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Development and evaluation of a liposomal gel containing *Ajuga bracteosa* for topical treatment of gout

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Abstract
This study evaluates a liposomal gel containing *Ajuga bracteosa* leaf extract for the topical treatment of gout and inflammation. The extract was encapsulated using the thin-film hydration method and incorporated into a carbopol gel base. The formulation was assessed for physicochemical properties, drug release, and anti-inflammatory activity using the carrageenan-induced paw edema model in rats. The liposomal gel significantly reduced paw edema, joint swelling, and pain compared to controls, indicating improved bioavailability and therapeutic potential. This suggests it may offer a promising herbal alternative for localized gout and inflammation management.

Keywords: *Ajuga bracteosa*, liposomal gel, gout, carrageenan-induced paw edema

Introduction
Herbal plants have played a significant role in human health and medicine for thousands of years. They are widely used in traditional systems of medicine such as Ayurveda, Unani, and Traditional Chinese Medicine. These plants contain natural compounds that have therapeutic properties, which can help treat and prevent various diseases [1]. *Ajuga bracteosa* is highly valued in traditional medicine systems such as Ayurveda and Unani. It is known for its: Anti-inflammatory, Antimicrobial, Antioxidant, Hepatoprotective, Analgesic and Antipyretic properties. *Ajuga bracteosa*, belonging to the Lamiaceae family of plants, presented promising anti-inflammatory activity that stemmed from its triterpenoid, flavonoid, and phenolic derivatives, as well as saponin and tannin contents [2]. A combination of diverse mechanisms explains this anti-inflammatory bioactivity. In experimental studies, *Ajuga bracteosa* has shown a marked decrease in inflammation in both *in vitro* cell cultures and *in vivo* animal models, including arthritis and edema [3]. Liposomes are small, spherical vesicles composed of one or more phospholipid bilayers, closely resembling the structure of natural cell membranes. Most common and traditional method Lipids are dissolved in an organic solvent such as chloroform or methanol. The solvent is evaporated under vacuum (using a rotary evaporator), leaving a thin lipid film on the inner wall of a round-bottom flask. The dry lipid film is hydrated with an aqueous solution (such as buffer or water) under agitation. The lipids spontaneously swell and form multilamellar vesicles (MLVs) [4].

Material and Methods
Authentication and Collection of Plant:
The plant specimen was authenticated by Associate professor Mr. Jagdeep Verma of Sardar Patel University, Mandi Himachal Pradesh, as per letter number: BOT/304.6. Plant leaves were collected from Devidarh, Mandi Himachal Pradesh. The leaves were washed with water to remove dirt, then shade dried. The plant was preserved in the advanced pharmaceutical museum of Abhilashi University.

Soxhlet Extraction
First, the dried and powdered leaves of *Ajuga bracteosa* were accurately weighed and placed in a thimble made of chromatography paper.

This thimble was then inserted into the main chamber of the Soxhlet apparatus. Methanol was selected based on the desired phytochemical profile and filled into a round-bottom flask attached to the apparatus. The system was assembled and heated to allow the solvent to evaporate and condense through a reflux condenser. As the solvent condensed, it dripped onto the plant material and extracted the soluble compounds. Once the chamber filled, the solution was siphoned back into the flask, and the cycle repeated continuously for several hours (typically 6-8 hours) until complete extraction was achieved. After completion, the solvent extract was filtered and concentrated using a rotary evaporator under reduced pressure to obtain the crude extract, which was then stored for further analysis [6].

Percentage Yield

The percentage yield of a herbal plant refers to the amount of usable extract (such as essential oil, active compounds, or dry matter) obtained from the plant material, expressed as a percentage of the original weight of the raw plant material [7].

$$\% \text{ Yield} = \frac{(\text{Weight of extract obtained}) \times 100}{(\text{Weight of plant powder taken})}$$

Qualitative phytochemical evaluation

The methanolic extract of *Ajuga bracteosa Za* leaves was subjected to standard phytochemical tests to evaluate its bioactive composition. The analysis revealed the presence of phenolics, flavonoids, coumarins, sesquiterpene lactones, triterpenes, alkaloids, tannins, saponins, and polysaccharides. These compounds are associated with significant therapeutic properties, including antimicrobial, antioxidant, and anti-inflammatory activities, underscoring the medicinal value of *Ajuga bracteosa* leaves [8].

Formulation of Liposomal dispersion

The liposomes were prepared by thin film hydration method using Rotary evaporator. In this process, lipids such as soya lecithin and cholesterol was accurately weighed and dissolved in chloroform and methanol (4:1). This solution ensures complete solubilization of the lipid components [9]. The flask is then connected to a rotary evaporator at 80 rpm, and the solvent is slowly evaporated under reduced pressure at a controlled temperature (around 40-45 ℃). As the solvent evaporates, a thin and uniform lipid film forms on the inner wall of the flask. In a separate conical flask, an accurately weighed amount of plant extract was dissolved in the necessary quantity of phosphate buffer saline (PBS, pH 7.4). The thin film was hydrated with the 10 ml solution of extract. The hydration continues for 1 hour while the flask was kept rotating at 55-60 ℃. The hydrated liposomes were sonicated for 20 min using a bath sonicator to obtain liposomal dispersion [10].

Optimization of liposomes

To develop effective liposomes, five different formulations (F1-F5) were prepared using the thin-film hydration method. The optimization process involved varying the concentration of phospholipid (soya lecithin) and adjusting the ratio of phospholipid to cholesterol, while keeping the solvent ratio of chloroform to methanol (4:1) constant. These formulations were then evaluated for entrapment efficiency and drug release. Based on the results, the formulation showing the highest entrapment efficiency and optimal drug release profile was selected for further study [11].

Table 1: Different formulations composition of liposomes for optimization

Sr. No	Formulation No	Phospholipid: Cholesterol (mg)	Solvent (Chloroform: Methanol)
1	F1	300:100	4:1
2	F2	250:150	4:1
3	F3	200:200	4:1
4	F4	150:250	4:1
5	F5	100:300	4:1

Entrapment efficacy

The entrapment efficiency of the prepared liposomal formulations was determined using the centrifugation method. A known volume of the freshly prepared liposomal dispersion was transferred into centrifuge tubes and centrifuged at 3000 rpm for 30 minutes at a controlled temperature of 4 ℃ using a refrigerated centrifuge. This process allowed the separation of liposomes (containing the entrapped drug) from the supernatant, which contained the unentrapped (free) drug. From the collected supernatant, 1 mL was withdrawn and diluted with phosphate-buffered saline (PBS) to achieve a concentration of 1000 µg/mL. The entrapment efficiency (EE%) was then calculated using the following formula. The entrapment efficiency (EE%) was then determined using the following equation [12].

$$\text{EE (\%)} = \frac{(\text{Total drug} - \text{Free drug})}{\text{Total drug}} \times 100$$

***In vitro* drug permeation studies**

In vitro drug permeation studies were carried out using an egg membrane, which mimics the stratum corneum layer of human skin. The egg membrane was isolated by immersing a fresh egg in hydrochloric acid (HCl) solution. After decalcification, the contents of the egg were discarded, and the remaining membrane was carefully washed with distilled water. The prepared formulation was placed onto the egg membrane, which was then tied securely around the mouth of a small beaker. This beaker was immersed in a larger beaker containing phosphate buffer (pH 7.4), ensuring that the egg membrane was in direct contact with the buffer, simulating physiological conditions [13].

At predetermined time intervals, fixed volumes of the release medium were withdrawn and replaced with fresh buffer to maintain sink conditions. The collected samples were analyzed for drug content using a Shimadzu UV-Visible spectrophotometer [14].

Formulation of liposomal gel

The dispersion method is a commonly used technique for the preparation of gel. Distilled water was added to a beaker and placed on a magnetic stirrer. Carbopol 934, serving the gelling agent was gradually sprinkled into the water while stirring continuously. The temperature was maintained at 50 ℃ and the mixture was stirred until the gel was completely hydrated and uniform [15].

In a separate container, methylparaben, glycerine and propylene glycol were mixed thoroughly and then incorporated into the gel. Finally, the pH of the gel was adjusted using triethanolamine (TEA) to achieve the desired

consistency. For the preparation of the final liposomal gel, liposomes were selected based on their entrapment efficiency and stability.

This selection was guided by preliminary evaluation results, which demonstrated that the liposomal vesicles could be effectively incorporated into a stable and homogeneous gel matrix. The resulting formulation allowed for ease of topical application, controlled drug release, and enhanced drug retention, as the liposomes exhibited optimal physicochemical characteristics [16].

Table 2: Formulation composition of gel base

Chemicals	F1	F2	F3	F4	F5
Carbopol 934	0.5 g	1 g	2 g	1.5 g	2.5 g
Propylene glycol	1 ml	1 ml	1 ml	1 ml	1 ml
Methyl paraben	0.02 g	0.02 g	0.02 g	0.02 g	0.02g
Triethanolamine	0.2 ml	0.2 ml	0.2 ml	0.2 ml	0.2 ml
Glycerine	2 ml	2 ml	2 ml	2 ml	2 ml
Distilled water up to 50 ml	QS	QS	QS	QS	QS

Evaluation of Liposomal gel

- **Organoleptic:** The liposomal gel was evaluated for its organoleptic properties, including color, odor, and appearance. It was found to be smooth, uniform in texture, and free from any unpleasant odor or visible impurities [17].
- **Homogeneity:** Homogeneity refers to the uniform distributiom of all components drug, excipients, gelling agents etc. within the gel formulation, ensuring that there are no lumps, air bubbles, phase separation or aggregates. Take small amount of gel on glass slide and visualize under normal light. The foreign particles, air bubbles or lumps in the gel formulations was observed [18].
- **pH determination:** The pH of the prepared liposomal gel was measured using a digital pH meter to ensure compatibility with the skin and to maintain the stability of the formulations. To measure the accurate pH, the pH meter was calibrated using standard buffer solutions of pH 4.0 and pH 7.0 [19].
- **Spreadability:** The spreadability of the prepared liposomal gel was determined by the method Slip and Drag. An excess of sample liposomal gel (1g) was applied in between two glass slides and compressed to a uniform thickness by placing 150-200 g weight on the slides for 5 mins. The time required to separate two slides, i.e. time in which the upper glass slide moves over the lower plate was taken as a measure of spreadability.

$$\textbf{Formula: } S = \frac{M \times L}{T}$$

Where,
S = Spreadbility
M = mass tied to upper plate
L = length of the slide
T = time taken to move the slide

- **Viscosity:** Viscosity was determined by using Brookfield viscometer. The viscosity of gels play an important role in controlling how the drug is released from the formulations. Viscosity measurements were carried out at room temperature (25-27 ℃) using a Brookfield viscometer (model LMVD-60). The base level of the instrument was set using level indicator. The spindle was cleaned and attached to the instrument. Then the spindle was rotated in the sample until a constant reading displaced on the viscometer. The viscosity in cps was directly read. The method was repeated for three times and average value was found and noted to determine viscosity.
- **Washability:** It is an important parameter for topical gels. It determines how easily the formulation can be removed from the site of application using water. Apply a small amount of gel on glass slide or skin. Allow it to dry for 5-10 minutes and then wash under running water for 1-2 minutes without soap and rub gently using fingertips [20].

Characterization of liposomal gel

- **Particle Size:** Particle size analysis is an essential parameters in evaluating liposomal formulations, as the vesicle size directly affects their stability, drug release profile, and bioavailability. The particle size of liposomes was determined by dynamic light scattering.
- **Zeta potential:** Zeta potential is a parameter for evaluating surface charge and stability of the liposomal formulations. Zeta potential indicates the degree of repulsion between adjacent, similar charged particles in the dispersion [21].

***In vivo* study: Carrageenan-induced paw edema model in rats**

The *in vivo* study was conducted at the Animal House of Abhilashi University with prior approval from the Institutional Animal Ethics Committee (Approval No: IAEC/AU/2025-004). Male Wistar albino rats (200-250 g) were housed under standard laboratory conditions and acclimatized for one week before the experiment.

The animals were divided into three groups (N=5 per group). Group I received 1% diclofenac gel (standard), while Groups II and III were treated with 2% and 4% *Ajuga bracteosa* liposomal gel, respectively. Paw edema was induced by subplantar injection of 0.1 mL of 1% w/v carrageenan solution in saline into the right hind paw. Paw volume was measured at 0, 15, 30 minutes, and 1 hour post-injection using a plethysmometer. After 1 hour, the respective gels were topically applied, and the anti-inflammatory effect was assessed by comparing changes in paw volume across the groups. All procedures were carried out in accordance with CPCSEA guidelines to ensure ethical handling and care of animals.

Results

Percentage yield of *Ajuga bracteosa* leaves extract

The percentage yield of a herbal plant refers to the amount of usable extract obtained from the plant material, expressed as a percentage of the original weight of the raw plant material.

$$\% \text{ Yield} = \frac{(\text{Weight of extract obtained}) \times 100}{(\text{Weight of plant powder taken})}$$

Table 3: Percentage yield

Sr. No	Plant name	Solvent (g)	Theoretical weight (g)	Yield	% Yield
1.	*Ajuga bracteosa*	Methanol	1000	75	7.5

$$\% \text{ Yield} = \frac{(\text{Weight of extract obtained}) \times 100}{(\text{Weight of Plant powder taken})}$$

$$\% \text{ Yield} = \frac{75 \times 100}{1000} = 7.5\%$$

Table 4: Preliminary phytochemical analysis of *Ajuga bracteosa* leaves

Phytochemical constituents	Test	Result
Test for alkaloids	Drangandroffs test Mayers test	+ve +ve
Test for carbohydrates	Fehlings test	+ve
Test for tannins	Gelatin test	+ve
Test for Saponin	Bromine water Foam examine	+ve +ve
Test for cardiac glycosides	Killer Kelani	+ve
Test for Flavonoids	Lead acetate test Alkaline reagent	-ve +ve

Optimization of different formulations of liposomes

Table 5: Percentage drug release of different formulations

Sr. No	Time (min)	% drug release				
		F1	F2	F3	F4	F5
1	15	0.35	0.33	2.9	2.8	3.1
2	30	1.9	1.5	4.4	4.31	4.6
3	60	2.9	2.1	6.94	8.1	8.25
4	120	3.77	3.25	11.16	13.05	13.5
5	240	6.81	6.5	17.04	20.25	21.5
6	360	12.41	12.4	24.26	26.72	28.5
7	480	19.87	19.21	33.03	36.5	38.8
8	720	29.25	29.1	44.51	60.45	62.08
9	960	43.15	42.1	59.21	65.45	70.05
10	1440	66.2	59.15	76.55	80.05	87.14

Table 6: Entrapment efficacy and drug release of different formulations

Sr. No	Formulation No	Soya lecithin (mg)	Cholesterol (mg)	Entrapment Efficacy (EE%)	Percent drug release after 24 hours
1	F1	300	100	78.5%	66.2%
2	F2	250	150	84.2%	59.15%
3	F3	200	200	80.0%	76.55%
4	F4	150	250	68.4%	80.05%
5	F5	100	300	55.9%	87.14%

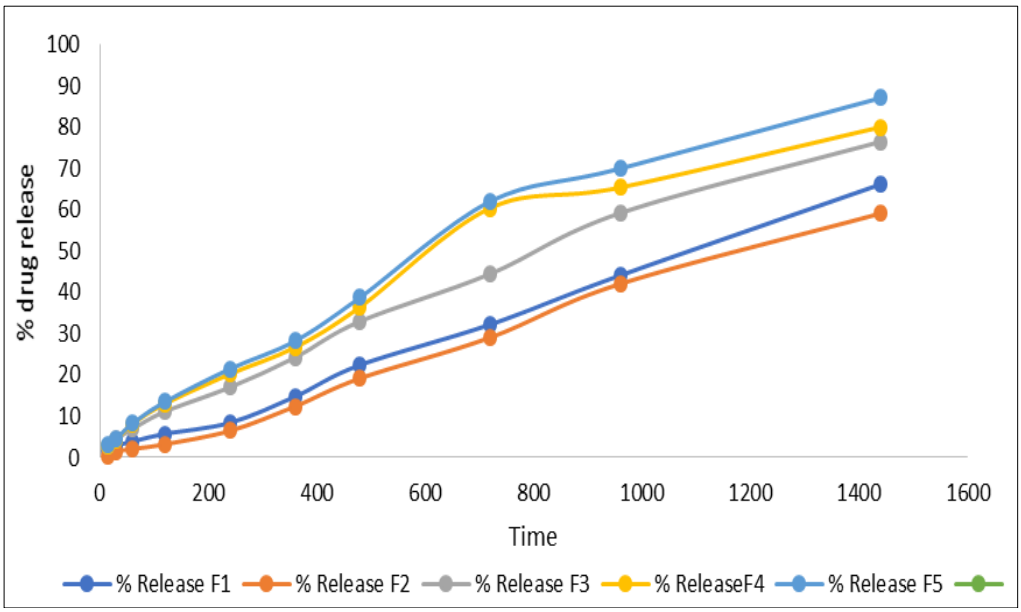

Fig 1: Percentage of drug release

Formulation F2 showed the highest entrapment efficiency (84.2%) and sustained drug release (59.15%), which indicated that a larger amount of the drug was successfully trapped inside the liposomes and indicating a good balance between vesicle stability and permeability, which was crucial for effective drug delivery in liposomal formulations. Based on these results, batch F2 was selected to formulate liposomes.

Incorporation of liposomes into gel

Formulation F2 of the liposomes was selected for the preparation of the final liposomal gel due to its high entrapment efficiency and stability.

Table 7: Optimization of liposomal gel

Formulation No	Texture	Homogeneity	Viscosity (Cps)	Spreadability (gm.sm/sec)	pH
G1	Gritty	Lumps	3671±0.58	18.36	6
G2	Smooth	Homogeneous	4124±0.43	21.38	6.3
G3	Smooth	Homogeneous	3714±0.21	20.6	6.2
G4	Rough	Small lumps	3688±0.69	19.37	6.1
G5	Smooth	Homogeneous	4237±0.11	22.16	6.5

Formulation G5 was the best among all the tested formulations because it showed better physical qualities (appearance, homogeneity, viscosity, spreadability & pH) and was safe for the skin, making it the most suitable option as a topical gel.

Characterization of liposomal gel

Particle Size

Particle size analysis is an essential parameters in evaluating liposomal formulations, as the vesicle size directly affects their stability, drug release profile, and bioavailability. The particle size distribution ranged from approximately 10 nm to 250 nm, with a standard deviation of 141.5 nm and a peak at 109 nm.

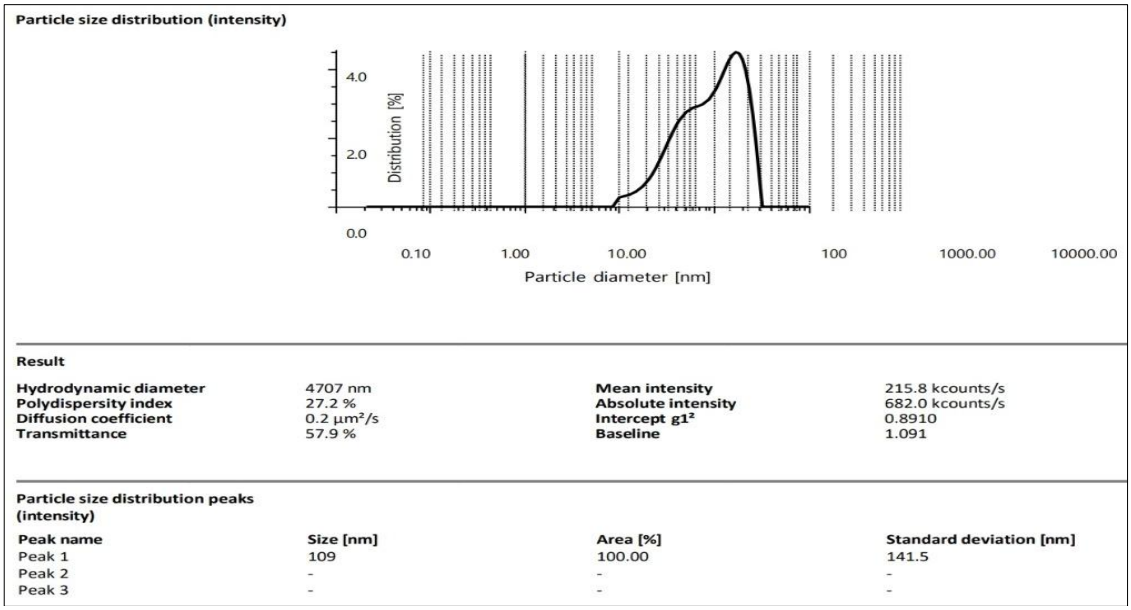

Fig 2: Particle size analysis

Zeta Potential: The Zeta potential distribution ranged from approximately -200 mV to 250 mV on the x-axis with a standard deviation of 6.63mV and a mean of -29 mV.

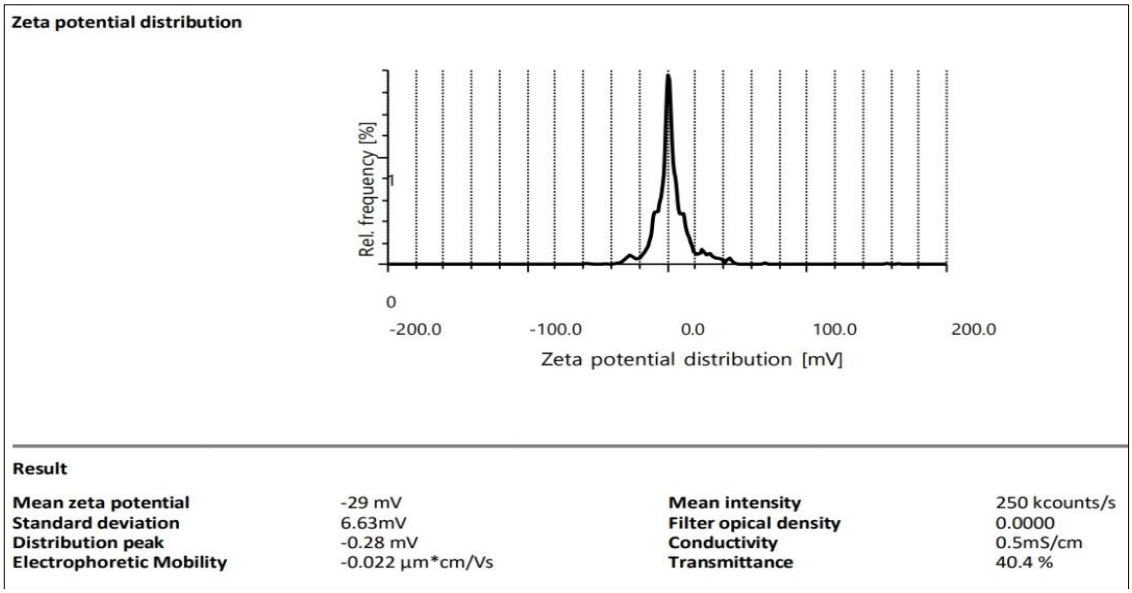

Fig 3: Zeta potential

***In vivo* study: Carrageenan-induced paw edema model in rats**

Wistar rats were divided into three groups to evaluate the anti-inflammatory effects of 1% Diclofenac gel and *Ajuga bracteosa* liposomal gels at concentrations of 2% and 4% by measuring inflammation withdrawal time. The anti-inflammatory activity was assessed using the carrageenan-induced paw edema model. Albino rats weighing 200-250 g were assigned to three groups, each consisting of five animals. Group I received 1% Diclofenac gel (standard), while Groups II and III were treated with liposomal gels containing 2% and 4% *Ajuga bracteosa*, respectively, applied to the right hind paw. Then, 0.1 ml of 1% carrageenan solution was injected into the sub-plantar region of the right hind paw to induce edema. Paw volume was measured at 0, 15, 30, and 60 minutes after carrageenan injection using a plethysmometer. The animals were treated with the liposomal gels, and the percentage inhibition of edema was calculated.

Table 8: Anti-inflammatory activity percentage inhibition against carrageenan induced paw edema model in rats

Sr. No	Groups	Dose	Time						
			30min	1hr	2hr	3hr	4hr	5hr	6hr
1	Standard	1%w/w Diclofenac gel	15%	20%	30%	43%	53%	59%	65%
2	Test I	2% w/w liposomal gel	2 %	4%	7%	9%	12%	14%	16%
3	Test II	3% w/w liposomal gel	3%	5%	8%	10%	13%	18%	23%

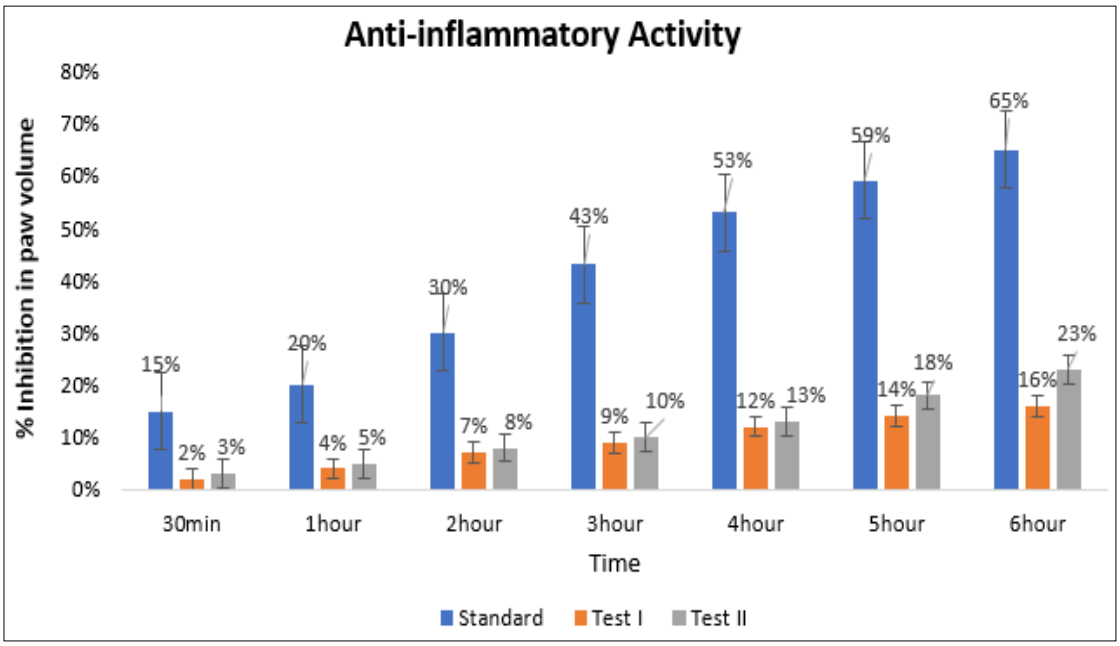

Fig 4: Graphical representation of Anti-inflammatory activity percentage inhibition against Carrageenan induced paw edema model in rats

Conclusion

The present study demonstrated the successful development and evaluation of a liposomal gel containing *Ajuga bracteosa* extract for the topical management of gout and inflammation. The optimized formulation (F2) exhibited high entrapment efficiency, sustained drug release, and favorable physicochemical characteristics. Incorporation into a carbopol gel base provided good spreadability, homogeneity, and skin-compatible pH. In vivo studies using the carrageenan-induced paw edema model confirmed significant anti-inflammatory activity, supporting the therapeutic potential of the formulation. These findings suggest that *Ajuga bracteosa* liposomal gel could serve as a promising herbal alternative to conventional therapies, offering localized action, reduced systemic side effects, and enhanced patient compliance. Further clinical investigations are recommended to validate its efficacy and safety.

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