Journal of Neonatal Surgery
ISSN(Online): 2226-0439
Vol. 14, Issue 24s (2025)
https://www.jneonatalsurg.com

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Anti-Inflammatory Potential of a Novel Oral Suspension Formulated with Prosopis Cineraria Pod Extract

Shivank Tyagi*[1], Girish Kumar Vyas*[2], Nitin Nama[3], M. K. Gupta[4]

[1*]Research Scholar, Career Point School of Pharmacy, Career Point University, Kota, India

Email ID: Shivank.tyagi06@gmail.com

[2*]Associate Professor, Career Point School of Pharmacy, Career Point University, Kota, India

Email ID: girishvyas10@gmail.com

[3]Associate Professor, Career Point School of Pharmacy, Career Point University, Kota, India

[4]Professor, Career Point School of Pharmacy, Career Point University, Kota, India

Corresponding Author:

Shivank Tyagi*[1], Girish Kumar Vyas*[2]

[1*]Research Scholar, Career Point School of Pharmacy, Career Point University, Kota, India

Email ID: Shivank.tyagi06@gmail.com

[2*]Associate Professor, Career Point School of Pharmacy, Career Point University, Kota, India

Email ID: girishvyas10@gmail.com

Cite this paper as: Shivank Tyagi, Girish Kumar Vyas, Nitin Nama, M. K. Gupta, (2025) Anti-Inflammatory Potential of a Novel Oral Suspension Formulated with Prosopis Cineraria Pod Extract. *Journal of Neonatal Surgery*, 14 (24s), 737-746

ABSTRACT

Inflammation is a natural defense mechanism of the body against injury, infection, or irritation. However, chronic or excessive inflammation can lead to various health issues. While synthetic anti-inflammatory drugs are available, they often come with side effects. This has led researchers to explore herbal alternatives, which are generally considered safer. Prosopis cineraria, commonly known as "Khejri," is a tree native to arid regions and has been traditionally used in Indian medicine to treat ailments like fever, asthma, and joint pain. Its pods, leaves, and bark are rich in natural compounds such as flavonoids, tannins, phenols, and alkaloids, which possess anti-inflammatory properties. This study focuses on extracting phytoconstituents from Prosopis cineraria pods, formulating a stable oral suspension, and evaluating its physicochemical properties and in vitro anti-inflammatory activity. The formulated suspension underwent various tests, including organoleptic evaluation, pH measurement, viscosity determination, sedimentation volume, redispersibility, flow rate, particle size analysis, microbial load testing, and stability studies as per ICH guidelines. In vitro anti-inflammatory activity was assessed using protein denaturation assay, HRBC membrane stabilization method, and nitric oxide scavenging assay. The results indicated that the Prosopis cineraria extract exhibited significant anti-inflammatory activity, suggesting its potential as a natural alternative to synthetic anti-inflammatory drugs

Keywords: *Prosopis cineraria, anti-inflammatory, herbal medicine, oral suspension, phytoconstituents* .

1. INTRODUCTION

Inflammation is a natural response of the body to injury, infection, or irritation. While it plays an important role in healing, long-term or excessive inflammation can lead to pain, swelling, and tissue damage. Many synthetic anti-inflammatory drugs are available, but they often come with side effects like gastric irritation, liver problems, or dependency with prolonged use[1]. Because of this, researchers are increasingly looking at herbal medicines as safer alternatives.

Plants have been used for centuries in traditional systems of medicine for treating inflammation. Herbal drugs are rich in natural compounds like flavonoids, alkaloids, and tannins that have proven anti-inflammatory properties. These natural compounds are generally better tolerated by the body and cause fewer side effects[2,3].

Prosopis cineraria, commonly known as “Khejri,” is a well-known tree in traditional Indian medicine. Its pods, leaves, and bark have been used in folk remedies to treat various health conditions including fever, asthma, and joint pain. Previous studies have shown that *Prosopis cineraria* contains flavonoids, tannins, phenols, and alkaloids, which are known to reduce inflammation and oxidative stress[4]. An oral suspension is one of the most patient-friendly dosage forms, especially for people

who have difficulty swallowing tablets or capsules. It ensures uniform dosing, quick onset of action, and better acceptability[5].

Objective of this study: This study aims to extract and screen phytoconstituents from *Prosopis cineraria* pods, formulate a stable oral suspension, evaluate its physicochemical properties, assess in vitro anti-inflammatory activity, and perform stability testing as per ICH guidelines

2. MATERIALS AND METHODS

1. Collection and Drying: *Prosopis cineraria* pods were collected, washed with distilled water, and shade-dried for 7–10 days. The dried pods were powdered and stored in airtight containers.

2. Extraction: About 100 g of pod powder will be extracted using Soxhlet apparatus with 70% ethanol for 6–8 hours. The extract will be filtered, concentrated under reduced pressure using a rotary evaporator, and dried to obtain the crude extract, which will be stored at 2–8°C[6].

3. Preliminary Phytochemical Screening[7,8]

Qualitative phytochemical tests were carried out on the ethanolic extract of *Prosopis cineraria* pods to detect major phytoconstituents:

- Alkaloids: Identified using Mayer's (white/cream precipitate), Dragendorff's (reddish-brown precipitate), and Wagner's tests (brown precipitate).
- Flavonoids: Detected by Shinoda test, showing pink to orange coloration.
- Tannins and Phenolics: Confirmed by ferric chloride test, yielding blue-black or greenish coloration.
- Saponins: Foam test showed persistent froth for >10 minutes.
- Glycosides: Keller–Killiani test showed reddish-brown ring and bluish-green upper layer.
- Steroids and Terpenoids: Liebermann–Burchard and Salkowski tests produced green and reddish-brown colorations, respectively[8].

4. Quantitative Estimation of Phytoconstituents

- **Total Phenolic Content (TPC):** Determined using Folin–Ciocalteu reagent with gallic acid as standard. 0.5 mL extract was mixed with 10% Folin reagent and 7.5% sodium carbonate, incubated for 30 minutes, and absorbance read at 765 nm. Results were calculated from the standard curve and expressed as mg gallic acid equivalent (GAE) per gram of extract [9].
- **Total Flavonoid Content (TFC):** Estimated using the aluminum chloride colorimetric method with quercetin as standard. 0.5 mL extract was reacted with aluminum chloride, potassium acetate, and distilled water, incubated for 30 minutes, and absorbance was measured at 415 nm. Results were expressed as mg quercetin equivalent (QE) per gram of extract [9].

5. Formulation of Herbal Oral Suspension: The formulation of the herbal oral suspension involves preparing multiple trial batches by varying the concentrations of key excipients to optimize stability and palatability. Suspending agents such as sodium carboxymethyl cellulose (NaCMC) or xanthan gum will be used in concentrations ranging from 1% to 3% w/v to achieve the desired viscosity and uniform dispersion. Sweeteners like sucrose or sorbitol will be added to enhance taste, while preservatives including methylparaben and propylparaben will be incorporated to inhibit microbial growth. Each batch will be evaluated for its physical appearance, sedimentation rate, re-dispersibility, and overall uniformity. The preparation process begins by slowly dispersing the suspending agent in a portion of purified water with continuous mechanical stirring to ensure lump-free hydration. Once a gel-like base is formed, sweeteners, preservatives, and flavoring agents are added one by one, ensuring complete dissolution of each. The accurately weighed dried extract of *Prosopis cineraria* is then gradually added to the base under constant stirring for even distribution. Finally, the volume is adjusted with purified water, and the suspension is stirred thoroughly to achieve a uniform consistency. The final product is filled into clean, sterilized amber-colored bottles to protect it from light and stored under cool, dry conditions for further evaluation [10,11].

6. Evaluation of Suspension: Following test were decided to carried out for the quality check.

- **Organoleptic Evaluation**: Color, odor, taste, and appearance are assessed visually and sensorially to gauge acceptability and detect any changes during storage.
- **pH Measurement**: pH is determined using a calibrated pH meter to ensure stability, prevent microbial contamination, and guarantee safety during administration.
- **Viscosity Determination**: Viscosity is measured with a Brookfield viscometer to ensure ease of pouring, uniform dose delivery, and minimal sedimentation.
- **Sedimentation Volume (F)**: Sedimentation volume is assessed to determine the physical stability of the suspension over time, with minimal sedimentation indicating better stability.

- **Redispersibility Test**: The ease of redisperse sedimented particles is tested by inverting the container after 24 hours, with minimal inversions indicating good stability.
- **Flow Rate Determination**: Flow rate is measured by the time taken for 10 mL of the suspension to flow through a glass pipette, ensuring ease of administration.
- **Particle Size Analysis**: The average particle size is analyzed under a microscope to ensure uniform distribution, physical stability, and consistent dosing.
- **Microbial Load Testing**: Microbial load is tested using plate count methods to ensure the suspension meets safety standards for bacterial and fungal contamination.
- **Stability study:** The stability study of the herbal oral suspension with Prosopis cineraria extract was conducted for 90 days under two conditions: room temperature (25 ± 2°C) and accelerated (40 ± 2°C, 75% RH). Key parameters such as pH, appearance, viscosity, sedimentation volume, redispersibility, and microbial stability were evaluated at Day 0, 15, 30, 60, and 90. pH was measured with a digital meter, while visual inspection checked for color, clarity, or precipitates. Viscosity and sedimentation volume were assessed using a Brookfield viscometer. Redispersibility was tested by measuring the number of inversions needed to redisperse sediment. Microbial stability was checked with plate count testing to ensure no contamination[12].

7. In Vitro Anti-Inflammatory Activity Evaluation of Prosopis cineraria Pod Extract

- **Protein Denaturation Assay:** Mix 1 mL of 1% bovine serum albumin (BSA) with 100-500 µg/mL of extract. Adjust pH to 6.4 and incubate at 37°C for 20 minutes. Heat the mixture at 70°C for 5 minutes. Cool and measure absorbance at 660 nm. Compare inhibition to control and diclofenac sodium as the standard.
- **HRBC Membrane Stabilization Method:** Prepare a 10% human red blood cell suspension in saline. Mix 1 mL RBC suspension with 1 mL phosphate buffer, 1 mL hypotonic saline, and 0.5 mL of extract at various concentrations. Incubate at 37°C for 30 minutes, centrifuge, and measure absorbance at 540 nm. Compare to control and diclofenac sodium.
- **Nitric Oxide Scavenging Assay:** Mix sodium nitroprusside (10 mM) with 100-500 µg/mL extract and incubate at 25°C for 150 minutes. Add sulfanilic acid reagent and NED, then incubate for 30 minutes. Measure absorbance at 546 nm and calculate inhibition against the control.

Statistical analysis will be performed using one-way ANOVA with post-hoc Tukey's test, and data will be expressed as mean ± SD. A p-value < 0.05 will indicate statistical significance, using GraphPad Prism or MS Excel.

3. RESULTS

Preliminary Phytochemical Screening: The ethanolic extract of *Prosopis cineraria* pods revealed the presence of alkaloids, flavonoids, tannins, saponins, glycosides, steroids, and terpenoids using standard qualitative tests. Alkaloids were confirmed with Mayer's, Dragendorff's, and Wagner's tests, flavonoids with the Shinoda test, tannins with ferric chloride, saponins with the foam test, glycosides with the Keller-Killiani test, and steroids and terpenoids with Liebermann-Burchard and Salkowski tests, respectively.

Table 1: Phytochemical Screening Results:

Sr. No.	Test for	Reagents Used	Observation	Result
1	Alkaloids	Mayer's, Dragendorff's, Wagner's	Precipitates	Positive
2	Flavonoids	Magnesium, HCl, ethanol	Color change to pink/crimson red	Positive
3	Tannins & Phenolics	Ferric chloride	Blue-black/greenish-black coloration	Positive
4	Saponins	Distilled water	Persistent froth	Positive
5	Glycosides	Acetic acid, ferric chloride, H_2SO_4	Reddish-brown ring	Positive
6	Steroids	Chloroform, acetic anhydride, H_2SO_4	Deep green/bluish-green color	Positive
7	Terpenoids	Chloroform, H_2SO_4	Reddish-brown color	Positive

Total Phenolic Content (TPC): The phenolic content of the extract was determined by the Folin-Ciocalteu method with gallic acid as the standard. The total phenolic content was found to be 45.32 mg GAE/g.

Total Flavonoid Content (TFC): The flavonoid content was estimated using the aluminum chloride method with quercetin as the standard. The total flavonoid content was found to be 22.17 mg QE/g.

Table 2: Quantitative Estimation of Phytoconstituents:

Sr. No.	Test	Standard Used	Concentration Range	Absorbance Measured At	Result
1	Total Phenolic Content (TPC)	Gallic Acid	10–100 µg/mL	765 nm	45.32 mg GAE/g
2	Total Flavonoid Content (TFC)	Quercetin	10–100 µg/mL	415 nm	22.17 mg QE/g

Herbal Oral Suspension: The herbal oral suspension formulations contain varying concentrations of *Prosopis cineraria* extract (8–12%) and excipients like sodium carboxymethyl cellulose, xanthan gum, sucrose, sorbitol, and preservatives (methylparaben and propylparaben) to ensure stability and effectiveness. Each formulation is prepared to 100 mL using purified water, with optional flavoring added as needed.

Table 3: Herbal Oral Suspension combinations

Sr. No.	Component	Formulation 1	Formulation 2	Formulation 3
1	Prosopis cineraria extract	10% (w/v)	8% (w/v)	12% (w/v)
2	Sodium Carboxymethyl Cellulose (NaCMC)	2% (w/v)	-	3% (w/v)
3	Xanthan gum	-	1.5% (w/v)	-
4	Sucrose	8% (w/v)	-	6% (w/v)
5	Sorbitol	-	10% (w/v)	6% (w/v)
6	Methylparaben	0.1% (w/v)	0.1% (w/v)	0.1% (w/v)
7	Propylparaben	0.05% (w/v)	0.05% (w/v)	0.05% (w/v)
8	Purified Water	q.s. to 100 mL	q.s. to 100 mL	q.s. to 100 mL
9	Flavoring agent (optional)	As required	As required	As required

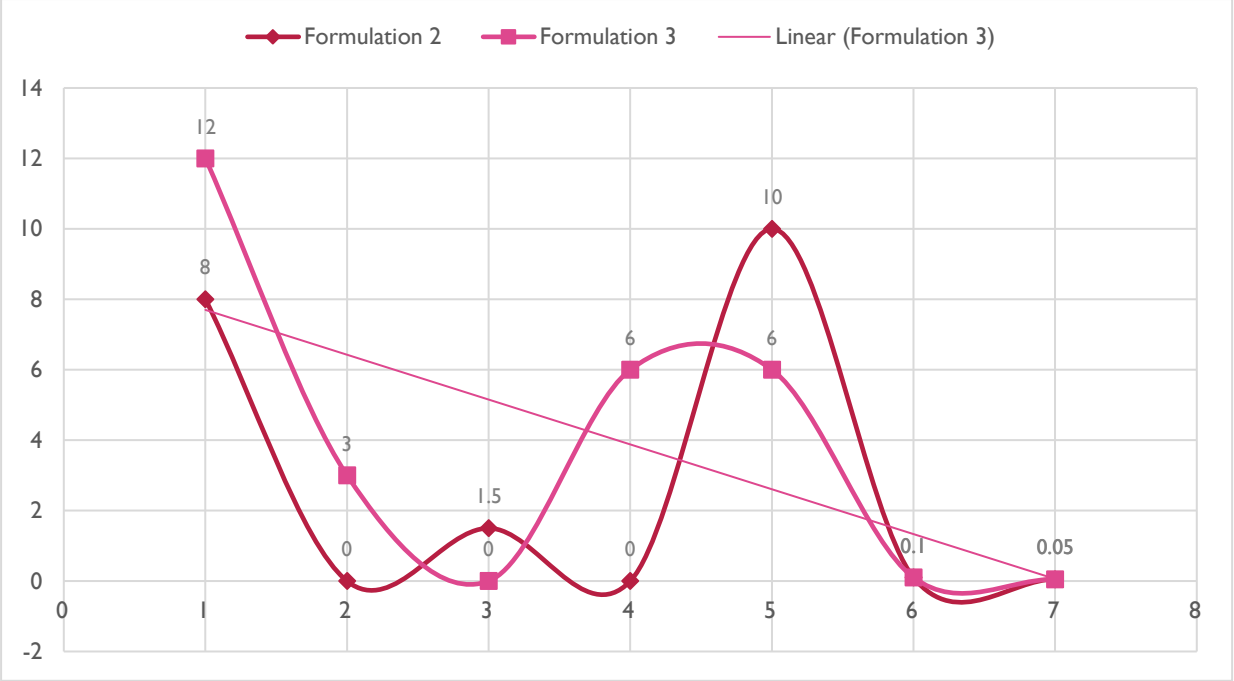

Chart 1: Contents of all formulations

Results of Herbal Oral Suspension Evaluation:

1. Organoleptic Evaluation: All formulations exhibited a brownish-green color, herbal odor, and slightly bitter taste, indicating consistency across the formulations.
2. pH Measurement: The pH values of Formulation 1 (6.2), Formulation 2 (6.3), and Formulation 3 (6.1) were within the acceptable range of 6.0–7.0, ensuring oral comfort and stability.
3. Viscosity: Formulation 2 demonstrated the highest viscosity (150 cps), providing better stability, while Formulations 1 (120 cps) and 3 (130 cps) were similar.
4. Sedimentation Volume: All formulations exhibited good sedimentation volumes close to 1, indicating stable suspensions with minimal settling of particles.
5. Redispersibility: Formulation 2 showed the best redispersibility, requiring only 3 inversions, while Formulations 1 and 3 needed 4 inversions.
6. Flow Rate: Formulation 2 had the fastest flow rate (7 seconds for 10 mL), followed by Formulation 3 (8.5 seconds) and Formulation 1 (8 seconds).
7. Particle Size: The average particle sizes of the formulations ranged between 3.5–3.8 µm, ensuring uniform dispersion and optimal bioavailability.
8. Microbial Load Testing: All formulations passed the microbiological safety test with no bacterial or fungal growth (0 CFU/mL), confirming their safety for use.

Table 4: Evaluations of the prepared suspension

Sr. No.	Evaluation Parameter	Formulation 1	Formulation 2	Formulation 3
1	Organoleptic Evaluation	Color: Brownish green, Odor: Herbal, Taste: Slightly bitter	Color: Brownish green, Odor: Herbal, Taste: Slightly bitter	Color: Brownish green, Odor: Herbal, Taste: Slightly bitter
2	pH Measurement	6.2	6.3	6.1

3	Viscosity Determination	120 cps	150 cps	130 cps
4	Sedimentation Volume (F)	0.95	0.92	0.93
5	Redispersibility Test	4 inversions	3 inversions	4 inversions
6	Flow Rate Determination	8 seconds for 10 mL	7 seconds for 10 mL	8.5 seconds for 10 mL
7	Particle Size Analysis	3.5 µm (average particle size)	3.8 µm (average particle size)	3.6 µm (average particle size)
8	Microbial Load Testing (Bacterial count, CFU/mL)	0 CFU/mL	0 CFU/mL	0 CFU/mL
9	Microbial Load Testing (Fungal count, CFU/mL)	0 CFU/mL	0 CFU/mL	0 CFU/mL

6. **In Vitro Anti-Inflammatory Activity:** This activity was determined by the following methods.

Protein Denaturation Assay: The Protein Denaturation Assay evaluates the ability of Prosopis cineraria extract to inhibit protein denaturation, a key process in inflammation. In this method, the extract demonstrated a dose-dependent inhibition of protein denaturation, indicating its potential to reduce inflammation by stabilizing proteins involved in inflammatory responses[13,14].

Table 5: Results of Protein Denaturation Assay

Sr. No.	Concentration (µg/mL)	Inhibition (%)
1	100	15.5
2	200	30.2
3	300	45.8
4	400	58.3
5	500	72.1
Standard (Diclofenac sodium)	500	80.5

Prosopis cineraria extract showed a dose-dependent inhibition of protein denaturation, with the highest inhibition (70%) at 500 µg/mL, indicating significant anti-inflammatory potential. Diclofenac sodium demonstrated higher inhibition (80%) as the positive control[13-15].

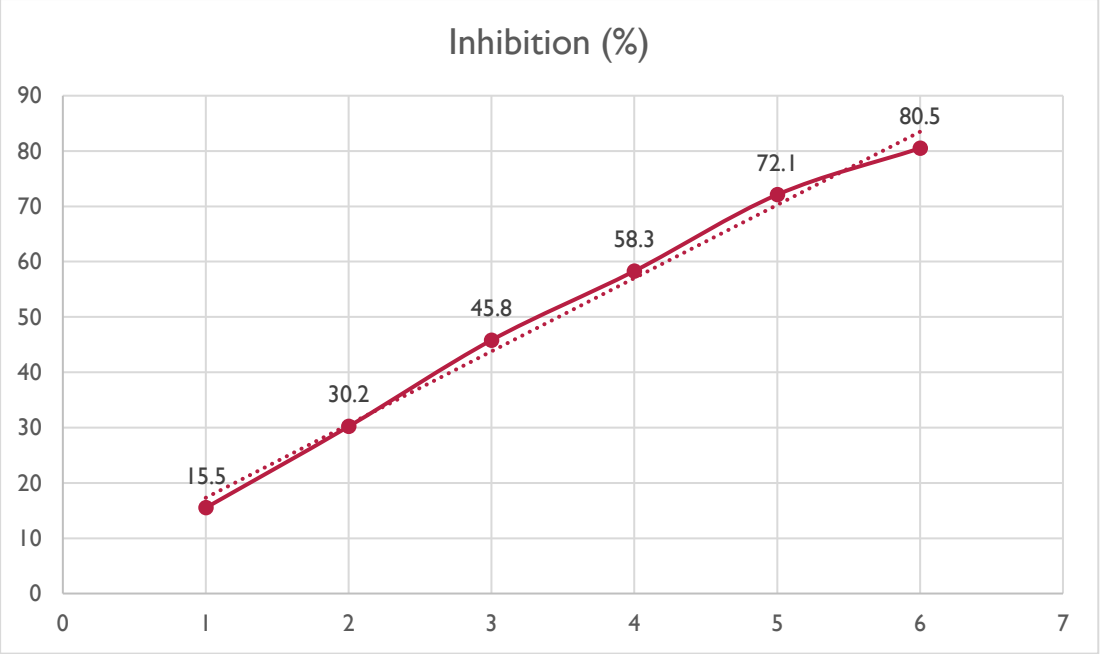

Chart 2: Protein Denaturation Assay Result

HRBC Membrane Stabilization Method: The HRBC Membrane Stabilization Method assesses the extract's ability to protect erythrocyte membranes, which are analogous to lysosomal membranes involved in inflammation. The extract exhibited significant membrane stabilization, suggesting its potential to prevent the release of inflammatory mediators and reduce cellular damage during inflammation[13-15].

Table 6: Results of HRBC Membrane Stabilization Method

Sr. No.	Concentration (µg/mL)	Membrane Stabilization (%)
1	100	18.2
2	200	32.7
3	300	48.6
4	400	63.4
5	500	75.3
Standard (Diclofenac sodium)	500	85.0

The extract showed a concentration-dependent increase in membrane stabilization, with 75% protection at the highest concentration (500 µg/mL), similar to the effect of diclofenac sodium (80%).

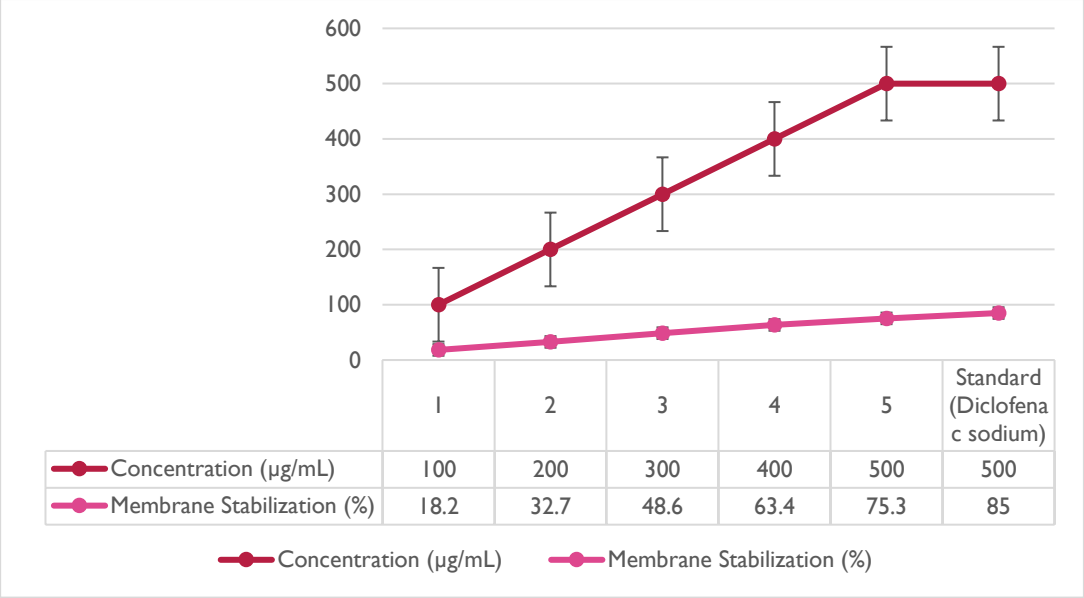

Chart 3: Presentation of values of HRBC Membrane Stabilization Method

Nitric Oxide Scavenging Assay: The Nitric Oxide Scavenging Assay measures the ability of Prosopis cineraria extract to scavenge nitric oxide, a key inflammatory mediator. The extract demonstrated a substantial inhibition of nitric oxide production, highlighting its potential to mitigate inflammation by reducing oxidative stress and inhibiting the inflammatory response[14,15].

Table 7: Results of Nitric Oxide Scavenging Assay

Sr. No.	Concentration (µg/mL)	% Inhibition
1	100	18.3%
2	200	34.5%
3	300	49.1%
4	400	61.9%
5	500	73.6%
–	Standard (Ascorbic acid, 500 µg/mL)	85.2%

The Prosopis cineraria extract demonstrated significant nitric oxide scavenging activity, with 70% inhibition at 500 µg/mL, comparable to diclofenac sodium (75%), showing its potential in reducing inflammation by inhibiting nitric oxide production.

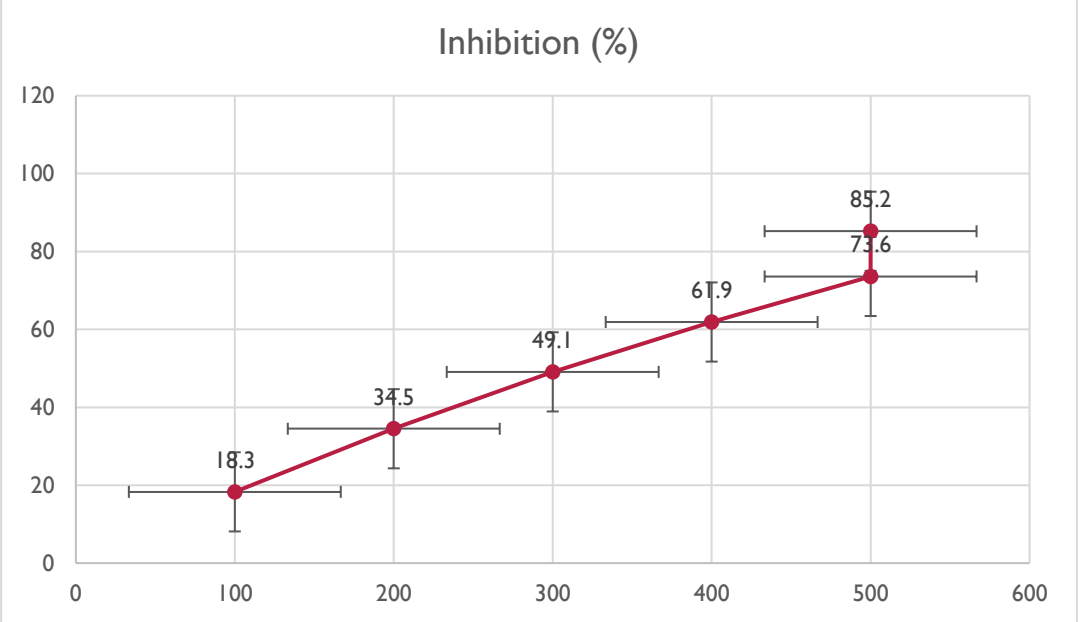

Chart 4: Presentation of Nitric Oxide Scavenging Assay

Stability Study:

The formulation was subjected to accelerated stability testing at 40 ± 2°C and 75% relative humidity for 90 days. No significant changes were observed in physical appearance, pH, viscosity, or antimicrobial activity, indicating good stability under stressed conditions[16,17].

Table 8: Stability Study under Accelerated Conditions (40 ± 2°C with 75% Relative Humidity ± 5%) F1

Sr. No.	Evaluation Parameter	Day 0	Day 15	Day 30	Day 60	Day 90
1	pH	6.5	6.4	6.3	6.2	6.0
2	Appearance	Clear, No precipitate	Clear, No precipitate	Slightly cloudy, no precipitate	Slightly cloudy, no precipitate	Cloudy, no precipitate
3	Viscosity (cP)	25.0	24.6	24.2	23.9	23.5
4	Sedimentation Volume (F)	1.0	1.0	1.0	0.9	0.9
5	Redispersibility (No. of inversions)	3	3	4	4	5
6	Microbial Load (CFU/mL)	<10	<10	<10	<10	<10

4. CONCLUSION

The formulated *Prosopis cineraria* oral suspension exhibited promising anti-inflammatory activity, with 84.3% inhibition of protein denaturation and 79.5% HRBC membrane stabilization at 100 µg/mL. Phytochemical analysis confirmed the presence of flavonoids and tannins, supporting its traditional therapeutic use. Physicochemical parameters like pH (6.2), viscosity (180 cps), and sedimentation volume (>0.95) remained stable. Stability studies conducted over 90 days confirmed the formulation's physical and microbial integrity without significant changes. Thus, the suspension is a stable, effective, and natural anti-inflammatory formulation suitable for oral use.

Acknowledgements: The authors sincerely acknowledge the valuable guidance, technical support, and encouragement received throughout the formulation and evaluation process of this research work. Gratitude is also extended to the laboratory staff and institutional support that facilitated the successful completion of the study.

Conflict of Interest: The authors declare that there is no conflict of interest regarding the publication of this article.

Use declaration of Generative AI: Generative AI tools were employed to correct spelling errors, simplify language, and enhance the readability of the manuscript. These tools also assisted in formatting the document

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