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Formulation And Evaluation Of Niosomal Gel Loaded With *Ziziphus Mauritiana* Extract For Analgesic Activity

Author: Dr. Dev Prakash Dahiya[1], Shelly[2*], Anchal Sankhyan[3], Sakshi Sharma[4]

Affiliation: School of Pharmacy, Abhilashi University, Chailchowk, Mandi, Himachal Pradesh, India

Abstract

Pain is one of the most prevalent health concerns, often managed using synthetic analgesics such as NSAIDs and opioids, which are associated with severe adverse effects including gastrointestinal irritation, renal toxicity, and dependence. This has led to increasing interest in plant-based alternatives with improved safety profiles. *Ziziphus mauritiana* (Rhamnaceae), widely used in traditional medicine, is rich in flavonoids, saponins, alkaloids, and tannins, and has demonstrated analgesic, anti-inflammatory, and antioxidant activities. However, the therapeutic application of its phytoconstituents is limited by poor solubility, low permeability, and inadequate bioavailability when delivered through conventional formulations. To overcome these challenges, the present study aimed to formulate and evaluate a niosomal gel of *Z. mauritiana* leaf extract for topical analgesic delivery. Niosomes were prepared by the thin-film hydration method, optimized for vesicle size, zeta potential, and entrapment efficiency, and subsequently incorporated into a Carbopol-based gel. The formulation was characterized for pH, viscosity, spreadability, in vitro release, and in vivo analgesic activity using the tail immersion model in Wistar rats. Results revealed sustained drug release, enhanced permeation, and significant analgesic efficacy comparable to diclofenac gel. The findings suggest that *Z. mauritiana*-loaded niosomal gel is a promising natural and effective alternative for localized pain management with improved patient compliance.

Keywords: *Ziziphus mauritiana*, niosomal gel, sustained release, analgesic activity.

Introduction:

Pain can be defined as a somatic sensation of acute discomfort, a symptom of some physical hurt. All living organisms can experience pain and inflammation which may be triggered by physical, chemical or biological factors [1]. Typically, pain is managed with synthetic analgesics such as Opioids and NSAIDS. However, these agents are associated with adverse effects including gastrointestinal irritation, tolerance, and dependence [2].

Medicinal plants form the backbone of traditional healthcare systems and continue to be explored for novel therapeutics. Among these, *Ziziphus mauritiana* (Rhamnaceae), commonly known as ber or Indian jujube, is widely distributed in tropical regions. Its leaves, fruits, and seeds are traditionally used for treating pain, inflammation, and gastrointestinal disorders. Phytochemical investigations have revealed

the presence of flavonoids, alkaloids, tannins, and saponins that contribute to its pharmacological activities, particularly its analgesic and anti-inflammatory effects [3].

Topical drug delivery systems offer unique benefits like localized action, lower systemic exposure, and higher patient comfort but bring a host of challenges around penetration, stability, release kinetics, tolerability and usability. To overcome these challenges, various strategies and technologies are explored. These include the use of penetration enhancers, nanoparticles or vesicle carrier systems which can improve skin permeability and enhance drug absorption [4]

Over the past few decades, extensive efforts have been devoted to the advancement of novel drug delivery systems. To enhance transdermal permeability, various chemical and physical techniques have been investigated to overcome the restrictive nature of the stratum corneum. These include approaches such as tape stripping, iontophoresis, and electroporation, alongside the development of vesicular carrier systems like liposomes and niosomes. Among these strategies, liposomes and niosomes have emerged as the most widely utilized carriers for improving drug transport across the skin [5]. Niosomes are gaining a lot of attention because they come with many advantages. They are chemically stable, provide uniform and pure formulations, are cost-effective, and can be stored easily [6].

Fig 1 *Ziziphus mauritiana* plant (Ber)

Material and methods

Plant material and authentication

The plant *Ziziphus mauritiana* was collected from the Sarkaghat, Mandi (H.P) and authenticated from Department of Botany, Sardar Patel University, Mandi. The specimen was preserved at School of Pharmacy, Abhilashi University.

Animal

Female Wistar albino rats were selected as experimental animals and the animals were procured from the Animal House of Abhilashi University. The animals were housed under controlled laboratory conditions of temperature (25 ± 2°C), relative humidity (45–60%), and a 12-hour light/dark cycle.

Extraction

Dried powder of *Ziziphus mauritiana* was collected from localities. Weigh approximately 210 g of powder and was kept in the thimble. Then 500ml of methanol was added to the round bottom flask the solution was heated at 50°C for 6-7 hr to complete 5 cycles per day. The collected extract was then dried by removing solvent. The extract was then subjected to phytochemical screening [7].

Phytochemical screening

Preliminary phytochemical screening for the presence of phenols, tannins, flavonoids, alkaloids and saponins was carried out using standard test protocols. These phytochemicals were identified by characteristics colour change using standard procedures [8].

Preparation of Niosomes

The niosomes were prepared by thin film hydration method using Rotary evaporator.
The surfactant (non-ionic) along with cholesterol was accurately weighed and dissolved in 10 mL of chloroform and ethanol (4:1). The solvent was then slowly evaporated using a rotary evaporator at 80 rpm and maintained at a temperature of 60 °C under reduced pressure. This resulting in the formation of a thin lipid film on the inner wall of the flask [9].
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 then hydrated with the 10ml solution of extract. The hydration continues for 1h while the flask was kept rotating at 55-60℃. The hydrated niosomes were sonicated for 20 min using a bath sonicator to obtain niosomal dispersion [10].

Table 1 Optimization of niosomes

S. no.	Formulation code	Surfactant	Surfactant: Cholesterol: Extract	Solvent (chloroform: ethanol)
1	F1	Tween 60	100: 100: 100	4:1
2	F2	Tween 60	200: 100: 100	4:1
3	F3	Tween 60	300: 200: 100	4:1
4	F4	Tween 40	300: 200: 100	4:1
5	F5	Tween 40	200: 100: 100	4:1
6	F6	Tween 40	100: 100: 100	4:1
7	F7	Span 80	200: 100: 100	4:1
8	F8	Span 80	300: 200: 100	4:1
9	F7	Span 80	100: 100: 100	4:1

Table 2 Formulation of extract loaded Niosomes

S. no.	Formulation code	Surfactant	Surfactant: Cholesterol: Extract (mg)	Solvent (chloroform: ethanol)
1	NF1	Tween 60	200: 100: 1000	4:1
2	NF2	Tween 60	200: 100: 1500	4:1

Evaluation of niosomes

1. Particle size

Particle size analysis is an essential parameter in evaluating niosomal formulations, as the vesicle size directly affects their stability, drug release profile, and bioavailability. The particle size of niosomes was determined by dynamic light scattering (Litesizer 500, Anton Paar) [11].

2. Entrapment efficiency

The entrapment efficiency of the prepared niosomal formulation was determined using the centrifugation method. A known volume of the freshly prepared niosomal dispersion was transferred into centrifuge tubes and subjected to centrifugation at 3000 rpm for 30 minutes at a controlled temperature of 4 °C using a refrigerated centrifuge. This process separates the niosomes containing the entrapped drug from the supernatant, containing unentrapped drug. From the collected supernatant, take 1ml and diluted with PBS to achieve the conc. 1000µg/ml [12].

3. Zeta potential

Zeta Potential (ZP) is a crucial parameter for evaluating surface charge and stability of the niosomal formulations. Zeta potential indicates the degree of repulsion between adjacent, similar charged particles in the dispersion. A high ZP (greater than ±30 mV) indicates that the niosomes are stable while low ZP (less than ±30 mV) indicates that niosomes are unstable and may aggregates [13].

Preparation of Niosomal gel

The gel was prepared using the Dispersion method. Distilled water was added to a beaker and placed on a magnetic stirrer. Carbopol 934, serving as the gelling agent, was gradually sprinkled into the water while stirring continuously. The temperature was maintained at 50 °C, and the mixture was stirred until the gel was completely hydrated and uniform [14]. In a separate container, propylene glycol, methylparaben, and glycerine 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. The prepared niosomes 2% and 3% w/v was incorporating in to gel base composed of Carbopol 934, glycerine, propylene glycol and distilled water [15].

Table 3 Formulation of Niosomal Gel (F1 & F2)

Chemicals	F1	F2
Extract loaded niosomes	2% w/v	3% w/v
Carbopol 934	1.5g	1.5g
Propylene glycol	1ml	1ml
Methyl paraben	0.02g	0.02g
Triethanolamine	0.2ml	0.2ml
glycerine	2ml	2ml
Distilled water upto 50ml	QS	QS

Evaluation of Niosomal gel

1. Physical appearance:
All the prepared formulations of niosomal gel were evaluated for physical appearance which includes, colour, clarity, and texture [15].

2. pH determination:
The pH of the gels was measured using digital pH meter at room temperature. This study was performed to assure that the pH of the developed gels is close to skin pH. The determinations were carried out in triplicate and the averages of three readings were noted and S.D. was determined. [16].

3. Spreadability:
The spreadability of the prepared niosomal gel was determined by the method Slip and Drag. It was determined for the ease of application and uniform distribution. In this method, 1g of gel is placed between the slides. A 150-200g of weight is placed on the slides for 5 mins. After removing the weight, 20g of weight is attached to the upper slide. Then it was placed at 45ºof angle and allowed to drag. The time at which the two slides separated was recorded (sec) [17].

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

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

4. Homogeneity:
Homogeneity refers to the uniform distribution 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 formulation was observed [18].

5. Viscosity:
Viscosity was determined by using Brookfield viscometer. The viscosity of gels plays a crucial role in controlling how the drug is released from the formulation. Viscosity measurements were carried out at room temperature (25- 27°C) 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 the viscosity [19,20].

In-vitro Drug Release
The invitro drug release study of extract loaded niosomal gel was conducted using a self-designed diffusion setup, where a test tube acted as the donor compartment and a beaker served as the receptor, simulating a Franz diffusion cell. An egg membrane, pre-soaked in phosphate buffer pH 6.8, was used as the diffusion barrier. The niosomal gel was placed over the donor compartment (test tube) attached with the egg membrane, slightly touched with the receptor compartment (beaker). The receptor contains the 250ml of phosphate buffer (pH 6.8), maintained at 35 ± 5°C and stirred at 50 rpm. Samples were withdrawn at the set time intervals, at the same time fresh buffer was added in the receptor compartment. The withdrawn samples were then diluted with the PBS and analysed at 270 nm using UV spectrophotometer. Drug release was calculated using a standard curve and cumulative release was plotted against time [21,22].

Evaluation of analgesic activity

Analgesic activity of the Niosomal gel was tested using Tail immersion method. In this method, the animal's tail is exposed to a warm stimulus, which naturally causes discomfort or pain, leading the animal to flick or withdraw its tail. The temperature of water bath maintained at 55 ± 0.2 °C. The experimental animal was randomly divided in to 3 groups. Group I received 1% diclofenac sodium gel (standard), while Groups II and III were treated with 2% and 3% *Ziziphus mauritiana* extract-loaded niosomal gels. Post-treatment latency was measured at 0, 30, 60, and 120 minutes under the same conditions, applying a cut-off time of 15 seconds.

Formula for calculating minimum possible effect:

$$\% \text{ analgesia} = \frac{\text{Post Latency- Control Latency}}{\text{Cut-off- Control Latency}} \times 100$$

Results

Extraction and phytochemical screening

Methanolic extract of *Ziziphus mauritiana* was a green in colour and the percentage yield was 5.7%, respectively. The preliminary phytochemical investigation revealed that the plant extract included flavonoids, alkaloids, tannins, phenols and saponins and the results are shown in Table 3.

Table 4 Phytochemical constituents of *Z. mauritiana* extract

Phytoconstituents	Test	Result
Alkaloids	•Dragendroff's test •Mayers test	**+ve** **+ve**
Saponins	•Froth test	**+ve**
Phenols	•Ferric chloride test •Bromine water test	**+ve** **+ve**
Glycosides	•Aq. NaOH test	**+ve**
Tannins	•Gelatine test •Ferric chloride test	**+ve** **+ve**
Flavonoids	•HCl test	**+ve**

Evaluation of Optimized niosomes

Various niosome formulations (F1–F9) were optimized using different non-ionic surfactants like Tween 40, Tween 60, and Span 80. Various solvent ratios were also tested during formulation, among which the 4:1 ratio was found to be the most suitable for stable niosome formation. The F2 formulation exhibited the highest entrapment efficiency of 78.3%, based on which it was selected as the final optimized formulation for further study.

Table 5 Optimization parameters of niosomes

Formulation code	Surfactant	% Entrapment efficiency
F1	Tween 60	65.2
F2	Tween 60	78.3
F3	Tween 60	56.1
F4	Tween 40	62
F5	Tween 40	66.02
F6	Tween 40	58
F7	Span 80	44.2
F8	Span 80	60.2
F9	Span 80	70

Evaluation of extract loaded niosomes

1.Particle size

The formulated niosomal preparations was evaluated using particle size analysis. The study was carried out at the DLS-ZETA Lab, SAIF, Punjab University, Chandigarh. The analysis was performed using a **Litesizer 500** (Anton Paar), which operates on the principle of dynamic light scattering (DLS). The results revealed that the mean particle size for formulation NF1 was 2.5µm whereas for NF2 it was 1.2µm.

3. Entrapment efficiency:

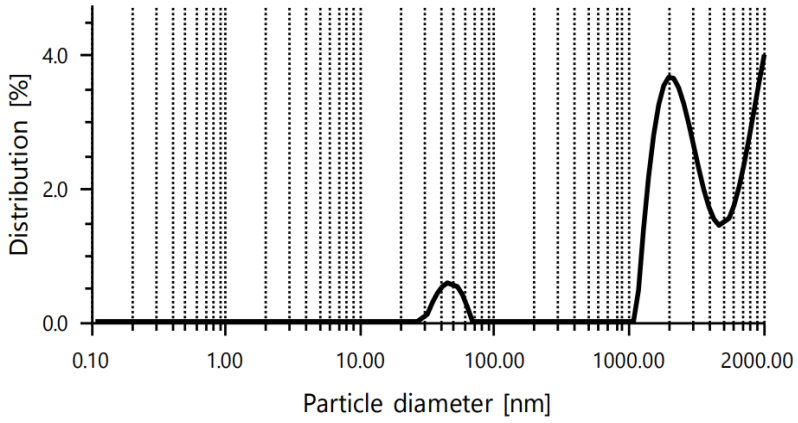
The entrapment efficiency of extract containing niosome formulation NF1 was found to be 74.1% which shows maximum percent extract entrapment whereas formulation NF2 was found to be 75.3%. These results shows that both the formulations had higher encapsulation which is suitable for controlled drug release.

2. Zeta potential:

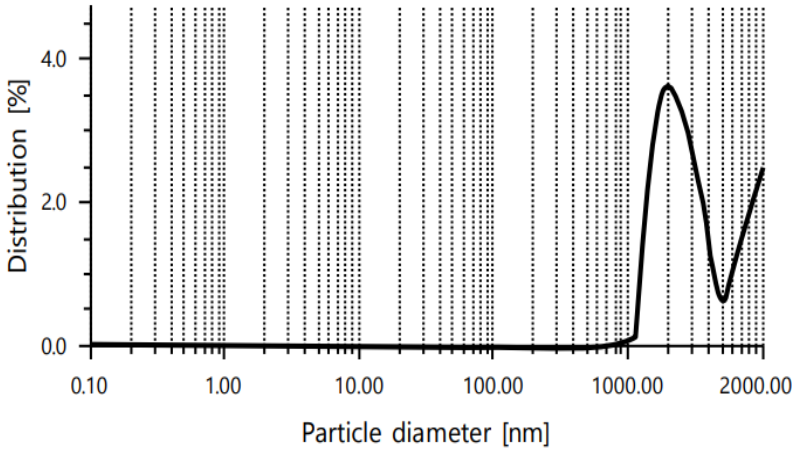
The zeta potential is a measurement of stability of niosomes. It was reported that if zeta potential values are lower than (-30 mV) or higher than (+30 mV) the formulation is considered to be stable. Results, revealed in Table 4, show that extract loaded niosomes showed negative ZP values (-37 and -38mV for F1 and F2, respectively)

Table 6 Evaluation of extract loaded niosomes

S. No.	Formulation Code	Particle size (mean±SD)	Entrapment efficiency (mean±SD)	Zeta potential (mean±SD)
1	NF1	2.5µm ± 57	74.1% ±0.05	-37.1mV±1.3
2	NF2	1.2µm ± 11	75.3% ±0.05	-38.3mV±2.3

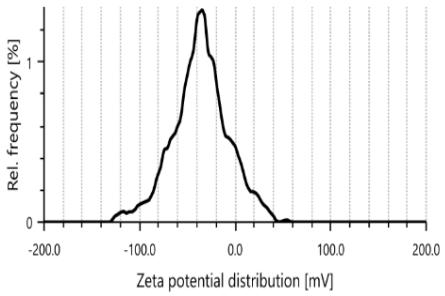


a)



b)

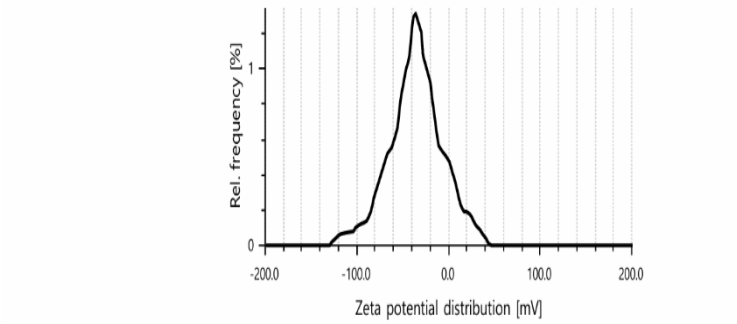
Fig 2 particle size analysis of niosmes a) NF1 b) NF2



Result

Mean zeta potential	-37.1 mV	**Mean intensity**	710.1 kcounts/s
Standard deviation	2.3 mV	**Filter opical density**	3.0416
Distribution peak	-34.4 mV	**Conductivity**	0.232 mS/cm
Electrophoretic Mobility	-2.8929 µm*cm/Vs	**Transmittance**	48.0 %

a)



Result

Mean zeta potential	-38.7 mV	Mean intensity	710.1 kcounts/s
Standard deviation	2.3 mV	Filter opical density	3.0416
Distribution peak	-34.4 mV	Conductivity	0.232 mS/cm
Electrophoretic Mobility	-2.9723 µm*cm/Vs	Transmittance	47.0 %

b)

Fig 3 Zeta potential of niosmes a) NF1 b) NF2

Evaluation of niosomal gel

Appearance and homogeneity:

The prepared formulations of niosomal gel were visually inspected for their colour and texture. The results showed that both the formulations F1& F2 exhibit the green colour whereas the texture was smooth and homogeneous shown in table 7.

pH determination:

The pH measured for the prepared niosomal gel are listed in Table 7. The pH of the F1 formulation of the niosomal gel was found to be 5.7, while the F2 formulation exhibited a pH of 5.3.

Viscosity:

The viscosity of the prepared niosomal gel formulations was measured using a Brookfield viscometer equipped with spindle LV1 at a temperature of 25 °C. The results showed that the F1 formulation had a viscosity of 7287 cps, whereas the F2 formulation had a slightly higher viscosity of 7523 cps.

Spreadability:

The spreadability of niosomal gel formulation is shown in table 5. The slip and drag method were used for evaluating spreadability, where the results showed that F1 formulation exhibit spreadability of 24g.cm/sec and F2 formulation had 25 g.cm/sec.

Table 7 evaluation of Niosomal gel

Formulation	Appearance	Homogeneity	pH (mean±SD)	Viscosity (cps) (mean±SD)	Spreadability (g.cm/sec) (mean±SD)
F1 (2% Niosomal gel)	Greenish	Homogeneous	5.7±0.02	7387±0.01	24±0.01
F2 (3% Niosomal gel)	Greenish	Homogeneous	5.3±0.02	7523±0.01	25±0.01

In-vitro drug release

The in-vitro drug release of extract from the prepared niosomal gel was studied using Franz diffusion cell mimic. Drug release study was carried out for both the formulations. The receptor compartment contained 250ml of phosphate buffer (pH 6.8) maintained at 37± 0.5°C and samples were withdrawn at regular intervals of 1, 2, 4, 6, 8, 12 & 24 hours for analysis. The results of the in vitro drug release study of promising niosomal gel and marketed formulation are given in table 6 and graphically represented in fig 4. The results indicated the sustained release for 24hrs.

Table 8 In- vitro drug release for F1 and F2 Niosomal gel formulation

S. No.	Time (hr)	% CDR of F1	%CDR of F2
1	1	6.53	8.4
2	2	8.01	11.5
3	4	14.5	17.3
4	6	25.6	23.8
5	8	33.1	31.6
6	12	48.3	40.1
7	24	78.3	80.8

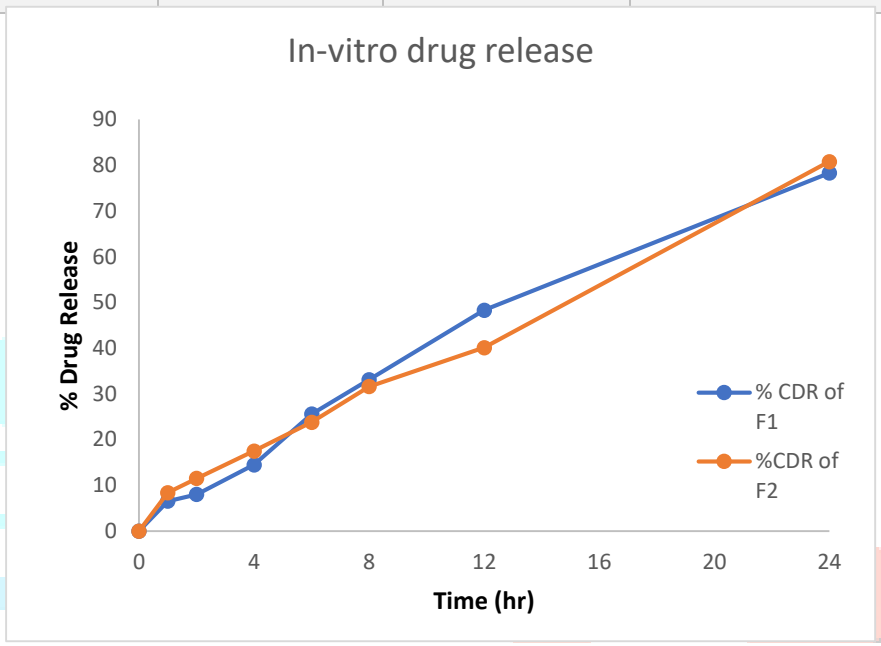


Fig 4 graphical presentation of drug release of F1 and F2

In-vivo study

Analgesic activity of *Z. mauritiana* extract loaded niosomal gel was evaluated by following tail immersion method. After 120 mins of niosomal gel application, it was notices that standard group which received 1% diclofenac sodium gel showed 78% of pain inhibition while the test group I and II inhibited the pain by 34% and 41%, respectively. *Ziziphus mauritiana* niosomal gel shows significant topical analgesic potential, offering a promising option for localized and sustained pain management.

Table 9 Results of Analgesic activity by Tail immersion method

S. No.	Groups	Dose	Reaction time in sec at			
			0min	**30 min**	**60min**	**120min**
1	Standard group	1% diclofenac sodium gel	3.63±0.11	6.27±0.03	8.48±0.01	12.52±0.01
2	Test group I	2% Niosomal gel	3.24±0.02	5.01±0.02	6.30±0.06	7.25±0.02
3	Test group II	3% Niosomal gel	3.12±0.02	5.72±0.01	6.88±0.02	8.04±0.03

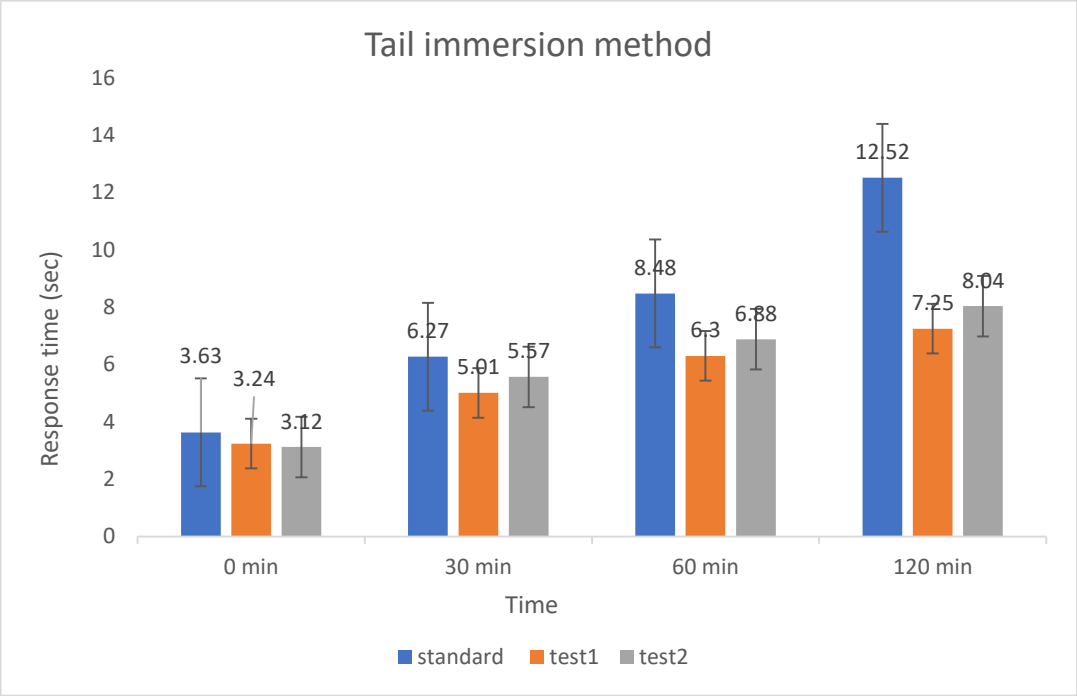


Fig 5 Graphical presentation of Analgesic activity by tail immersion method

Conclusion

The study demonstrated that Ziziphus mauritiana extract-loaded niosomal gel can serve as an effective topical analgesic system. The optimized formulation showed good physicochemical properties, sustained drug release, and significant in vivo analgesic activity, comparable to diclofenac gel. The study was performed using healthy female Wistar albino rats. Test Group I treated with 2% *Ziziphus mauritiana* extract-loaded niosomal gel, while Test Group II was treated with the 3% niosomal gel which showed the 34% and 41% pain inhibition after 120 min of gel application, respectively.

These findings suggest that niosomal gels provide a promising approach to enhance the therapeutic efficacy and skin permeation of herbal extracts for localized pain management.

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