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Research on: Ethosomal Drug Delivery System for White Flower *Clitoria ternatea* Leaf Extract: Formulation, Characterization, and Anti-inflammatory Evaluation

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Abstract:

This study evaluates the antioxidant and anti-inflammatory potential of *Clitoria ternatea* and its enhancement through an ethosomal gel formulation. Phytochemical and GC–MS analysis confirmed the presence of bioactive compounds. SEM revealed a porous structure with micro- to submicron-sized particles. The ethosomal gel showed significantly higher antioxidant and anti-inflammatory activity than the crude extract in both in vitro and in vivo studies. The improved efficacy is attributed to enhanced solubility, stability, and skin penetration. The formulation demonstrates promising potential for the topical treatment of inflammatory skin disorders

Materials and Methods: *Clitoria ternatea* extract was prepared and subjected to phytochemical and GC–MS analysis. Ethosomes were formulated and incorporated into a Carbopol gel. SEM analysis was performed to study morphology. Antioxidant and anti-inflammatory activities were evaluated using in vitro methods, followed by in vivo assessment using the formalin-induced paw edema model.

Results: SEM showed a porous structure with particle sizes ranging from nanometers to micrometers. The ethosomal gel exhibited higher antioxidant and anti-inflammatory activity than the crude extract in a dose-dependent manner. In vivo studies confirmed significant and sustained anti-inflammatory effects comparable to those of the standard drug.

Conclusion: Ethosomal encapsulation significantly enhances the therapeutic efficacy of *Clitoria ternatea* extract. The developed gel is a promising candidate for topical anti-inflammatory applications.

Keywords: *Clitoria ternatea*, ethosomes, antioxidant, anti-inflammatory, SEM, and topical delivery.

1. Introduction:

An increasing number of people worldwide are turning to medicinal plants and herbs for their health benefits. As a result, it is crucial to scientifically assess their therapeutic potential, biological properties, and safety to support informed choices. [1] Many vital drugs and bioactive compounds originate from traditional medicinal plants. These plants exhibit diverse pharmacological effects, including antimicrobial, antioxidant,

anticancer, hypolipidemic, cardiovascular, nervous system, respiratory, immune, anti-inflammatory, analgesic, and antipyretic actions.[2] Phytochemical studies show that Clitoria ternatea contains tannins, phlobatannins, carbohydrates, saponins, triterpenoids, phenols, flavonoids, flavonol glycosides, proteins, alkaloids, anthraquinones, anthocyanins, cardiac glycosides, Stigmast-4-ene-3,6-dione, volatile oils, and steroids.

The plant exhibits a wide range of pharmacological properties, including antioxidant, hypolipidemic, anticancer, anti-inflammatory, analgesic, fever-reducing, anti-diabetic, central nervous system, antimicrobial, gastrointestinal antiparasitic, and insecticidal effects. This review covers the chemical constituents and pharmacological activities of Clitoria ternatea. The extraction process involves dried leaves of C. ternatea. [3]

2. Material and Method

India's Botanical Survey of India Arid Zone Regional Center confirmed C. ternatea, with registration number A.12012/Tech./2023-24(PI.I.) 540. After this confirmation, the extraction process began, starting with the preparation of the plant material. The dried C. ternatea leaves were then ground into a coarse powder. Subsequently, 150 grams of the powdered material was placed in a Soxhlet apparatus, with 500 mL of ethanol as the solvent. The extraction procedure involved continuous hot percolation, comprising 9 to 10 cycles, for 24 hours at a controlled temperature ranging from 50 to 60 degrees Celsius. The successful completion of the extraction was indicated by a color change from light green to a dark, resinous substance. [3]

2.1. Formulation study

Table 1: Formulation of *Clitorea ternatea* extract-loaded Ethosomes

Formulation Code	Lecithin (mg)	Ethanol (%V/V) ml	Extract (gm)	Cholesterol (%w/w)	PG (%V/V)	Water QS up to ml
XXET 1	100	5	5	0.1	0.1	100
XXET 2	200	15	5	0.1	0.1	100
XXET 3	300	10	5	0.1	0.1	100
XXET 4	200	5	5	0.1	0.1	100
XXET 5	100	15	5	0.1	0.1	100
XXET 6	300	15	5	0.1	0.1	100
XXET 7	200	10	5	0.1	0.1	100
XXET 8	300	5	5	0.1	0.1	100
XXET 9	100	10	5	0.1	0.1	100

2.1.1. Preparation of *extract-loaded* Ethosomes gel

The ethosomes were prepared by a simple cold method. Briefly, lecthin (100–300mg) was dissolved in a mixture of ethanol (5–15%) and propylene glycol (1%) in a completely closed flask using a magnetic stirrer at 30°C. Next, the *Clitorea ternatea* extract (5%) dissolved in ethanol was introduced slowly as a fine stream using a syringe, and the volume was adjusted with distilled water. The entire system was stirred at 700-900 rpm for 15-20 minutes, then sonicated for 30 minutes. The nanoethosomes loaded with *Clitorea ternatea* extract were then stored at room temperature. A total of nine batches (XXET 1-XXET 9) of ethosomes were prepared by

varying the ethanol and lectin concentrations, as described in Table 1.

2.1.2. **Preparation of ethosomal gel**:

Ethosomal vesicle suspensions were incorporated by the dispersion method using Carbopol 940 as a dispersing and gelling agent. The specified amount of Carbopol 934 powder was allowed to swell overnight. Tri-ethanolamine was added to the drop-by-drop neutralized mixture, and optimized ethosomal dispersion was added and mixed properly. Mixing was continued until a transparent gel appeared, and a preservative was added. The prepared gels were stored in a glass container at 4-8 °C. [4]

Table 2: Effect of Lecithin Concentration and Ethanol Volume on Particle Size and Zeta Potential of Formulated Nanoemulsions

Formulation code	Lecithin (mg)	Ethanol (v/v) ml	Particle size Nm	Zeta potential
XXET 1	100	5	1.153	-41.8
XXET 2	200	15	0.752	-40.1
XXET 3	300	10	0.827	-34.7
XXET 4	200	5	0.973	-43.5
XXET 5	100	15	0.869	-38.4
XXET 6	300	15	0.543	-29.2
XXET 7	200	10	0.883	-37.8
XXET 8	300	5	0.991	-40.5
XXET 9	100	10	0.868	-37.3

This table presents the characterization of nine nanoemulsion formulations (XXET 1–9), showing how varying lecithin concentrations (100–300 mg) and ethanol volumes (5–15% v/v) affect the resulting particle size (nm) and zeta potential (mV). The formulations demonstrate how both parameters affect colloidal stability and droplet size distribution

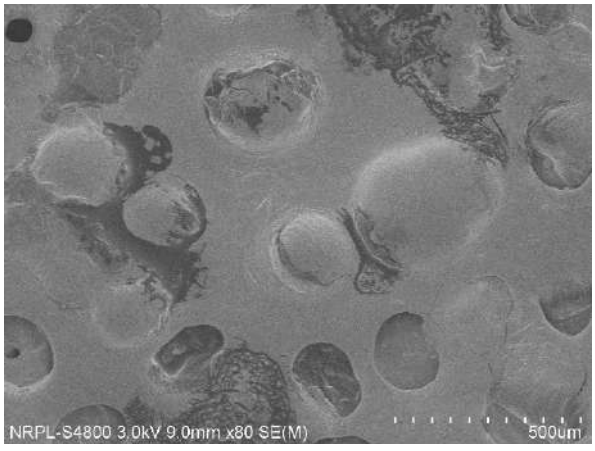

Figure 1: SEM image of dried nanoemulsion surface, showing porous structures with spherical indentations, indicating droplet evaporation and matrix porosity. Captured with Hitachi NRPL-S4800 at 3.0 kV, 9.0 mm working distance, 80× magnification. Scale bar: 500 µm.

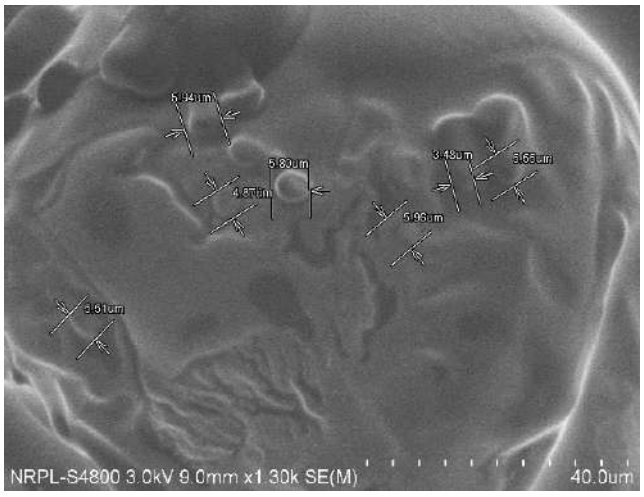

Figure 2: SEM image showing surface morphology and particle size distribution, ranging from ~3.4 µm to ~5.9 µm (NRPL-S4800, 3.0 kV, 9.0 mm, ×1.30k SE).

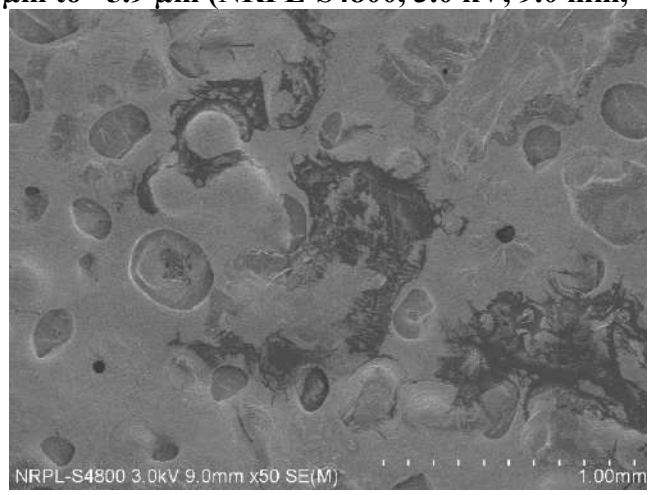

Figure 3: SEM micrograph showing the sample's surface morphology and pore structure at ×50 magnification (NRPL-S4800, 3.0 kV, 9.0 mm, SE).

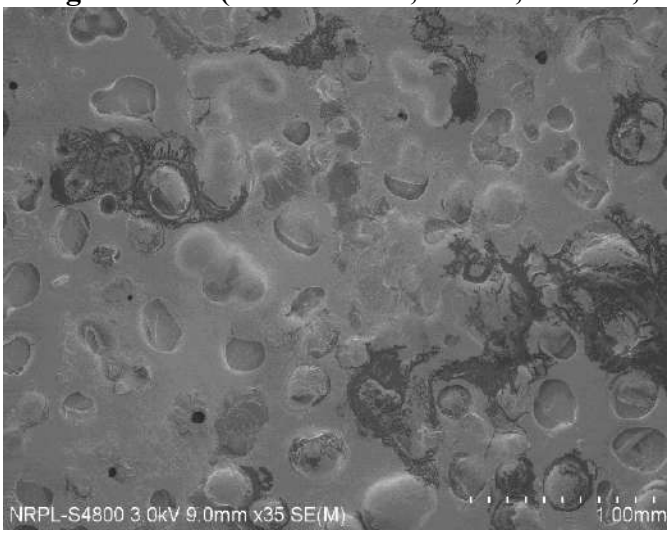

Figure 4: SEM image showing surface morphology and porosity at ×35 magnification (NRPL-S4800, 3.0 kV, 9.0 mm, SE)

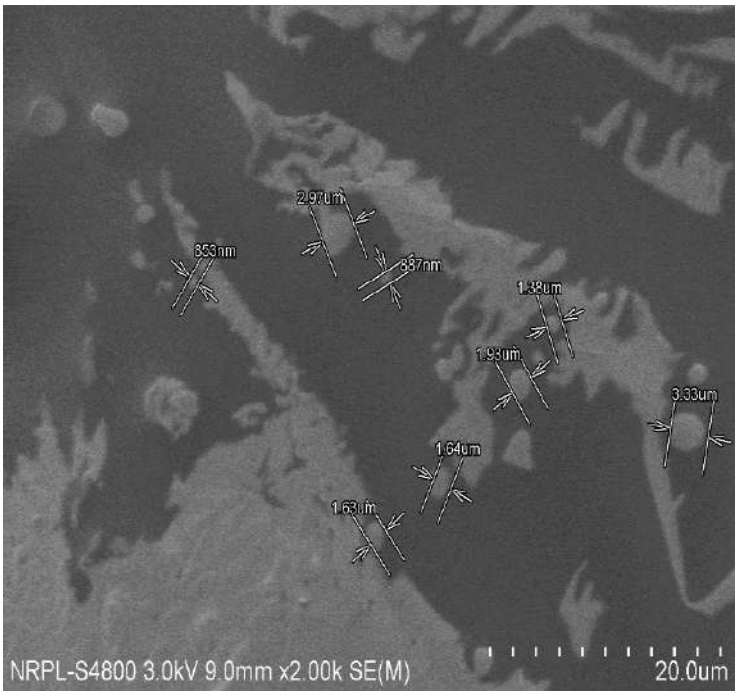

Figure 5: SEM micrograph showing surface features and particle size distribution of the sample, ranging from ~853 nm to ~3.33 µm (NRPL-S4800, 3.0 kV, 9.0 mm, ×2.00k SE)

Result:

1. Figure 23 shows the SEM image of the dried nanoemulsion surface, revealing a highly porous matrix with spherical indentations, indicating droplet evaporation and pore formation during the drying process.
2. The surface morphology observed suggests uniform distribution of droplets within the matrix and the development of interconnected porosity.
3. Figure 24 demonstrates the surface morphology and particle size distribution, with particle sizes ranging from approximately 3.4 µm to 5.9 µm, indicating micro-sized aggregates formed after drying.
4. Figure 25 illustrates the overall surface texture and pore structure at lower magnification, confirming the presence of irregular pores and rough surface morphology.
5. Figure 26 further highlights the porous nature of the sample, showing well-developed voids and surface heterogeneity at ×35 magnification
6. Figure 27 presents a higher-magnification SEM micrograph showing distinct particles with a size distribution ranging from ~853 nm to ~3.33 µm, indicating the presence of submicron to micron-sized particles.
7. Overall, SEM analysis confirms that the dried nanoemulsion exhibits porous surface morphology, spherical indentations, and broad particle size distribution, characteristic of nanoemulsion-derived dried systems.

3. In vitro study of formulation:

Table 3. Comparative antioxidant activity (IC_{50} values) of Ascorbic acid, *Clitoria ternatea* extract, and extract-loaded ethosomal gel at different concentrations (50–300 µg/mL).

Dose µg/ml	Ascorbic acid		Extract		Extract the ethosome gel	
	Mean	SD	Mean	SD	Mean	SD
50µg/ml	3.86	0.11	1.54	0.12	2.89	0.18
100µg/ml	8.54	0.14	3.65	0.19	6.92	0.23
150µg/ml	12.59	0.17	5.97	0.17	9.87	0.29
200µg/ml	15.38	0.19	9.72	0.23	13.96	0.33
250µg/ml	18.51	0.21	12.16	0.21	16.58	0.35
300µg/ml	20.28	0.23	13.64	0.23	19.13	0.37

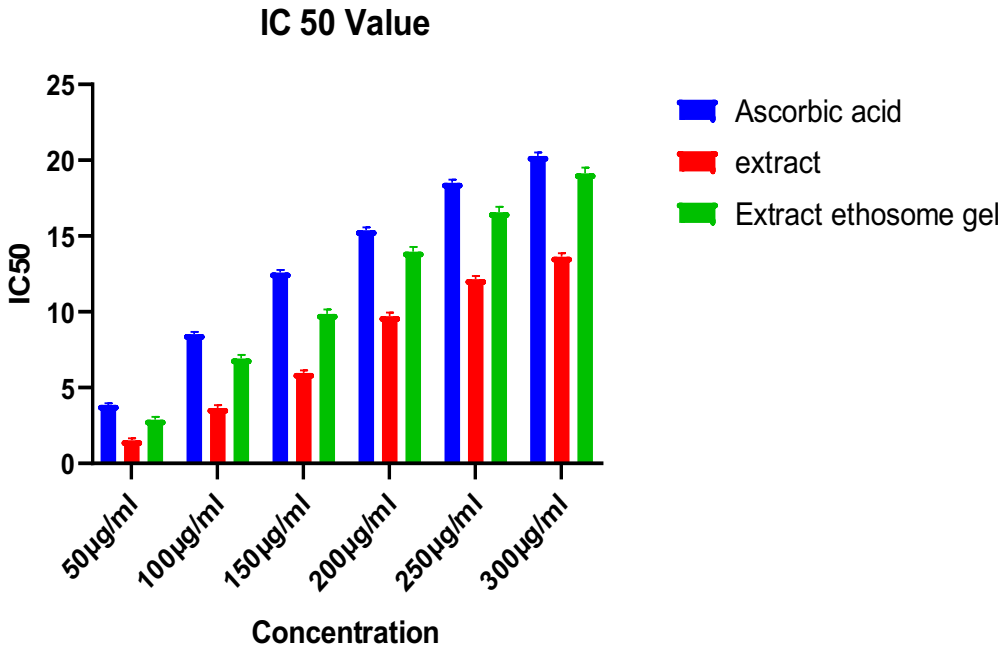

Fig 6: Comparison of IC_{50} values for ascorbic acid, plant extract, and ethosome gel at concentrations ranging from 50 to 300 µg/ml. The graph shows a dose-dependent increase in antioxidant activity, with ascorbic acid exhibiting the lowest IC_{50} values, followed by the ethosome gel extract and the extract. This indicates that ascorbic acid has the highest free-radical-scavenging efficiency.

Result: Antioxidant activity increased in a concentration-dependent manner across all samples. Among the tested groups, **Ascorbic acid** exhibited the highest radical scavenging activity, followed by the **ethosomal gel loaded with the extract**, and then the **plain extract**. At 300 µg/mL, Ascorbic acid showed the maximum IC_{50} inhibition (20.28 ± 0.23%), whereas the extract and extract-loaded ethosomal gel recorded 13.64 ± 0.23% and 19.13 ± 0.37%, respectively. The **ethosomal gel formulation significantly enhanced the antioxidant potential** of the extract compared to the crude extract alone, likely due to improved solubility and bioavailability of active constituents within the ethosomal system.

Table 4 Anti-inflammatory activity (% protection) of Aspirin, *Clitoria ternatea* extract, and extract-loaded ethosomal gel at different concentrations (62.5–1000 µg/mL).

Dose µg/ml	Asprin		Extract		Extract the ethosome gel	
	Mean	SD	Mean	SD	Mean	SD
62.5 µg/ml	29.53	0.79	14.17	0.69	21.29	0.59
125 µg/ml	37.17	0.58	21.23	0.71	32.47	0.78
250 µg/ml	53.18	0.62	32.39	0.62	46.24	0.58
500 µg/ml	69.14	0.89	43.71	0.78	58.56	0.83
1000 µg/ml	85.13	0.87	53.12	0.82	76.36	0.75

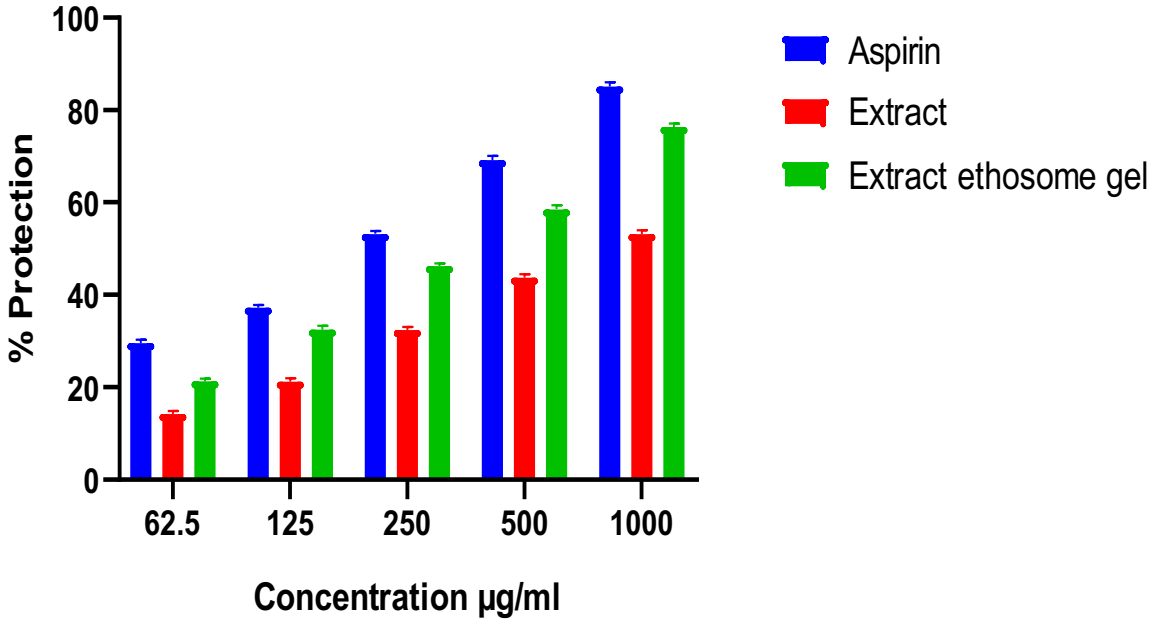

Fig 7: Comparison of the percentage protection at different concentrations (62.5–1000 µg/ml) for aspirin, plant extract, and ethosome gel. The data reveal a dose-dependent increase in anti-inflammatory activity, with aspirin showing the highest % protection, followed by the ethosome gel extract and the extract. This suggests that the ethosomal formulation is more effective than the crude extract.

Result: All tested samples demonstrated a **dose-dependent increase in % protection**, indicating greater anti-inflammatory activity at higher concentrations. Among the groups, **Aspirin (standard drug)** showed the highest % protection at all concentrations, reaching **85.13 ± 0.87%** at 1000 µg/mL. The **extract-loaded ethosomal gel** exhibited markedly greater anti-inflammatory activity (76.36 ± 0.75%) than the plain **extract** (**53.12 ± 0.82%**) at the same dose.

This enhancement suggests that **ethosomal encapsulation improved the delivery and efficacy** of bioactive compounds from the Clitoria ternatea extract, possibly by enhancing skin penetration and sustaining release at the target site.

Table 5. Percentage Radical Scavenging Activity (%RSA) of Ascorbic acid, *Clitoria ternatea* extract, and extract-loaded ethosomal gel at different concentrations (50–300 µg/mL).

Dose µg/ml	Ascorbic acid		Extract		Extract the ethosome gel	
	Mean	SD	Mean	SD	Mean	SD
50 µg/ml	14.98	0.55	9.17	0.32	12.09	0.29
100 µg/ml	26.12	0.62	14.16	0.49	19.23	0.37
150 µg/ml	33.97	0.37	24.16	0.36	30.19	0.27
200 µg/ml	43.05	0.48	30.17	0.48	37.11	0.37
250 µg/ml	54.28	0.34	36.18	0.51	46.14	0.58
300 µg/ml	57.16	0.48	39.15	0.62	53.21	0.68

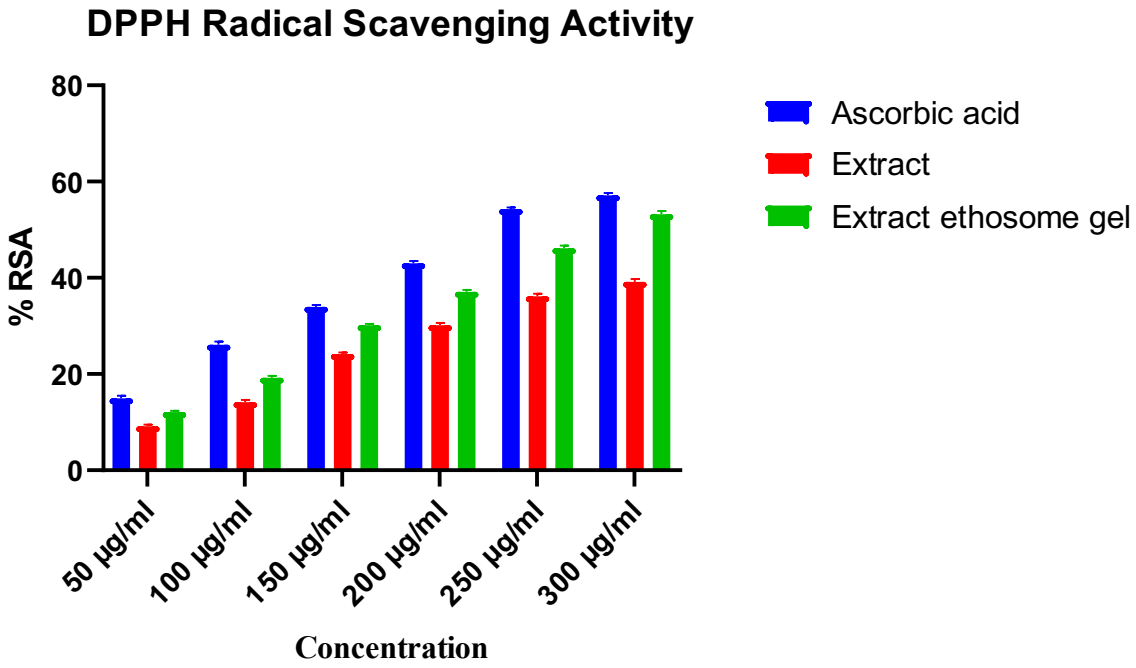

Figure 8 illustrates the DPPH radical-scavenging activity of ascorbic acid, plant extract, and the extract ethosome gel across different concentrations (50–300 µg/ml). The results show a dose-dependent increase in %RSA, with ascorbic acid exhibiting the highest antioxidant activity, followed by the ethosome gel extract and the crude extract. This indicates that the ethosomal formulation improves free-radical-scavenging capabilities compared to the crude extract.

Result:

The %RSA increased in a dose-dependent manner across all samples, confirming concentration-related enhancement of antioxidant activity. At every concentration tested, Ascorbic acid demonstrated the highest radical scavenging potential, reaching 57.16 ± 0.48% at 300 µg/mL. The extract-loaded ethosomal gel consistently showed a higher percentage of relative solubility (RSA) than the plain extract, with values of 53.21 ± 0.68% and 39.15 ± 0.62%,

respectively, at 300 µg/mL. These findings suggest that the ethosomal formulation significantly improves the antioxidant efficacy of Clitoria ternatea extract, likely by increasing the stability and bioavailability of active phytoconstituents, such as flavonoids and anthocyanins.

4. In vivo study:

4.1 Experimental animals procured

Adult Wistar rats of male 9 to 11 weeks of age, weighing 160–180g, were procured from Mahaveera Enterprises, Hyderabad. Animals were housed in standard laboratory conditions at 25 °C with a 12-hour light-dark cycle, with free access to chow and water *ad libitum*. The research protocol was approved by the IAEC, Pacific College of Pharmacy, Udaipur, Rajasthan. Registration no. 1622/PO/Re/S/12/CPCSEA.

4.1.1 Formalin-Induced Rat Paw Edema

The formalin-induced inflammation test was conducted according to Turner's method. Edema was induced in the left hind paw of rats in all groups with subplantar injection of 20 µL of freshly prepared 2% formalin in the hind paw. The right hind paw was maintained as a negative control. The anti-inflammatory activity was compared with that of the control and treated groups using the plethysmograph method.

To all groups of rats, on both hind paws, to ensure constant paw volume. In all groups, the left and right paw volume was measured at zero time (normal paw volume) and at 1, 2, 3, 5,12, and 24h after induction of inflammation. [5]

The percent inhibition of edema was calculated as follows:

$$\% \text{ Edema inhibition} = \frac{(VL-VR)\ \text{control} - (VL-VR)\ \text{treated} \times 100}{(VL-VR)\ \text{control} \times 100}$$

Whereas

VL: represents the mean of the left paw displacement volume and

VR: represents the mean of the right paw displacement volume.

Group I: Control

Group-II: Formalin-induced

Group-III: Extract

Group-IV: ETGs

Group-V: Diclofenac gel

4.2 Acute Dermal Toxicity Study in Mice

Acute dermal toxicity is the **adverse effect occurring within 24 hours** after a **single dermal (skin) application** of a test substance, with observation for **14 days**. [6]

OECD 434 (dose) **5, 50, 200, 2000 mg/kg**.

Observation Period

1**4 days** after exposure.

Observe for:

- Mortality
- Skin reactions (erythema, edema)
- Behavioral changes
- Body weight changes
- Systemic toxicity signs

Table no 9: Acute Dermal Toxicity Study in Mice

S No.	Dose required	Mortality observed/ not observed
1.	5mg/kg	Not observed
2.	50mg/kg	Not observed
3.	200mg/kg	Not observed
4.	2000mg/kg	Not observed

Result: In the acute dermal toxicity study, no toxicity was found.

Table 10: Effect of different treatments on paw edema volume (mL) at various time intervals (0–5 hrs)

Treatments	0 hrs	1 hrs	2 hrs	3hrs	5hrs
Control	0.0± 0.0	0.0± 0.0	0.0± 0.0	0.0±0.0	0.0±0.0
Formalin	1.07±0.021	1.65± 0.63	2.01±0.81	2.11 ±0.56	1.93±0.54
Extract	1.04 ± 0.011	1.44±0.51ns	1.76± 0.72ns	1.80 ±0.60*	1.61±0.41*
ETs gel	1.05±0.01	1.43 ±0.39ns	1.71±0.71*	1.66± 0.55**	1.41±0.25***
Standard	1.05±0.01	1.42±0.31ns	1.68±0.69**	1.70±0.49***	1.25±0.21***

One-way ANOVA followed by Tukey–Kramer multiple comparison test showed * $p < 0.05$ ** $p < 0.01$ and *** $p < 0.00$ as compared to the positive control group and the reference group.

Fig 9 Control Group

One-way ANOVA followed by Tukey–Kramer multiple comparison test showed * $p < 0.05$ ** $p < 0.01$ and *** $p < 0.00$ as compared to the positive control group and the reference group

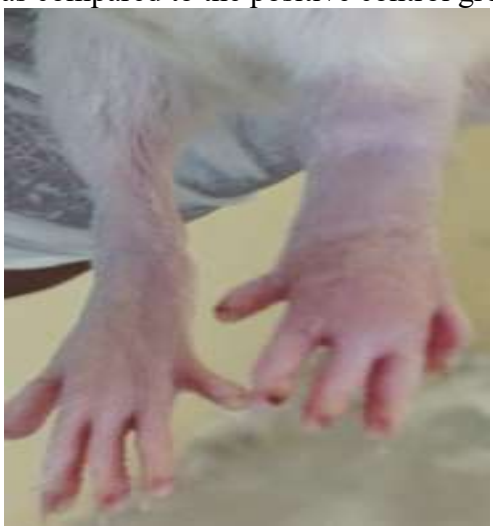

Fig 10 Formalin-induced group

One-way ANOVA followed by Tukey–Kramer multiple comparison test showed * $p < 0.05$ ** $p < 0.01$ and *** $p < 0.00$ as compared to the positive control group and the reference group

Fig 11: Extract treated group

One-way ANOVA followed by Tukey–Kramer multiple comparison test showed * $p < 0.05$ ** $p < 0.01$ and *** $p < 0.00$ as compared to the positive control group and the reference group

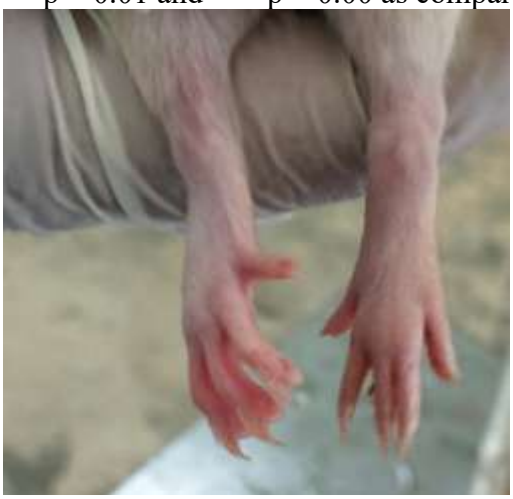

Fig 12 Ethosomal gel-treated group

One-way ANOVA followed by Tukey–Kramer multiple comparison test showed * $p < 0.05$ ** $p < 0.01$ and *** $p < 0.00$ as compared to the positive control group and the reference group.

Fig. 13 Standard drug-treated group

One-way ANOVA followed by Tukey–Kramer multiple comparison test showed * $p < 0.05$ ** $p < 0.01$ and *** $p < 0.00$ as compared to the positive control group and the reference group.

4.3 Statistical Analysis of Results

The volume measured by the plethysmometer test and the thickness of the dermis were expressed as mean ± Standard Error of Mean. Paired *t*-test and One-Way ANOVA test followed by Tukey–Kramer multiple comparison tests were used to compare the mean between different groups, and the result was considered significant at *p*-value < 0.05.

Result: The effect of different treatments on the measured parameter at 0, 1, 2, 3, and 5 hours is presented in the table.

The control group showed no response throughout the experimental period (0.0 ± 0.0 at all time points).

The formalin-treated group showed a progressive increase in response, peaking at 3 hours (2.11 ± 0.56), followed by a slight reduction at 5 hours (1.93 ± 0.54), indicating sustained induction of the inflammatory/pain response.

Treatment with the extract showed a lower response than the formalin group at all time points. Although the reduction was not statistically significant at 1 and 2 hours (ns), a significant decrease was observed at 3 hours ($p < 0.05$) and 5 hours ($p < 0.05$), suggesting a time-dependent therapeutic effect.

The ETs gel formulation demonstrated a more pronounced effect than the extract alone. A significant reduction was observed at 2 hours ($p < 0.05$), which became highly significant at 3 hours ($*p < 0.01$) and very highly significant at 5 hours ($**p < 0.001$), indicating enhanced efficacy of the gel formulation.

The standard treatment group showed activity comparable to or slightly better than that of the ET gel. While no significant effect was observed at 1 hour, significant inhibition was observed at 2 hours ($p < 0.01$), with highly significant effects at 3 hours ($p < 0.001$) and 5 hours ($p < 0.001$). The maximum inhibition was observed at 5 hours (1.25 ± 0.21).

Overall, the results indicate that both the extract and ETs gel significantly reduced the formalin-induced response in a time-dependent manner, with the gel formulation showing enhanced activity comparable to the standard drug.

5. Histopathological analysis

Following the euthanasia of the rat, the paw was extracted for histopathological analysis. The tissue samples were cut into 4 µm-thick sections, fixed in 10% neutral-buffered formalin, and embedded in paraffin wax. Each specimen was then stained with hematoxylin and eosin. [7,8].

Result

Hematoxylin and eosin (H&E) -stained sections of the rat's hind paw:

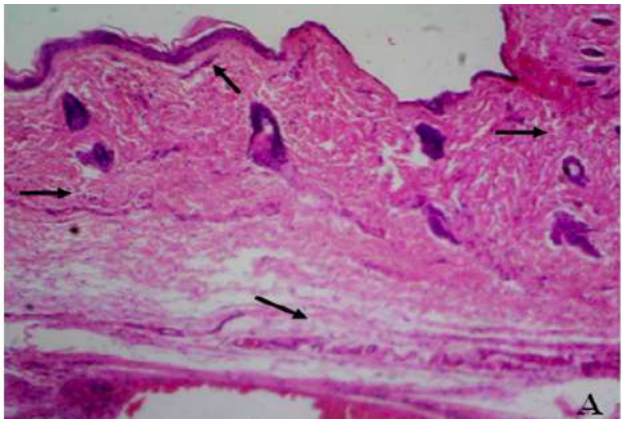

Fig 14: Control Group: Figure: (A) Rat's paw showed normal morphological appearance of epithelial tissue

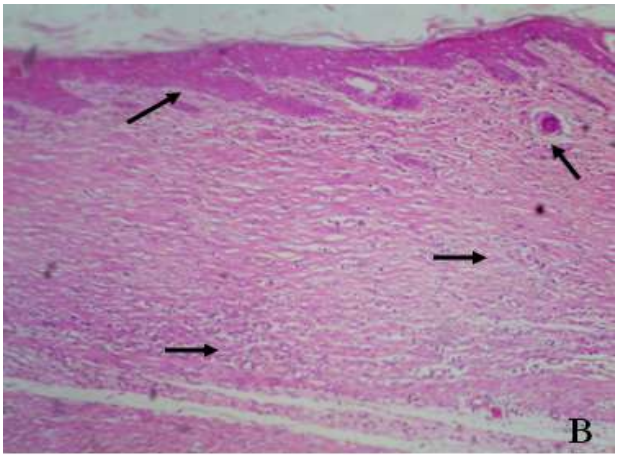

Fig 15: Formalin Induced Group: induced rat (Fig. B) showing areas of severe dermal inflammation, extending into deep dermis with inflammatory cell infiltration, marked edema, and loss of epithelial integrity

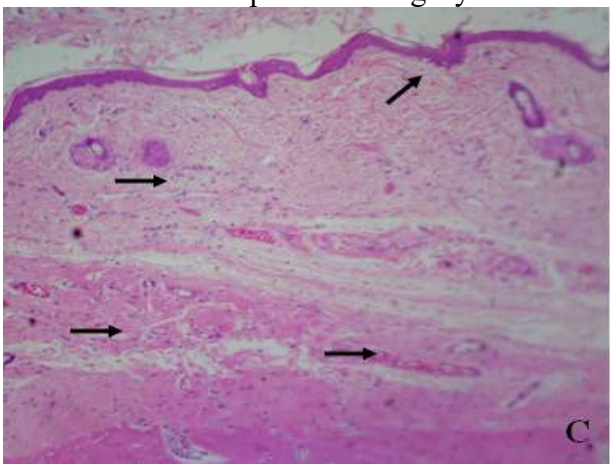

Fig 16: Extract Treated Group: The extract (Fig. C) from the treated animal showed improvements in epithelial cells, with mild to moderate epithelial disruption, cellular infiltration, degeneration, mild edema, and epithelial hyperplasia.

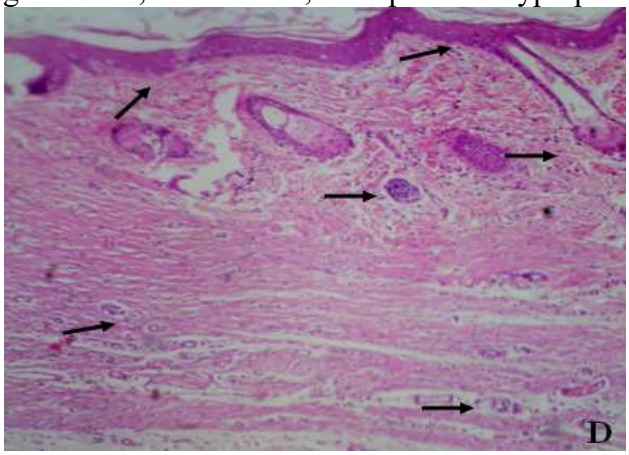

Fig 17: ETHOSOMAL GEL Treated Group: Ethosomal Gel (Fig. D) treated animal showed restoration of epithelial cells with an organized architectural appearance, mild degeneration, and epithelial hyperplasia

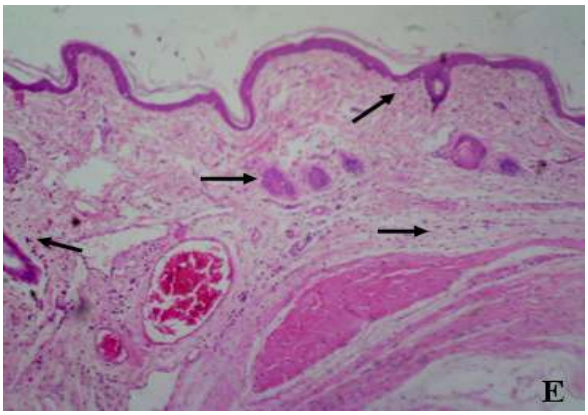

Fig 18: Standard Group: Standard (Fig. E) exhibited restoration of epithelial cells with improvement in epidermis and dermis resembling the normal architectural appearance of epithelium

6. CONCLUSION

The present work systematically explored Clitoria ternatea L. as a phytopharmaceutical candidate and successfully translated its bioactive potential into a novel ethosomal gel for topical delivery. The findings from phytochemical, in vitro, in silico, formulation, and in vivo studies collectively support the central hypothesis that ethosomal encapsulation can enhance the therapeutic performance of C. ternatea extract on the skin.

In vitro antioxidant assays demonstrated an apparent concentration-dependent increase in radical-scavenging activity. Although the standard antioxidant (ascorbic acid) remained the most potent, the ethosomal gel loaded with the extract consistently outperformed the crude extract at equivalent concentrations, indicating that the ethosomal carrier enhanced the apparent antioxidant efficacy, likely through improved solubilization, stabilization, and controlled release of active phytoconstituents.

Similarly, in vitro anti-inflammatory models, such as protein denaturation and membrane stabilization, revealed marked inhibition of inflammatory processes by the extract. Notably, the ethosomal gel formulation produced significantly higher percentage protection than the plain extract, with activity approaching that of the standard NSAID used for comparison. These results highlight the ability of the ethosomal system to potentiate and prolong the anti-inflammatory effect of the encapsulated phytochemicals.

The development and optimization of the ethosomal formulation were key outcomes of this thesis. By systematically varying the lecithin concentration and ethanol content, an optimized ethosomal dispersion with a sub-micrometer particle size and a sufficiently negative zeta potential was obtained, indicating good colloidal stability. SEM analysis confirmed a largely uniform morphology. Incorporation of the optimized ethosomes into a carbopol-based gel yielded a transparent, homogeneous preparation with acceptable pH, viscosity, spreadability, and drug content, satisfying pharmaceutically relevant criteria for topical application.

In vitro release studies suggested that the ethosomal gel provided more controlled and sustained release of the extract than non-ethosomal formulations. This behavior is desirable for maintaining adequate drug levels at the site of action while potentially reducing dosing frequency. The in vivo formalin-induced rat paw edema model further substantiated these findings: animals treated with the extract-loaded ethosomal gel showed significant and sustained inhibition of edema compared with the control and

plain extract groups, with efficacy comparable to, though not exceeding, that of the standard anti-inflammatory drug. Together, these data confirm that ethosomal encapsulation improves percutaneous delivery and in vivo performance of C. ternatea extract.

In conclusion, the thesis provides a robust scientific basis for the traditional use of Clitoria ternatea. It establishes an ethosomal gel of its extract as a promising candidate for the topical management of inflammatory and oxidative stress-mediated skin disorders. With further optimization and clinical validation, this formulation has the potential to evolve into a valuable phytopharmaceutical product.

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