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FORMULATION AND CHARACTERIZATION OF DIOSMIN LOADED PRONIOSOMES FOR ANTI INFLAMMATORY AND ANTIOXIDANT ACTIVITY

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ABSTRACT
Diosmin, a naturally occurring flavonoid glycoside with proven vasoprotective, anti-inflammatory, and antioxidant properties, is clinically limited by poor aqueous solubility and low oral bioavailability. The present study focuses on the formulation and comprehensive characterization of diosmin-loaded proniosomes as a novel vesicular drug delivery system to overcome these limitations. Proniosomes were prepared using the slurry method with non-ionic surfactants (Span 60 or Span 80), cholesterol, and maltodextrin as carrier material. The developed formulations were evaluated for drug–excipient compatibility, entrapment efficiency, surface morphology, in-vitro drug release, and biological performance. FT-IR analysis confirmed the absence of chemical interactions, indicating formulation stability. Entrapment efficiency was remarkably high (95.80–99.82%), with Span 60–based formulations showing superior drug loading. SEM analysis revealed nearly spherical, uniform vesicles with smooth surfaces. Optimized formulations demonstrated a sustained drug release profile, achieving up to 99% release over 24 hours. In-vitro pharmacological evaluation showed significant anti-inflammatory activity via protein denaturation inhibition (75.70% at 100 µg/mL) and strong antioxidant potential in the DPPH assay (72.17% inhibition at 100 µg/mL), comparable to standard drugs. Overall, diosmin-loaded proniosomes represent a promising, stable, and efficient delivery platform for enhancing the therapeutic efficacy of poorly soluble flavonoids.

Keywords: *Diosmin; Proniosomes; Vesicular drug delivery; Anti-inflammatory activity; Antioxidant activity; Sustained release.*

1. INTRODUCTION

Proniosomes are dry, free-flowing formulations that upon hydration form niosomes. They overcome stability issues of niosomes and are particularly relevant for oral delivery. Proniosomal gels have been explored for improving the solubility and controlled release of drugs with poor bioavailability [1]. This system is gaining attention for oral peptides and poorly soluble drugs because of its enhanced stability and ease of handling. Proniosomes can be stored in a stable form, easily reconstituted before use, and are more convenient for handling, transport, and industrial processing. In oral delivery, proniosomes improve the solubility and dissolution rate of poorly water-soluble drugs. Their gel-based forms, known as proniosomal gels, have received significant attention for enhancing drug absorption across the gastrointestinal tract. These systems are particularly valuable for drugs with poor bioavailability, where sustained and controlled release can further improve therapeutic outcomes. Proniosomes thus represent a practical and efficient vesicular system with strong translational potential for commercial products [2].

Inflammation is a natural defense mechanism of the body, characterized by redness, swelling, pain, and heat, which occurs in response to injury, infection, or chemical stimuli. While acute inflammation is protective, chronic inflammation contributes to the progression of various diseases including arthritis, cardiovascular disorders, neurodegenerative

conditions, and cancer. Anti-inflammatory agents are therefore crucial in therapeutic management3. Oxidative stress contributes to the pathogenesis of chronic diseases such as diabetes, atherosclerosis, neurodegenerative disorders, cancer, and aging-related conditions. Formulation of antioxidant agents into novel drug delivery systems like proniosomes, liposomes, and nanoparticles can enhance solubility, stability, and targeted delivery to tissues undergoing oxidative damage [4]. This approach not only improves therapeutic efficacy but also minimizes the required dose and potential side effects. Diosmin is a naturally occurring flavonoid glycoside, first isolated from *Scrophularia nodosa* in 1925. It is widely used as a vasoprotective and venotonic agent. Due to its pharmacological properties, diosmin has found extensive therapeutic applications in the treatment of vascular disorders such as chronic venous insufficiency and haemorrhoids [5].

The present work aims to develop and systematically characterize diosmin-loaded proniosomal formulation as an advanced vesicular drug delivery system, with the objective of overcoming diosmin's inherent limitations of poor solubility and low oral bioavailability. The study further seeks to evaluate their therapeutic potential through comprehensive in vitro assessments of antioxidant and anti-inflammatory activity.

2. MATERIALS AND METHODS

Materials

Diosmoin and Cholesterol were procured from Tychos Therapeutics Pvt Ltd, Chennai. Lecithin was procured from HiMedia Laboratories Pvt. Ltd., Mumbai, Span (60, 80) was procured from Spectrum Reagents and Chemicals Edavar, Cochin and were of an analytical and pharmacopeial grade.

Methods

Preparation of Phosphate Buffer (pH 7.4)

Phosphate buffer of pH 7.4 was prepared by dissolving 6.8g of Potassium dihydrogen orthophosphate (KH_2PO_4) and 1.56g of Sodium hydroxide (NaOH) in about 900ml of distilled water. The solution was stirred continuously until complete dissolution. The pH was checked and adjusted if necessary. Finally the volume was made up to 1000ml with distilled water.6

Preparation of Working Standard Solutions

1mg of diosmin was added to a 10ml of standard flask and make up it with 0.5M NaOH. From the stock solution, 0.2ml was pipetted out and transferred into another 10ml standard volumetric flask. The volume was then made up to 10ml using phosphate buffer pH 7.4 to obtain the working standard solution. This working solution was scanned in the range of 200-400 nm using UV-visible spectrometer to determine the wavelength of maximum absorption (λmax). Based on the λmax obtained, further dilutions were prepared by pipetting 0.4, 0.6, 0.8, 1.0 ml of the stock solution into a separate 10ml standard flasks and making up each to volume with phosphate buffer pH 7.4 for the construction of the calibration curve [7].

Preformulation studies

i. Solubility Studies

The sample was qualitatively tested for its solubility in various solvents. It was determined by taking 2 mg of drug sample in 5 ml of solvent such as water, methanol, ethanol, phosphate buffer pH 7.4, DMSO, Chloroform, 0.5M Sodium Hydroxide, 0.1N Hydrochloric acid, etc., in small test tubes and well solubilized by shaking.

ii. Fourier transform infrared spectroscopy (FT-IR) studies

Compatibility of diosmin with excipients was confirmed by FTIR studies. The FTIR study was conducted using potassium bromide (KBr) disc (pellet) method to analyze pure drug diosmin, Cholesterol, Lecithin, non-ionic surfactants (Span 60, Span 80) and their physical mixture in the same amount.

Formulation of Diosmin loaded proniosomes

Six set of formulations were prepared (to select the best one) which is denoted by DPF (Diosmin proniosome granules) with different ratios. In this, 3 sets contained diosmin, cholesterol, span60 and maltodextrin. The amount of drug and maltodextrin was kept constant. In the other 3 sets, instead of span 60, span 80 was used.

Slurry method was used for the preparation of proniosomes. Table 1 represents the composition of different proniosomal formulations. Lipid mixture comprising of span 60/span 80 and cholesterol were accurately weighed at various molar ratios and drug (10 mg) was dissolved in ethanol. Then maltodextrin was added to the resultant solution to form slurry and was then transferred into a round bottom flask. Attached to a rotary flash, the organic solvent was then evaporated under reduced pressure, at a temperature of 45±2°C, and thereafter to ensure complete removal of solvent. Product the resultant powders were further dried overnight in a vacuum oven at room temperature to obtain dry, free-flowing and stored in a tightly closed container at 5 °C for further evaluation [8].

Table 1. Composition of Diosmin loaded proniosome using span 60 & span 80

Formulation Code	Diosmin in mg	Cholesterol in mg	Maltodextrin in mg	Span 60 in mg	Span 80 in mg	Molar ratio	
						Span 60/ Span 80 in µmol	Cholesterol in µmol
DPF 1	10	96.5	500	86	0	250	250
DPF 2	10	77.33	500	107.65	0	200	250
DPF 3	10	96.6	500	86.12	0	250	200
DPF 4	10	96	500	0	107.15	250	250
DPF 5	10	77.33	500	0	107.15	250	200
DPF 6	10	96.66	500	0	85.72	200	250

Evaluation of Diosmin loaded proniosomes

i. Entrapment efficiency

A sample of 0.2 g of proniosomes was taken in a glass tube, and 10 ml of phosphate buffer (pH 7.4) was added. This aqueous suspension was sonicated in a sonicator bath followed by centrifugation at 9000 rpm at 20°C for 30 min. The supernatant was collected and assayed by using ultraviolet (UV) method for unentrapped Diosmin content at 264 nm.9 The percentage of drug encapsulation (entrapment efficiency percentage [EE%]) was calculated by the following equation:

$$EE\% = (\text{Total amount of drug} - \text{Unentrapped drug/Total amount of drug}) \times 100$$

ii. Scanning Electron Microscopy (SEM)

The surface morphology and size distribution of the proniosomes were analyzed using SEM. A drop of proniosomal suspension was placed on a specimen stub coated with carbon and then with gold vapor using a Hitachi vacuum evaporator. The samples were examined under a scanning electron microscope to observe the vesicular shape and were then photographed [10].

iii. Dissolution study of Proniosomes

Diosmin-loaded proniosome granules DPF3 and DPF4 were accurately weighed (equivalent to 10 mg of diosmin). The granules were placed inside capsules and transferred into the dissolution basket, immersed in 900 ml of phosphate buffer. The rotation speed was set to 50 rpm. 2 ml of sample was withdrawn at specific time intervals (15min, 30min, 1h, 2h, 4h, 5h, 12h, 24h) and replenished with fresh dissolution medium to maintain constant volume. The samples were filtered and the absorbance was measured at λmax 265 nm using UV spectrophotometer [11].

iv. *In vitro* anti-inflammatory activity - Protein Denaturation Assay

The reaction mixture (3 mL) consisted of 1 mL of 1% bovine albumin, 1 mL of phosphate buffered saline (PBS, pH 6.4), and 1 mL of various concentration of samples and standard drug diclofenac sodium (20-100 µg/ml) separately, and the mixture was mixed, and was incubated in a water bath (37 ◦C) for 15 min, and then the reaction mixture was heated at 70 ◦C for 5 min. After cooling, the turbidity was measured at 680 nm using a UV/VIS spectrometer (Optima, SP-3000, Tokyo, Japan). Phosphate buffer solution was used as the control [12, 13]. The percentage inhibition of protein denaturation was calculated by using the following formula:

$$\% \text{ inhibition} = \frac{\text{Abs control} - \text{Abs treated}}{\text{Abs treated}} \times 100$$

v. *In vitro* Antioxidant Activity

- **DPPH Assay Method**

The antioxidant activity of sample was examined by stable DPPH free radical activity. Ethanolic solution of DPPH (0.05 mM) (500µl) was added to 1000µl of plant sample with the different concentrations (20-100µl). The freshly prepared DPPH solution was kept in the dark at 4°C. The mixture was kept to stand for 5 minutes at 540nm, absorbance was measured spectrophotometrically. A blank sample contains the same amount of ethanol and DPPH was prepared. The radical activity of the tested samples, expressed as percentage of inhibition were calculated [14, 15].

$$\text{Percent (\%) inhibition of DPPH activity} = [(A-B)/A] \times 100.$$

Where A and B – absorbance values of blank and sample, respectively. A curve of concentration versus percentage inhibition was plotted and concentration required for 50% inhibition was determined.

3. RESULTS AND DISCUSSION

i. Calibration curve of Diosmin

Calibration curve of Diosmin was plotted in pH 7.4 phosphate buffer at λmax 265 nm and observed linear correlation with R2 value equals to 0.990 and the straight line equation obtained was y=0.055x+0.022 (Figure 1).

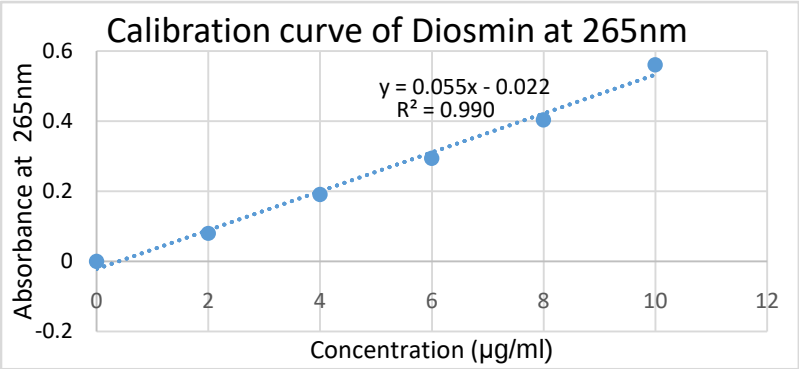

Fig. 1. Calibration curve of Diosmin

Preformulation studies

i. Solubility studies

Diosmin exhibited good solubility in dimethyl sulfoxide (DMSO) and 0.5M sodium hydroxide (NaOH). Other solvents such as ethanol, methanol, chloroform, water, phosphate buffer (pH 7.4), and 0.1 N HCl is showed poor solubility, with visible sedimentation and absorbance values.

Table 2. Solubility studies of Diosmin

Solvent	Concentration (µg/ml)	Solubility (mg/ml)
Ethanol	-	Below detection
Methanol	32.85	0.0329
0.1N HCl	16.25	0.0163
Phosphate buffer (7.4)	-	Below detection
Chloroform	-	Below detection
0.5 M NaOH	1585.7	1.586
DMSO	79.42	0.0794
Water	-	Below detection

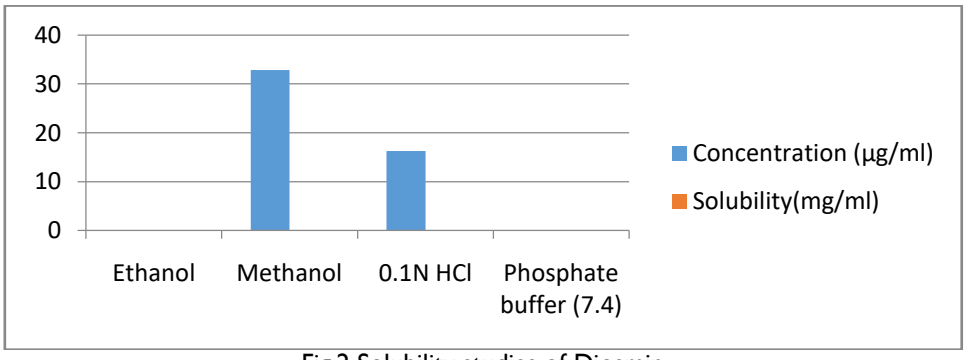

Fig.2 Solubility studies of Diosmin

Drug -Excipient Compatibility studies

FTIR studies were performed to know the chemical interactions between drug and excipients. The IR spectrum of pure drug and physical mixture of drug were studied by making a KBr pellet (Figure 3 to 7).

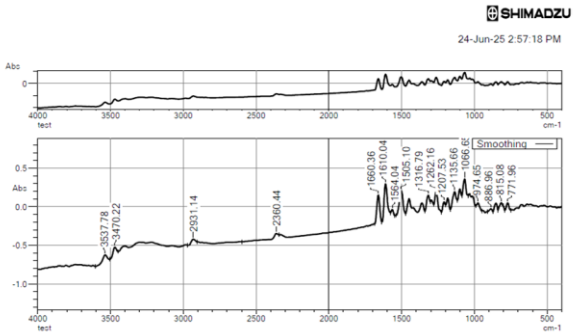

Fig.3 FTIR spectra of Diosmin

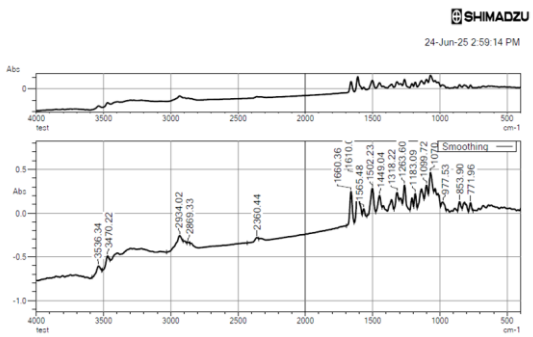

Fig.4. FTIR spectra of rug and Cholesterol

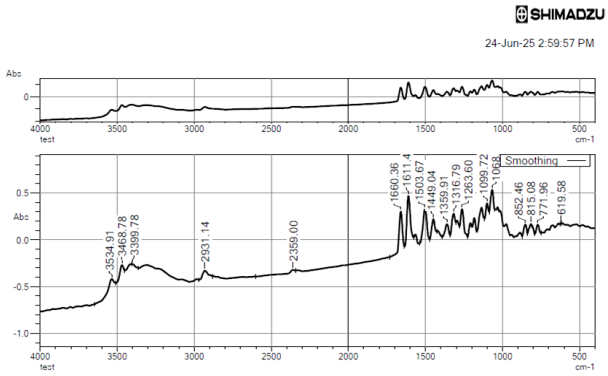

Fig.5. FTIR spectra of Drug and Maltodextrin

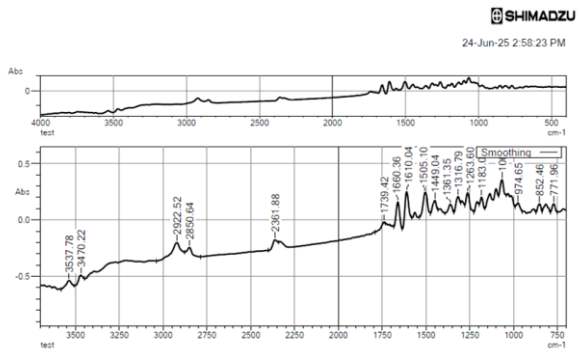

Fig.6. FTIR spectra of Drug and Span 60

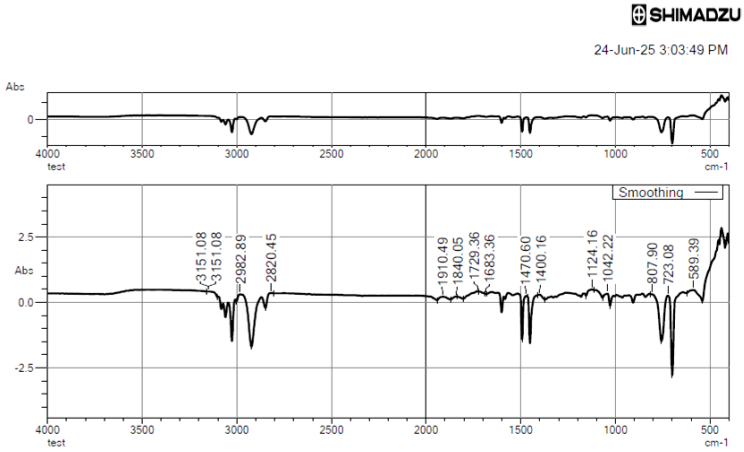

Fig.7. FTIR spectra of Drug and Span 80

Evaluation of Diosmin proniosome granules

i. Entrapment Efficiency (%EE)

The % EE of proniosome-derived niosomes was estimated by the UV-spectrophotometric method. % EE was found to be in the range of 95.80% to 99.82%. The maximum % EE achieved by batch DPF3 was 99.82%. From the above result, it was observed that as the concentration of span 60 increases, % EE also increased. The entrapment efficiency of Diosmin in various proniosomal granules is summarized in Table 3. The percentage entrapment efficiency of the vesicles was primarily influenced by the fundamental properties of the surfactant, such as its HLB (hydrophilic-lipophilic balance), phase transition temperature, structural orientation, and critical packing parameter. Proniosomes prepared using Span 60 showed moderately higher entrapment efficiency compared to those made with other surfactants. This can be attributed to Span 60's higher phase transition temperature (Tc, 53°C) and longer saturated alkyl chain (C16). Entrapment efficiency is a crucial parameter that reflects the stability of the vesicles and depends on the amounts of surfactant and cholesterol used. To prevent the formation of leaky and permeable proniosomes, cholesterol was added as a stabilizing agent. However, raising the cholesterol concentration beyond a certain level can disrupt the linear vesicular membrane structure, preventing the drug from being retained within the bilayers.

Table 3. Entrapment efficiency (%EE) of Diosmin Proniosome granules

S.No.	Formulation code	Percentage entrapment efficiency (%)
1	DPF 1	98.55
2	DPF 2	99.75
3	DPF 3	99.82
4	DPF 4	99.23
5	DPF 5	96.50
6	DPF 6	95.80

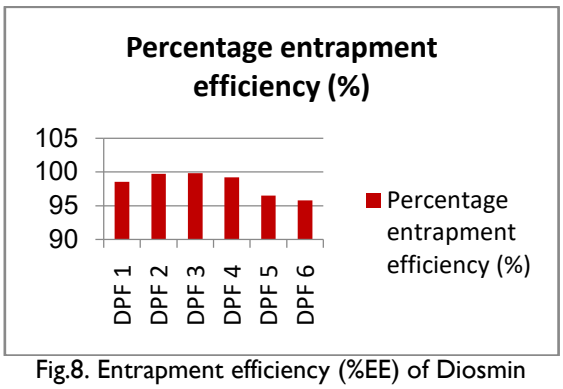

Fig.8. Entrapment efficiency (%EE) of Diosmin Proniosome granules

ii. Scanning Electron Microscopy Examination:

The surface morphology and size distribution of proniosomes were studied by SEM (as shown in Figure 7 & 10). The figures show the SEM image of the Diosmin proniosome granules. It has nearly spherical shape with a smooth surface showing that encapsulated drug vesicles are in nano-size range with no sign of aggregation, signifying the homogeneity of the produced Diosmin proniosome granules.

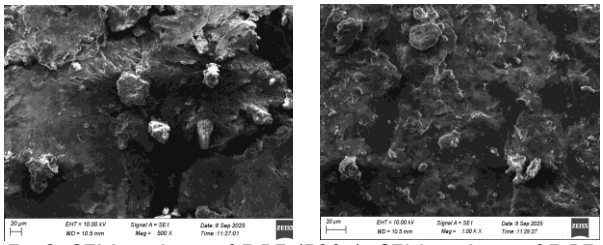

Fig 9. SEM analysis of DPF (500x); SEM analysis of DPF (1000x)

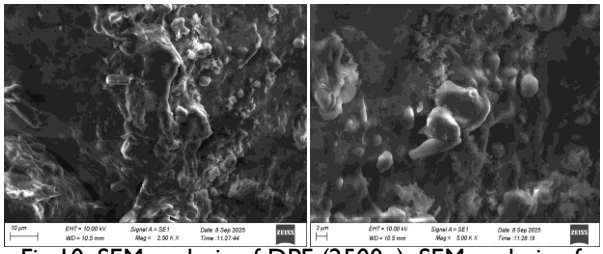

Fig 10. SEM analysis of DPF (2500x); SEM analysis of DPF (5000x)

iii. *In-vitro* Drug Release

Both the formulations was found to have a linear release and were found to provide more than 90% release with in a period of 24 h. Among both the formulations, DPF3 showed significant prolonged *in-vitro* drug release (99.0%) for a period of 24h.

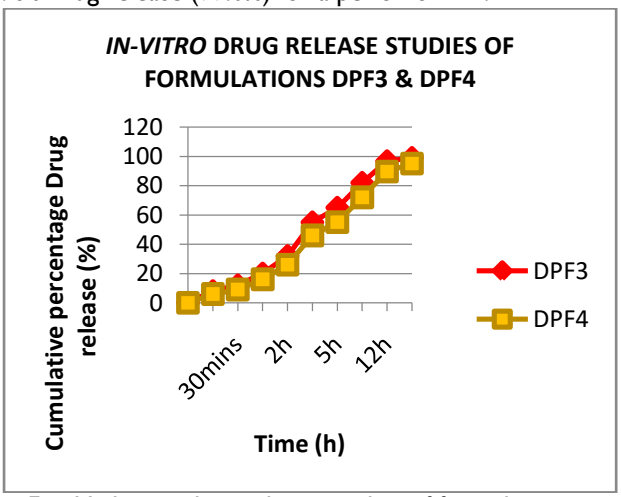

Fig. 11. *In-vitro* drug release studies of formulations DPF3 & DPF4

iv. *In vitro* anti-inflammatory activity

- **Inhibition of Albumin denaturation**

Protein denaturation is a process in which proteins lose their tertiary structure and secondary structure by external stress or compound such, as strong acid and base, a concentrated Inorganic salt, an organic solvent or heat. Most biological proteins lose their biological function when denaturated. Denaturation is a protein, it is well-inflammation activity, ability of plant extract to inhibit protein denaturation was studied. It was effective in inhibiting heat reduce albumin denaturation. The sample showed a maximum inhibition of 75.70% was observed at 100 µg\ml. Diclofenac sodium a standard anti-inflammation drug showed the maximum inhibition 81.30% at the concentration of 100 µg\ml compared with control.

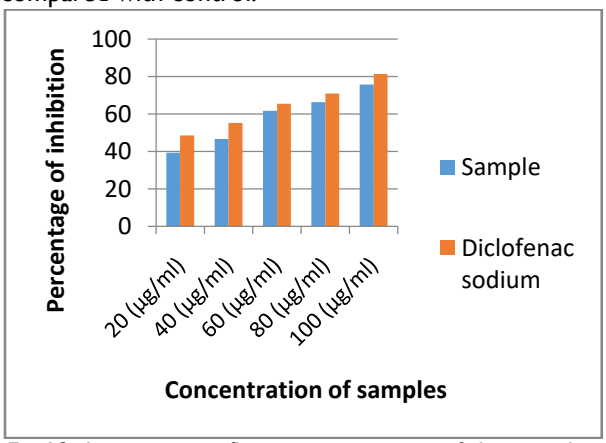

Fig 12. *In vitro* anti-inflammatory activity of the samples as compared with standard drug diclofenac sodium using protein denaturation method.

v. *In vitro* Anti Oxidant activity

- **DPPH Assay Method**

The result showed that the sample had better percentage antioxidant activities at high concentrations when compared with standard ascorbic acid. The sample showed 72.17% activity at concentration 100 µg/ml while ascorbic acid gave 80% at the same concentration.

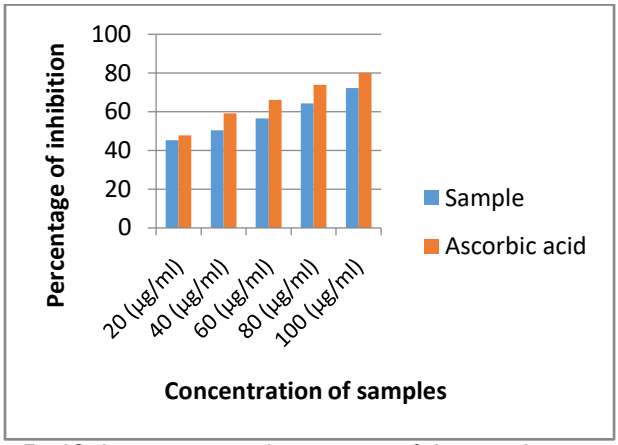

Fig 13. *In vitro* anti-oxidant activity of the samples as compared with standard drug ascorbic acid using DPPH method.

The calibration curve of diosmin in phosphate buffer pH 7.4 at 265 nm showed good linearity with an R^2 value of 0.990, establishing accuracy and reliability of the method for drug estimation throughout the study. The organoleptic evaluation revealed that all formulations were pale yellow and uniform in appearance, which indicated homogeneity and physical stability. Preformulation solubility studies showed that

diosmin exhibited poor solubility in aqueous and acidic media, while higher solubility was observed in dimethyl sulfoxide and sodium hydroxide, which confirmed the necessity of a carrier system to enhance its solubility. The FTIR spectrum demonstrated that there was no significant drug–excipient interaction, indicating compatibility and stability of the drug with selected formulation ingredients.

Entrapment efficiency studies showed that diosmin was successfully incorporated into the proniosomal vesicles, with values exceeding 90% in optimized formulations, which confirms effective loading and reduced loss of the active drug. The in-vitro dissolution studies revealed a biphasic release profile where an initial burst release was followed by sustained release over 24 hours, with cumulative release reaching up to 95–99%. This demonstrates that the proniosomal formulation is capable of maintaining prolonged drug availability. Such release kinetics are desirable for maintaining therapeutic drug levels over prolonged durations and minimizing the need for frequent dosing. The sample showed a maximum inhibition of 75.70% was observed at 100 µg\ml. Diclofenac sodium a standard anti-inflammation drug showed the maximum inhibition 81.30% at the concentration of 100 µg\ml compared with control. In Anti Oxidant activity, DPPH inhibition assay, the sample showed 72.17% activity at concentration 100 µg/ml while ascorbic acid gave 80% at the same concentration.

4. CONCLUSION

Diosmin is a natural flavonoid commonly found in citrus fruits and is known for its antioxidant, anti-inflammatory, and vasoprotective properties. However, its poor water solubility and low bioavailability limit its therapeutic effectiveness. To overcome these issues, proniosomes, a type of vesicular drug delivery system, are used. Proniosomes can convert into niosomes upon hydration, providing better stability, controlled drug release, and improved absorption. Therefore, the present study aims to formulate diosmin-loaded proniosomes and evaluate their antioxidant and anti-inflammatory activities to develop an efficient and stable drug delivery system for enhanced therapeutic action.

The proniosomes were prepared by the slurry method and characterized for Entrapment efficiency. The entrapment efficiency of the six formulations ranged from 95.80% to 99.82%, indicating excellent drug loading capacity. Invitro dissolution studies showed a sustained release profile, confirming the controlled release behaviour of the proniosomal system.In-vitro dissolution studies revealed a sustained release profile, with 95% to 99% of drug release observed after 24 hours, confirming efficient and controlled drug delivery. The sample showed a maximum inhibition of 75.70% was observed at 100 µg\ml. Diclofenac sodium a standard anti-inflammation drug showed the maximum inhibition 81.30% at the concentration of 100 µg\ml compared with control. In Anti Oxidant activity, DPPH inhibition assay, the sample showed 72.17% activity at concentration 100 µg/ml while ascorbic acid gave 80% at the same concentration.

5. ACKNOWLEDGEMENT

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6. CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

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