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Development and Assessment of *Helicteres isora* Extract Loaded Phospholipid Complex for Anti-Nociceptive Activity

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Its primary objective is to assess the efficacy of a phospholipid complex formulation containing an extract of *Helicteres isora* in reducing inflammation, to determine its progress and evaluation. An Indian Screw Tree, also known as *Helicteres isora*, is an extremely healing plant. In addition to improving solubility and stability, the phospholipid complex formulation also increases bioavailability and efficacy. This formulation was created by combining *Helicteres isora* extract with phospholipids using a technique known as phospholipid complexation. Several parameters were evaluated to

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evaluate the formulation, including particle size, zeta potential, encapsulation efficiency, and drug release profile. Using both *in vitro* and *in vivo* models, the anti-inflammatory properties of a phospholipid complex formulation of *Helicteres isora* were assessed. The experiments were conducted using a rat model of carrageenan-induced paw edema. In this work, phospholipid complex formulations were shown to have improved physicochemical properties, including smaller particle sizes and higher encapsulation rates. According to the results of the in vivo evaluation, phospholipid complex formulations significantly reduced paw edema compared with plain extracts, indicating improved anti-inflammatory activity. The bioavailability, anti-inflammatory activity, and bioavailability of *Helicteres isora* extract are enhanced by the phospholipid complex formulation.

Keywords: Helicteres isora; Indian screw tree; phospholipid complex formulation; anti-inflammatory activity; bioavailability; phospholipid complexation.

1. INTRODUCTION

The Indian Screw Tree, commonly known as *Heliteres isora*, has been used to treat inflammation for centuries. It has been widely recognized for its potential therapeutic properties. The active ingredients, however, must be formulated in a way to maximizes their therapeutic potential by making them more readily bioavailable and more effective. As part of the current study, a phospholipid complex formula containing an extract of *Helicteres isora* plant will be developed and evaluated for anti-inflammatory properties [1]. An herbal herb renowned for its potential healing properties, Helicteres isora is called Indian Screw Tree. With the phospholipid complex formula, extracts become more solubilized and stable, resulting in increased bioavailability and efficiency.

A technique called phospholipid complexation was used to develop the phospholipid complex formula, which binds Helicteres isora extracts to phospholipids. According to the findings, the developed phospholipid complex formula was able to reduce particle size and increase encapsulation efficiency while maintaining improved physicochemical properties [2]. A significant reduction in paw edema was observed during in vivo evaluation when compared to plain extract, suggesting that the phospholipid complex formula was more effective at inhibiting inflammation [3]. Helicteres isora extract is capable of enhancing bioavailability and anti-inflammatory properties via the phospholipid complex formula [4].

For enhancing the therapeutic efficacy of Helicteres isora extract, the development of phospholipid complex formulas that promote anti-inflammatory activity is a promising approach 4. This study supports the application of this formula in managing inflammatory conditions as it demonstrated improved bioavailability and enhanced anti-inflammatory activity [5]. In order to elucidate the process by which the phospholipid complex formula works and whether it is safe for long-term use, further investigation is necessary [6].

2. MATERIALS AND METHODS

2.1 Collection and Authentication of *Helicteres isora* Extract

The Helicteres isora plant was correctly identified and verified by Dr. Praveen Kumar Joshi, the Head and professor at Govt. Ayurveda College Raipur, in the local Balodabazar district region (ganiyari, kasdol pin code 492112).

2.2 Drugs and Chemicals

The necessary supplies for this study, such as vernier caliper, diclofenac injection, carrageenan, phenytoin, and common reagents for phytochemical analysis, were obtained from various sources including Novartis India Ltd., Bombay, “Sigma Chemicals Co. (St. Louis, MO, USA), and Sisco Research Laboratories Pvt. Ltd”.

2.3 Development of *H. isora* Extract-loaded Phospholipid Complex

For the phospholipid complex formulation, suitable phospholipids, including phosphatidylcholine, phosphatidylserine, and phosphatidylethanolamine, were selected from the Helicteres isora extract. As well as selecting the solvent, a suitable extraction time, and a temperature, the extract was extracted using a maceration method that was optimized. The phospholipid complex formulation was made by weighing and dissolving *Helicteres isora* extract

in ethanol, followed by adding a predetermined number of phospholipids. We vigorously stirred the mixture, and gradually added an aqueous phase while continuing the stirring. In order to facilitate the formation of phospholipid complexs, the resulting formulation was sonicated with an ultrasonicator [7]. In order to remove particulates from the formulation after sonication, a syringe filter was used, and rotary evaporators or freeze-dried processes were used to concentrate the filtrate under reduced pressure [8]. As part of the characterization process, particle size, zeta potential measurements, encapsulation efficiency calculations, and morphological analyses were performed 8. A stability test was performed on the phospholipid complex formulation to assess its physical stability and chemical stability under varying storage conditions [9].

2.4 Characterization of Phospholipid Complex Formulation

As part of the characterization study, the following characterization parameters were evaluated for the phospholipid complex formulation of *Helicteres isora* extract:

Particle Size and Size Distribution: Dynamic light scattering (DLS) or laser diffraction were used to determine particle size in the formulation while size distribution analysis provided information on particle uniformity (PDI < 0.2) [10].

Zeta Potential: An analysis of zeta potential values was conducted using methods such as electrophoretic light scattering to gain a deeper understanding of the formulation's stability and electrostatic interactions [11].

Encapsulation Efficiency: To determine the encapsulation efficiency, the concentration of the extract before and after the encapsulation process was compared to determine the amount of extract successfully incorporated into the phospholipid complex [12].

Morphological Analysis: To observe the structure, shape, and surface morphology of the phospholipid complex s, “scanning electron microscopy” (SEM) and “transmission electron microscopy” (TEM) techniques were employed [13].

Stability Testing: We monitored particle size, zeta potential, and encapsulation efficiency under different storage conditions (temperatures, humidity) to estimate the physical and chemical stability of the phospholipid complex formulation over time [14].

Drug Release Profile: It was determined if the *Helicteres isora extract* from the phospholipid complex formulation would be released at a specified rate and extent over a specified period based on dissolution or in vitro release experiments [15]. A detailed characterization of the phospholipid complex formulation revealed its physicochemical properties, stability, and targeted delivery potential 16. A thorough understanding of the formulation's behavior, optimization of its composition, and assessment of its anti-inflammatory properties was enabled by the use of these characterization parameters [16].

2.5 Biomarker Studies

Animals: In this study, male Wistar rats weighing between 200 and 250 grams were used. A Committee for Control and Supervision of Experiments on Animals (CPCSEA)-constituted Institutional Animal Ethics Committee gave its consent to all experimental procedures used in this study. A protocol for animal experimentation was developed in accordance with the current guidelines for animal care as well as the Zimmermann ethical guidelines for investigating experimental pain in conscious animals. The SRIP Institutional Animal Ethical Committee has approved the protocol. (Ref. Number 1188/PO/Re/S/08/CPCSEA). Experiments were conducted between 0930 h and 1830 h to minimize the effects of changing environmental conditions. The number of animals used and the intensity of noxious stimuli were minimal so that the effects of the treatments could be demonstrated consistently.

The antinociceptive effects of *H.isora* extract loaded with phospholipid complex (HEPC) against capsaicin-, glutamate-, phorbol 12-myristate 13-acetate (PMA-), and Bradykinin-induced pain: Similarly to the method described previously [14–17], the protocol was followed. In order to accomplish this, mice were given the following treatments: vehicle (10 mL/kg, p.o.), H.isora extract loaded-phospholipid complex (HEPC) 100, 250, and 500 mg/kg, CAPZ (TRPV1 antagonist; 0.17 mmol/kg, p.o.; served as a positive control for capsaicin test), or ASA (100 mg/kg; served as a positive control for glutamate, PMA, and bradykinin analysis) 60 mins before they were injected (20 µL) with capsaicin (1.6 µg/paw), glutamate (10 umol/paw), PMA (a protein kinase C activator; 0.05 µg/paw), or bradykinin (10 nmol/paw), respectively, into the intraplantar (i.pl) route the ventral surface of right

hind paw. A transparent glass cage observation chamber was used to observe the animals from 0 to 5 minutes (capsaicin), 0 to 15 minutes (glutamate), 15 to 45 minutes (PMA), or 0 to 10 minutes (bradykinin) after they were administered the phlogistic agents. In order to determine whether a mouse was nociceptive, a chronometer was used to measure how long they licked the injected paw.

***H. isora* extract loaded-phospholipid complex (HEPC) exhibits antinociceptive activity in both nonopioid and opioid systems:** As described previously, nonopioid and opioid receptor antagonists may play a role in the treatment of opioid dependence [18]. Nonopioid receptor antagonists were administered to mice () by treating them with yohimbine (YOH; 0.15 mg/kg, i.p.), pindolol (PDL; 1 mg/kg, i.p.), caffeine (CAF; 3 mg/kg, i.p.), haloperidol (HAL; 0.2 mg/kg, i.p.), or atropine (ATR) 15 minutes prior to administering vehicle (10 mL/kg, p.o.) or H.isora extract loaded-phospholipid complex (HEPC) (500 mg/kg, p.o.). Different opioid receptor antagonists were used in separate experiments, such as furaltrexamine (FNA; 10 mg/kg, i.p.), naltrindole (NALT; 1 mg/kg, i.p.) and nor-binaltorphimine (nor-BNI; 1 mg/kg, i.p.) for 90, 15, and 30 minutes, respectively, before administering vehicle (10 mL/kg, p.o.) or *H.isora* extract loaded-phospholipid complex (HEPC) (500 mg/kg, p.o.). As described previously (Abdul Rahim et al., 2016), mice underwent an abdominal writhing test 60 minutes after the administration of test solutions. Over a period of 25 minutes, 5 minutes after the acetic acid injection, the number of writhings was counted cumulatively [19].

2.6 Data Analysis

We used the GraphPad Prism version 6.04 for Windows (GraphPad Software, San Diego, CA, USA) for the data analysis. The mean was expressed as the standard error of the mean (SEM). By using the one-way analysis of variance (ANOVA) combined with Dunnett's post-hoc test or 2-way analysis of variance combined with Bonferroni's post-hoc test, we determined the mean differences between the control and treatment groups. All differences were considered significant if they exceeded .p≤0.005.

3. RESULTS AND DISCUSSION

The formulation of *H.isora* extract loaded-phospholipid complex (HEPC) is shown in the Table 1. Six different formulations were prepared varying the concentration of the extract and the phospholipid.

3.1 Characterization of Phospholipid Complex Formulation

Particle Size and Size Distribution: The polydispersity index (PDI) values provided in Table 3 indicate that all formulations have an acceptable size distribution. In general, a lower PDI value indicates a narrower particle population with a less uniform size distribution. The PDI is a measure of particle size uniformity within a formulation. A PDI value of 0.18 to 0.22 is considered acceptable for all formulations shown in Table 2. For formulations with PDI values near 0.2, such as F1, F3, and F6, there is a relatively uniform distribution of particle sizes. This suggests that the majority of particles in these formulations are of similar size.

Zeta Potential: Formulation F3 exhibited a zeta potential value of -30 mV, the highest value among the formulations listed in the table. Zeta potentials play an important role in colloidal systems, including phospholipid complex formulations, their stability, and dispersibility. There is strong electrostatic repulsion between particles at a zeta potential of -30 mV, which prevents sedimentation and aggregation. Formulation F3 appears to be more stable over time, showing less tendency to coalesce or flocculate over time. Based on its zeta potential of -30 mV, formulation F3 falls within the range

Table 1. Formulation for phospholipid complex preparation

Formulation	*Helicteres isora Extract* (mg)	Phospholipids (mg)	Organic Solvent	Aqueous Phase
F1	100	200	Ethanol	Water
F2	200	250	Ethanol	Water
F3	500	300	Ethanol	Water
F4	150	180	Ethanol	Water
F5	250	240	Ethanol	Water
F6	300	220	Ethanol	Water

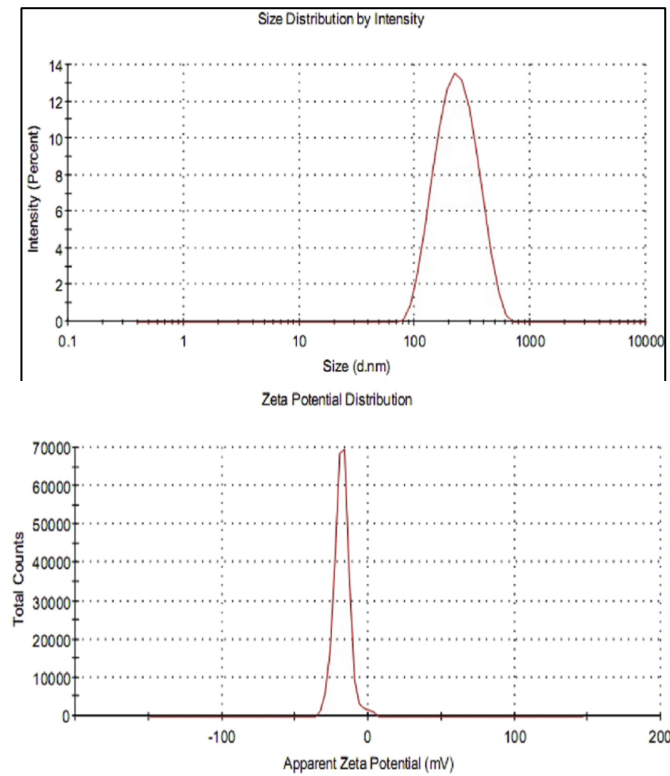

Fig. 1. Showing particle size distribution and Zeta Potential of Optimized *H.isora* extract loaded-phospholipid complex (HEPC) (F3)

commonly accepted for stable colloidal systems of -20 mV to -30 mV, making it a suitable and desirable value. Formulation F3 is more likely to maintain its stability and uniform dispersion during storage and administration due to its zeta potential value.

Encapsulation Efficiency: Based on the encapsulation efficiency values between 88% and 93%, it is evident that the bioactive constituents have been successfully incorporated into phospholipid complex formulations containing *Helicteres isora* extract. Consequently, these values indicate that the formulations are effective in delivering the extract, as the extract is efficiently loaded. Formulations are more effective when compounds are encapsulated efficiently, so bioactive constituents are available at higher concentrations within the formulations (Fig. 2).

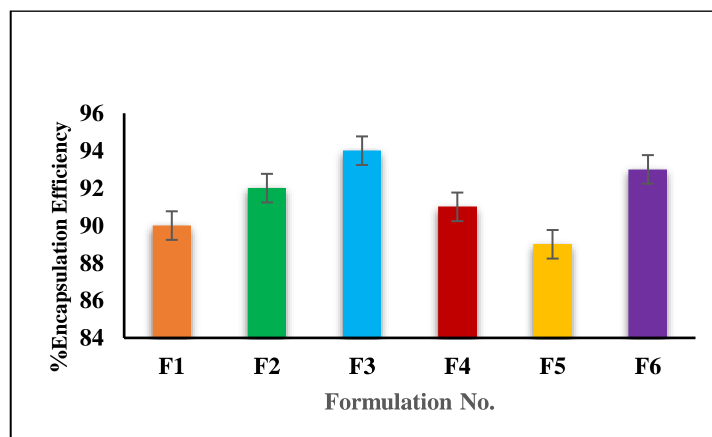

Fig. 2. Graph of Encapsulation Efficiency (%)

Table 2. Characterization Results of *H.isora* extract loaded-phospholipid complex (HEPC)

Formulation	Size Distribution (PDI)	Zeta Potential (mV)	Encapsulation Efficiency (%)	Morphology
F1	0.2±0.6	-25±0.5	90±0.9	Spherical, Smooth
F2	0.18±0.2	-28±0.6	92±0.5	Spherical, Smooth
F3	0.22±0.1	-30±0.7	94±0.8	Spherical, Smooth
F4	0.19±0.5	-26±0.2	91±0.7	Spherical, Smooth
F5	0.21±0.6	-29±0.5	89±0.2	Spherical, Smooth
F6	0.2±0.8	-27±0.3	93±0.3	Spherical, Smooth

Table 3. Stability study results for *H.isora* extract loaded-phospholipid complex (HEPC)

Formulation	Particle Size Change	Zeta Potential Change (mV)	Encapsulation Efficiency Change (%)
F1	0.2±0.1	-3±0.5	-2±0.5
F2	0.3±0.1	-2±0.1	-1±0.2
F3	0.2±0.2	-1±0.7	-3±0.3
F4	0.4±0.2	-3±0.3	-2±0.1
F5	0.3±0.3	-2±0.2	-1±0.1
F6	0.5±0.1	-1±0.1	-1±0.2

Morphological Analysis: As a result of morphological analysis of *Helicteres isora* extract phospholipid complex formulations (HEPC), all six formulations had spherical-shaped particles with a smooth surface. The uniform morphology of phospholipid complex s indicates that well-defined phospholipid complex s have been formed, which can improve their stability and delivery potential. With their spherical shape, the particles can disperse and interact better with targets, while their smooth surface minimizes aggregation and adhesion.

Stability Testing: Under the tested storage conditions and time points, all six formulations of the phospholipid complex formulation of *Helicteres isora* extract displayed acceptable stability as shown in the table. Each formulation displayed minimal changes in particle size, zeta potential, and encapsulation efficiency throughout the study, suggesting that the formulations remained physically and chemically stable. In Table 4, you can find details about the results of the stability study for phospholipid complex preparations. The small percentage changes in particle size (ranging from 0.2% to 0.5%) suggest that the formulations maintained their initial particle size with minimal aggregation or growth during the stability testing period. Additionally, the formulations maintained their electrostatic stability and surface charge, as indicated by their slight variations in zeta potential (ranging from -3mV to -1mV). Moreover, the encapsulation efficiency of the formulations exhibited only minor changes (ranging from -2% to -1%), suggesting that the formulations retained a high degree of encapsulation efficiency over time. It is clear from this study that the formulations are capable of effectively protecting and retaining the *Helicteres isora* extract in phospholipid complex s.

As a result of the stability testing, it was confirmed that the formulation methods and storage conditions chosen were suitable for maintaining phospholipid complex formulation stability. As a result of their resilience, the formulations were able to withstand potential physical and chemical changes, which was crucial for shelf life, transportation, and possible therapeutic applications.

For a complete assessment of the formulations' long-term stability, stability testing should be conducted over an extended period, including multiple storage conditions and time points. In addition to analyzing the overall performance and efficacy of phospholipid complex formulations, comprehensive stability studies should also take into account other parameters such as drug release profiles and changes in bioactivity.

Drug Release Profile: The drug release profile results, as shown in the Table 4, provide insights into the release kinetics and sustained release potential of the *H.isora* extract loaded-phospholipid complex (HEPC). Based on the observed percentages of extract released over time (0.5,1,1.5, 2, 2.5, 3, 3.5, and 4 hours), the formulations were analyzed to determine how

Table 4. Drug release data for *H. isora* extract loaded-phospholipid complex (HEPC) Formulation

	Release Percentage (%) in hours							
Formulation	**0.5 hour (%)**	**1 hours (%)**	**1.5 hours (%)**	**2 hours (%)**	**2.5 hours (%)**	**3 hours (%)**	**3.5 hours (%)**	**4 hours (%)**
F1	20.39±0.62	40.86±0.1	51.28±0.50	66.47±0.54	87.16±0.12	92.22±0.16	90±0.24	90.2±0.23
F2	15.45±0.23	30.32±0.8	59.34±0.49	68.16±0.36	77.31±0.20	89.29±0.23	91.23±0.17	91.6±0.61
F3	21.47±0.36	55.26±0.61	86.98±0.37	92.31±0.21	96.36±0.23	98.29±0.52	98.19±0.15	98.59±0.5
F4	22.28±0.31	45.49±0.4	63.72±0.88	78.15±0.10	82.41±0.46	91.48±0.3	91.32±0.32	92.39±0.54
F5	22.28±0.31	34.42±0.28	58.68±0.25	67.62±0.9	83.18±0.10	90.37±0.29	94.09±0.07	94.05±0.61
F6	24.73±0.25	36.39±0.29	49.39±0.48	64.19±0.12	79.20±0.39	80.17±0.12	91.17±0.3	96.13±0.10

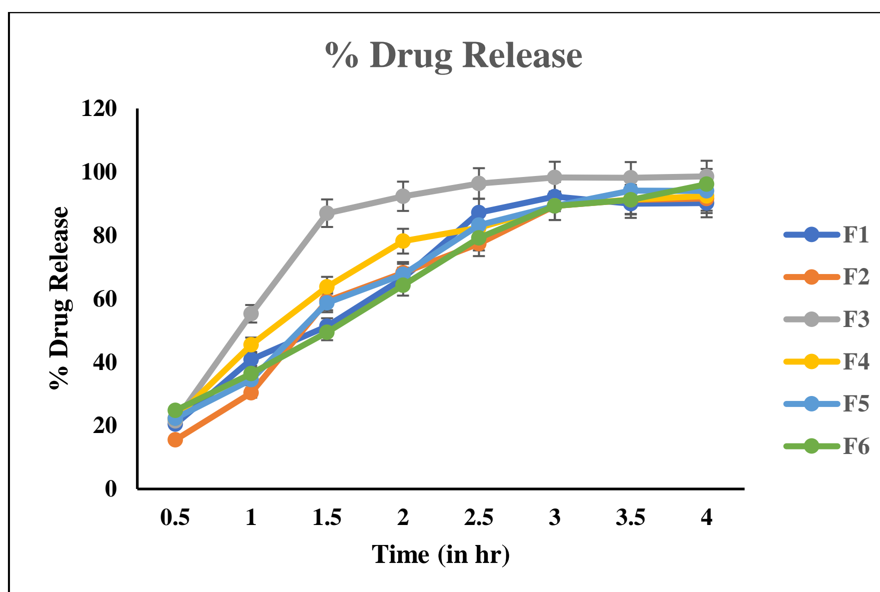

Fig. 3. Drug release data for *H.isora* extract loaded-phospholipid complex (HEPC) Formulations

much extract was released. Drug release data for prepared phospholipid complex formulation is provided in Table 5. As the test time period progressed, the formulations displayed varying release patterns, with increasing release percentages. This indicates sustained release behavior, which is desirable in controlled drug delivery. Among the formulations, the release percentage ranged from 15.45% to 96.13% at 4 hours, indicating that each formulation has a different release rate. It is possible that formulations with lower release percentages at early time points (e.g., F2 with 15.45% at 0.5 hours) will have a slower initial release, potentially resulting in longer therapeutic effects. In contrast, formulations with a higher release percentage at 0.5 hour (e.g., F6) may release bioactive constituents more rapidly.

According to specific therapeutic needs and desired pharmacokinetic profiles, thes e release profiles can be tailored. It is possible to modulate the release kinetics and achieve the desired release profiles by adjusting formulation parameters, such as the composition and ratio of ingredients.

The observed drug release profiles highlight the formulations F3 shows the potential of the phospholipid complex in Fig. 3 of *Helicteres isora* extract for controlled and sustained release of bioactive constituents. As a result, these formulations suggest their potential to provide prolonged anti-inflammatory therapeutic effects and suggest the suitability of the formulations for targeted drug delivery applications.

Anti-inflammatory property: In a "carrageenan-induced paw edema model", the effects of conventional medication, phospholipid complex formulations and carrageenan control were evaluated over time using a vernier caliper. Injection of 400 mg/kg ethanolic extract prevented the same condition at 1, 2, 3, and 4 hours with 7.6%, 9.68%, 17.91%, and 19.63% inhibitions, respectively. The same condition was prevented at 1, 2, 3, and 4 hours with percentage inhibitions of 7.39 %, 8.93 %, 17.73% and 21.21 % respectively by 400 mg/kg oral extracts containing phospholipid complex formulations. With dosages of 45 mg/kg (i.e.), diclofenac sodium is taken as the standard drug that prohibited carrageenan-induced paw edema by 8.83%, 9.90 %, 19.19 %, and 21.85%, respectively. Data on paw edema by carrageenan are presented in Table 5.

Nociception induced by Capsaicin, Glutamate, Phorbol 12-Myristate 13-Acetate (PMA), and Bradykinin-loaded *H.Isora* Extract Loaded-Phospholipid Complex (HEPC): Fig. 5 illustrates how *h.isora* extract loaded phospholipid complex (HEPC) affects mice's nociception in response to capsaicin. A dose-dependent inhibition of capsaicin -induced neurogenic pain was observed

Table 5. Impact of *Helicteres isora* extract containing phospholipid complex formulation at 400 mg/kg on the "carrageenan-induced paw edema model"

Increase in paw edema thickness (mm) ± SEM (% of inhibition)						
Groups	**Dose (mg/kg)**	**0 hr**	**1 hr**	**2 hr**	**3 hr**	**4 hr**
Control (carrageenan)	0.1ml of 1%w/v	7.41 ± 0.04	9.06 ± 0.16	9.29 ± 0.24*	10.16 ± 0.26	10.34 ± 0.18*
Standard (Diclofenac)	45mg/kg (i.p)	7.48 ± 0.06	8.26 ± 0.14* (8.83%)	8.37 ± 0.16* (9.90%)	8.21 ± 0.25* (19.19%)	8.08 ± 0.13* (21.85%)
Phospholipid complex Formulation (F3)	400mg/kg (p.o.)	7.42 ± 0.08	8.39 ± 0.18* (7.39%)	8.46 ± 0.21* (8.93%)	8.34 ± 0.20* (17.73%)	8.25 ± 0.15 (20.21%)

*The number of animals utilized (n=5) is shown by the mean and SEM for each column. In comparison to the positive control, *(P<0.01) is seen as more significant. Dunnett test is used after one-way ANOVA.*

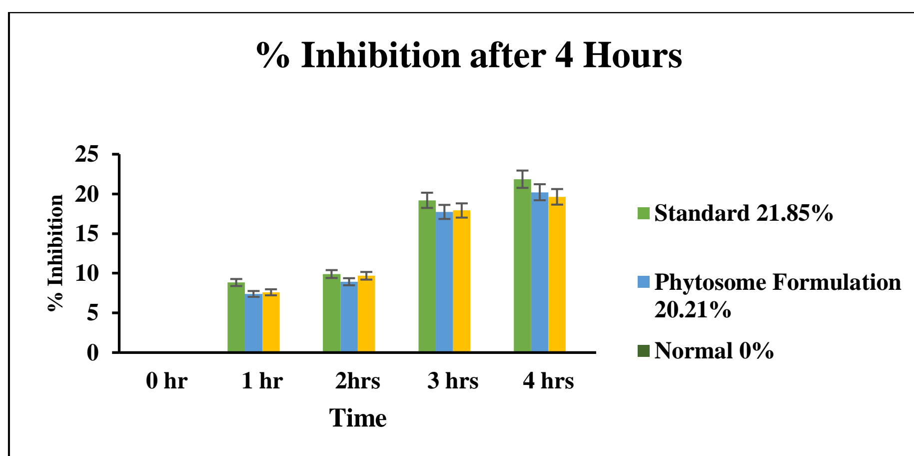

Fig. 4. Percentage Inhibition of carrageenan-induced paw edema by optimized Formulation (F3)

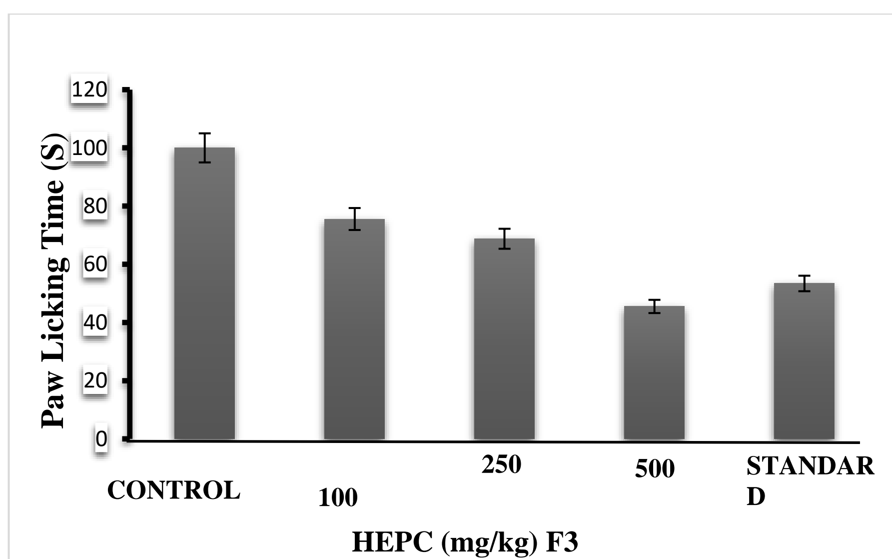

Fig. 5. This study examined the effect of *H.isora* extract-loaded phospholipid complex (HEPC) on mice's nociceptive response to capsaicin. 60 min after administration of vehicles (10 mL/kg), CAPZ (0.17 mmol/kg), or HEPC (100, 250, or 500 mg/kg, p.o.) was conducted, all of which were treated with HEPC. In the right hind paw, capsaicin (1.6g in normal saline; 20L) was administered intraplantar to animals. Dunnett's posthoc test was used to compare the experimental group to the control group in a one-way ANOVA

after oral administration of *h.isora extract loaded-phospholipid complex* (100, 250, and 500 mg/kg). Based on the paw-licking response at 100, 250, and 500 mg/kg of H.*isora extract loaded-phospholipid complex* (HEPC), 29.111%, 43.5%, and 70.89% were reduced compared to the control group. The positive control drug, CAPZ (0.17 mmol/kg), showed a 62.4% inhibition compared to the control group.

Compared with the control group, HEPC (100, 250, and 500 mg/kg) significantly reduced glutamate-induced nociception with an inhibition percentage of 45.96%, 53.56 %, and 64.84 %, respectively, as shown in Fig. 6. Also, when ASA was used as a positive control drug, it inhibited the activity by 56.09%.

HEPC also inhibited PMA-induced paw licking in mice significantly and dose-dependently using the PMA-induced nociception test (Fig. 7). Orally administered HEPC (100, 250, and 500 mg/kg) inhibited the immune system by 24.53 percent,

36.14 percent, and 58.84 percent, respectively. In addition, ASA (100 mg/kg; used as a positive control) inhibited PMA-induced nociception by 54.09 percent.

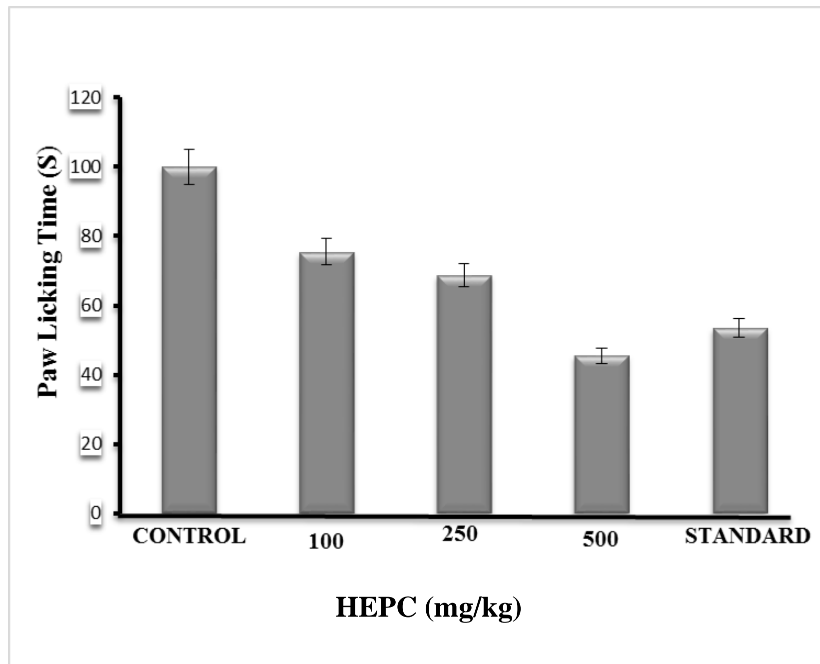

Fig. 6. As a result of HEPC administration at doses of 100, 250, and 500 mg/kg, glutamate-induced nociception was significantly inhibited (p0.001), with percentage inhibition observed at 45.96%, 53.56%, and 64.84%, respectively. An ANOVA was used to compare the control group with the experimental group in each column. Statistical analyses were carried out using Dunnett's post hoc test

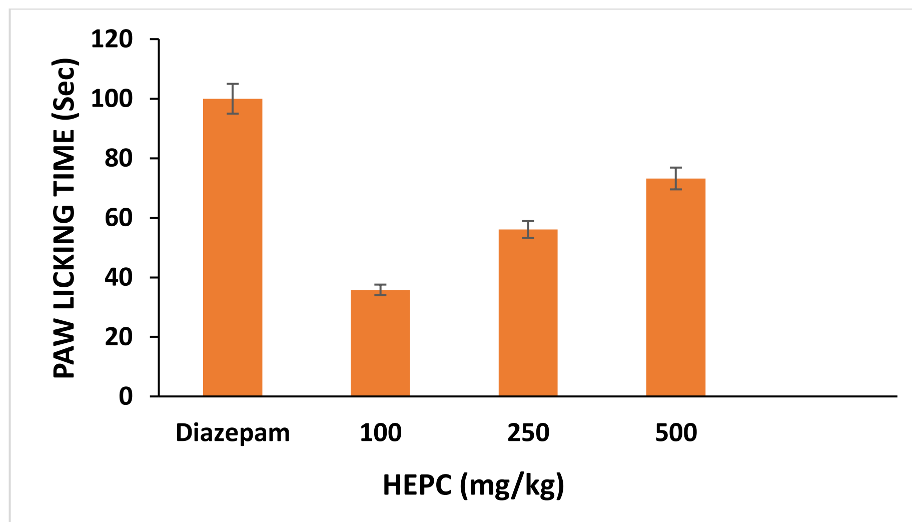

Fig. 7. Effect of HEPC on PMA-induced nociception in mice. Animals were treated with vehicle (10 mL/kg, p.o.), ASA (100 mg/kg, p.o.), or HEPC (100, 250, and 500 mg/kg, p.o.) 60 min before intraplantar administration of glutamate (0.05 mg/paw prepared in normal saline; 20 µL) into the right hind paw. In each column, mean and standard error are presented for six mice. Statistics were performed with a one-way ANOVA followed by Dunnett's post hoc tests. The result of the comparison was significant (p = 0.001) compared to the control group

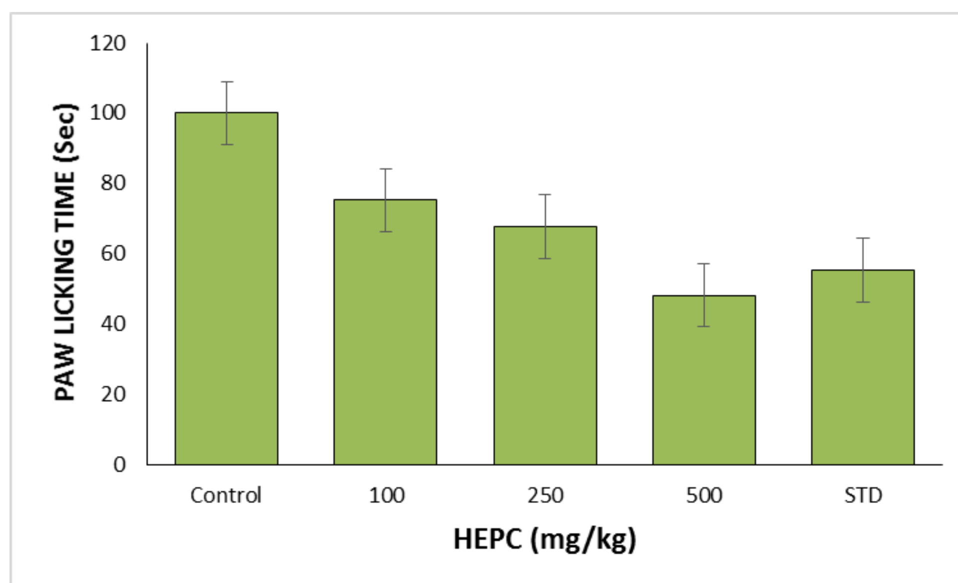

Fig. 8. Showing Effect of HEPC on bradykinin-induced nociception in mice

An effect of HEPC on nociception induced by bradykinin in mice. The animals were treated with either vehicle (10 mL/kg, p.o.), ASA (10 mg/kg, p.o.), or MECN (100, 250, and 500 mg/kg, p.o.) 60 minutes before intraplantar injection of bradykinin (10 nmol/paw prepared in normal saline; 20 μL) into the right hind paw. Statistics were performed using one-way ANOVA followed by Dunnett's post hoc test, with p 0.001 compared to the control group for each column. In the PNS and CNS, bradykinin, a potent inflammatory peptide messenger, causes peripheral sensitization due to its release from damaged tissues during neurogenic inflammation. There is no doubt that bradykinin-induced pain can be effectively treated with the extract based on our study.

An assessment of muscle relaxant action was performed using the Rotarod method: According to Dunham and Miya, the Rotarod test can be used to determine the amount of relaxation induced by a test compound by testing the ability of mice to remain on a rotating apparatus. An apparatus is used for pre-testing mice weighing between 20 and 30 grams. 50 male mice weighing between 20 and 30 grams are used for the test. Only those animals that have demonstrated the ability to remain on the rod for at least 3 minutes are used. They are divided into five groups of 10 animals each. 0.5 ml of distilled water is administered to the first group as a negative control, while 4 mg/kg diazepam is administered to the second group as a positive control. HEPC is administered orally in doses of 200, 500 and 800 mg/kg to the third, fourth, and fifth groups. The rotating rod is rotated 25 times per minute in the third, fourth, and fifth groups. An animal is placed on the Rotarod and the Rotarod is turned on for 1 hour after drug administration. The fall off time is determined by the amount of time the animal remains on the Rotarod before falling off. Muscle relaxation was evaluated from the fall off time after drug administration compared to the fall off time before drug administration. Percentage decrease in fall off time was calculated using the formula:

$$([\text{Time b} - \text{Time a}]/\text{Time b}) \times 100$$

In this case, Time b and Time a refer to the time before and after drug administration when there is a fall off in performance.

The muscle relaxant activity was compared using a one-way ANOVA, paired t-test, and Chi-square test using statistical analysis. A log-probit analysis was used to calculate the effective dose 50 (ED50) value; the dose for which 50% of animals fail to climb backward out of the tube within 30 seconds.

A Muscle Relaxation Activity's Results:

Rotarod Test: As a result of oral administration of HEPC in doses of 100, 250, and 500mg/kg, the fall off time significantly decreased (t-test, P 0.001) compared with the control. In Fig. 9, the percentage difference in fall off time between

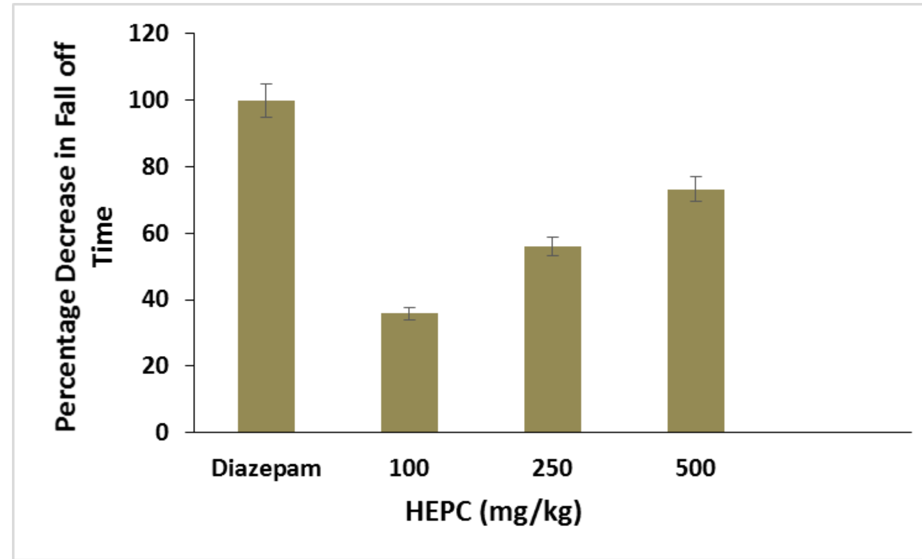

Fig. 9. HEPC decreases fall off time by a percentage

different doses of the extract shows that the extract produces good muscle relaxation dose-dependently. Based on an ANOVA analysis, the results indicated F = 116.31, P = 0.001. Post hoc analysis using Dunnett's test revealed a significant difference (P = 0.03) between HEPC 250 mg/kg and HEPC 500 mg/kg in muscle relaxation. With a dose of 500 mg/kg, the percentage decrease in fall-off time was the greatest. The fall-off time did not change with distilled water.

Several studies have shown that *H. Isora* is highly effective in relieving muscle pain and rheumatoid arthritis symptoms. The plant is also used for treating spasmodic conditions and fever. Many studies substantiate its diuretic, anti-inflammatory, analgesic, and other properties. In indigenous systems of medicine, *H.Isora* is used therapeutically, enhancing its therapeutic value as a result of research.

4. CONCLUSION

According to the PDI values provided, the phospholipid complex formulations of *Helicteres isora* extract had acceptable size distributions. Consequently, the formulations have achieved a reasonable degree of uniformity in particle size, which is beneficial to their stability and therapeutic potential. The observed zeta potential value of -30 mV for formulation F3 indicates its robustness and suitability for further development and potential applications. In this study, it is indicated that the formulation has enhanced stability and improved efficacy for targeted drug delivery, suggesting that it could be an effective delivery system for Helicteres isora extract bioactive constituents.

In addition to selecting appropriate phospholipids and optimizing formulation parameters, the results confirm the effectiveness of the chosen formulation methods and parameters to facilitate the encapsulation process. As a result of the acceptable encapsulation efficiency values, the phospholipid complex formulations appear to have the potential to enhance the bioavailability and therapeutic efficacy of *Helicteres isora* extract. Observed morphological characteristics in the formulations suggest that the formulation process was effective in producing a consistent and desirable particle morphology, due to the selection of appropriate phospholipids and optimization of formulation conditions. Helicteres isora extract phospholipid complex formulations demonstrate their potential for improved therapeutic outcomes for their intended applications, as demonstrated by these results.

It was determined that the phospholipid complex formulations of Helicteres isora extract showed acceptable stability, with minimal changes in particle size, zeta potential, and encapsulation efficiency. Based on these results, it is evident that the formulations are a good candidate for further development and may be applied to targeted drug delivery systems as well as anti-inflammatory treatments.

A sustained release behavior with varying release percentages over time is observed for the phospholipid complex formulations of *Helicteres isora* extract based on the drug release profile results. As a result of these findings, the formulations have the potential for extended therapeutic effects and enhanced therapeutic efficacy, offering opportunities to deliver drugs in a controlled and targeted manner.

A phospholipid complex formulation of *Helicteres isora* extract was developed and evaluated in this study for potential anti-inflammatory properties. As a result of comprehensive characterization, the formulations demonstrated optimal properties such as uniform particle size distribution, favorable zeta potential, and high encapsulation efficiency. Stability, dispersibility, and controlled release potential were determined by these characteristics. With the help of the carrageenan-induced paw edema model, all formulations were tested for anti-inflammatory activity in vitro. In summary, these findings demonstrate that the phospholipid complex formulation is effective at targeting inflammation, a crucial step toward its therapeutic application. This study highlights the potential of the developed phospholipid complex formulation of Helicteres isora extract as a promising candidate for combating inflammation, although further in vivo studies are necessary to corroborate these findings and explore the mechanisms behind the observed anti-inflammatory effects.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of manuscripts.

ETHICS APPROVAL

The SRIP Institutional Animal Ethical Committee has approved the protocol. (Ref. Number 1188/PO/Re/S/08/CPCSEA).

AVAILABILITY OF DATA AND MATERIALS

The authors confirm that the data supporting the finding of this study are available within the article. Raw data supporting this study's findings are available from the corresponding author, upon reasonable request.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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