids, steroids, tannins, glycosides, and volatile oils. These phytoconstituents may contribute to the biological activities observed in the present study, particularly the anti-inflammatory and anti-arthritic effects.

The ethanolic *Moringa oleifera* bark extract exhibited concentration-dependent inhibition of protein denaturation in the in-vitro assay. The extract showed $43.6 \pm 2.58\%$, $47.6 \pm 3.14\%$, and $55.3 \pm 2.58\%$ inhibition at concentrations of 5, 10, and 15 mg/mL, respectively. The highest inhibition was observed at 15 mg/mL. The calculated IC_{50} value of the extract was 11.01 mg/mL. This activity suggests that the extract may have the potential to inhibit protein denaturation, which is associated with inflammatory and arthritic conditions. The observed activity may be attributed to the presence of flavonoids, phenolic compounds, and other bioactive phytoconstituents. Thus, the findings indicate potential in-vitro anti-arthritic activity of the ethanolic bark extract.

At the fourth-hour assessment, the high-dose *Moringa oleifera* bark extract showed an inhibition of paw edema (36.34%) that was close to that of the standard drug indomethacin (37.40%), whereas the low-dose extract showed 27.48% inhibition. These findings indicate that the higher dose of the extract exhibited greater anti-inflammatory activity than the lower dose.

Conclusion

The present study demonstrated that ethanolic *Moringa oleifera* bark extract possesses potential in-vitro anti-arthritic and in-vivo anti-inflammatory activities. The extract inhibited protein denaturation and reduced egg albumin-induced paw edema in rats. The higher dose produced greater inhibition of paw edema than the lower dose and showed an effect comparable to the standard drug at the fourth hour. These findings suggest that *Moringa oleifera* bark may serve as a potential natural source of bioactive compounds with anti-inflammatory and anti-arthritic properties. Further studies are required to isolate the active constituents and establish their mechanisms of action.

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