S-04 : EVALUATING AN ECO-FRIENDLY LIPID NANOCARRIER LOADED WITH *COLEUS fORSKOHLII* FOR ANTI-INFLAMMATORY, ANTIMICROBIAL, AND WOUND HEALING EFFICACY

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INTRODUCTION

Natural products isolated from plants have been a successful source of potential drug leads with unique structural diversity. *Coleus forskohlii* (F) is considered a significant future medicinal crop due to its recently authenticated therapeutic properties and richness in secondary metabolites. However, its poor solubility hinders its application. The improvement of wound healing in clinical practice using nanoparticles is a relatively novel therapeutic concept. Due to their lipid core, lipid nanoparticles have proved to have enhanced efficacy, attributed to their ability to provide increased drug loading and stability, prolonged drug release, and great functionalities. Additionally, they exhibit greater adhesion to the skin and film formation, enabling hydration and maintenance of skin integrity, as well as presenting more effective penetration through the skin barrier. Lipid nanocarriers can increase pharmacological activity, alter the release profile of drugs, promote synergistic effects, and improve the sensory properties of the final formulation.

OBJECTIVE

The study aims to examine the *in vitro* and *in vivo* impact of the prepared lipid nanocarrier (LNC) loaded with *Coleus forskohlii* plant extract to expedite wound healing.

METHODOLOGY

1. Preparation of plant extract & LNCs

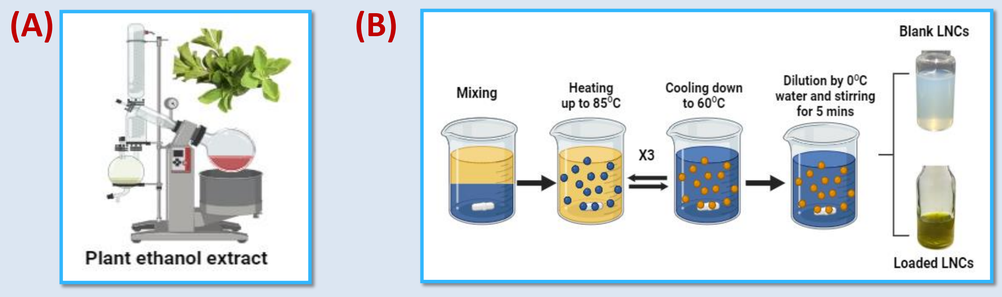

Fig. 1. (A) Preparation of plant extract (the powdered substance was extracted with 15 mL of 95% ethanol at 55°C for 2-hours followed by centrifugation at 9000 rpm for 5 minutes); (B) Blank (LNC) and loaded LNCs (FL) were prepared using the phase inversion technique followed by sudden dilution with cold water.

2. Characterization of plant extract & LNCs

- Ethanolic plant extracts was scanned using Fourier-transform infrared spectroscopy (FT-IR), Gas chromatography–mass spectrometry (GC-MS), and UV for the identification of different compounds.
- Characterization of LNCs: Physical properties, transmission electron microscopy (TEM), and entrapment efficiency (EE%) by ultrafiltration method .

3. Cytotoxicity and anti-inflammatory assays of the plant extract, LNCs and FL

- Cytotoxicity was assessed by MTT assay (A at 595 nm)
- *In vitro* anti-inflammatory activity was assessed by the human red blood corpuscles (HRBCs) membrane stabilizing method.

4. Antimicrobial assays

- The antimicrobial activity was evaluated by antibiofilm inhibition.

5. *in vitro* and *In vivo* wound healing assays

- Wound healing was assessed in vitro by cell scratching method. Scratches were created in the cell monolayer of human skin fibroblast cells (HSF) and observing the closure of the 'wound' edges to quantify cell migration rates.
- For in vivo experiment, a circular scar with a 10 mm diameter and a full-thickness open wound excision extending to the subcutaneous tissue was created in albino rats.

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RESULTS & DISCUSSION

1. Characterization of plant extract & LNCs

GC-MS for *Coleus forskohlii* (F) and FT-IR spectrum revealed the important reported characteristic bands .

UV scan for *Coleus forskohlii* (F) showed maximum UV absorption at 490 nm at which content and EE were determined for (FL)

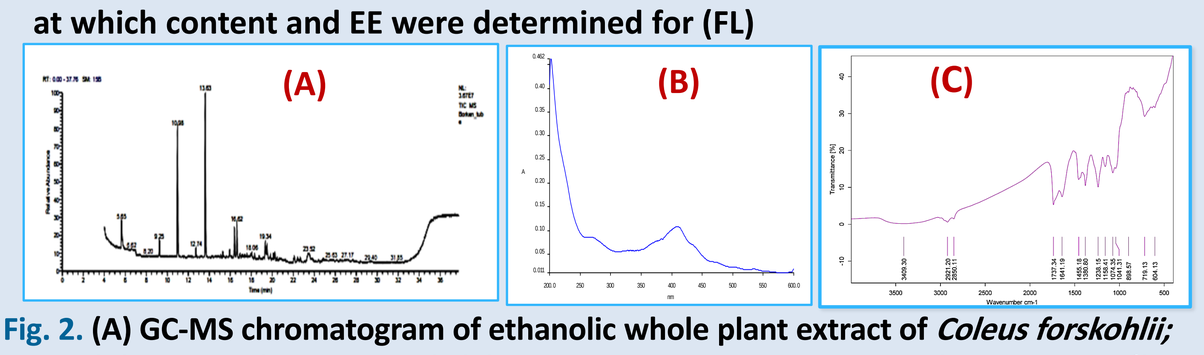

Fig. 2. (A) GC-MS chromatogram of ethanolic whole plant extract of *Coleus forskohlii;* (B) UV scan (200-600 nm); (C) FT-IR spectrum of plant extract (4000-500 cm^{-1}).

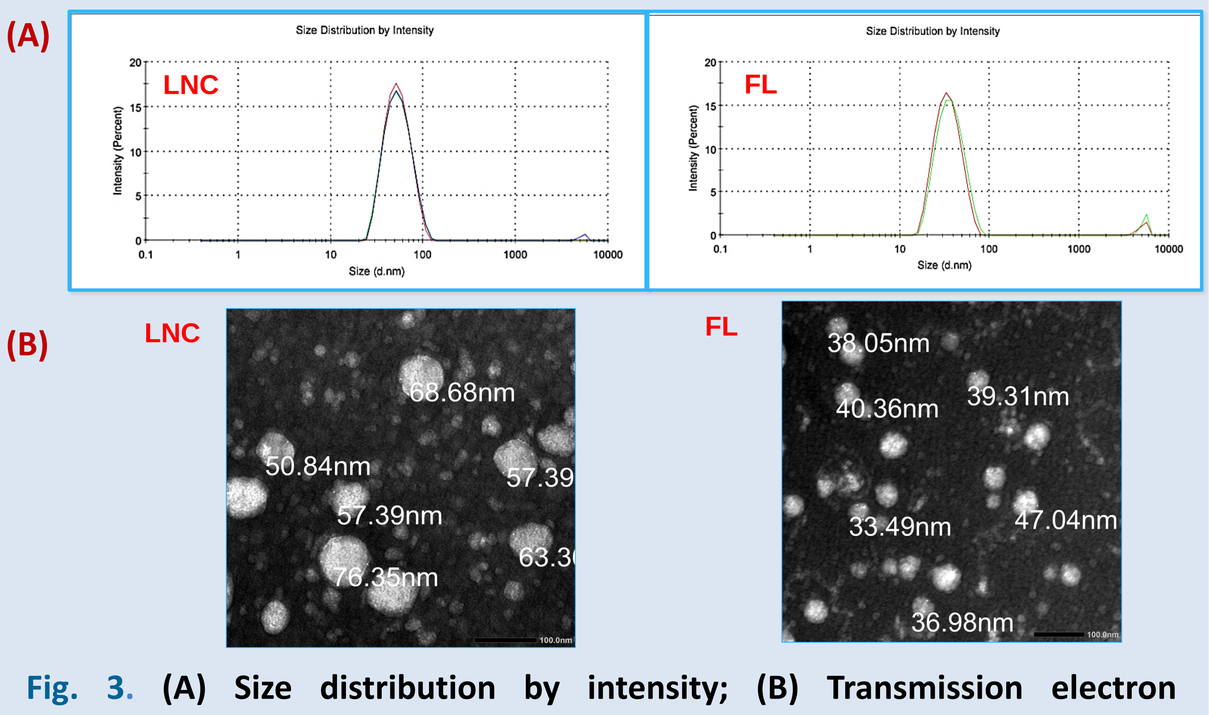

Fig. 3. (A) Size distribution by intensity; (B) Transmission electron microscope for blank LNCs and loaded LNCs (FL)

Table 1 : EE%, drug content and colloidal properties of blank LNCs and loaded LNCs.

Properties	Blank LNCs	Loaded LNCs (FL)
EE %	-	98.42%
Drug content	-	2.7mg/mL
Zeta potential	-7.76mv	-9.4mv
Size	50.50±1.38 nm	38.42±5.09nm
PDI	0.125±0.04	0.223±0.005

2. Cytotoxicity, Anti-inflammatory, and Antimicrobial assays

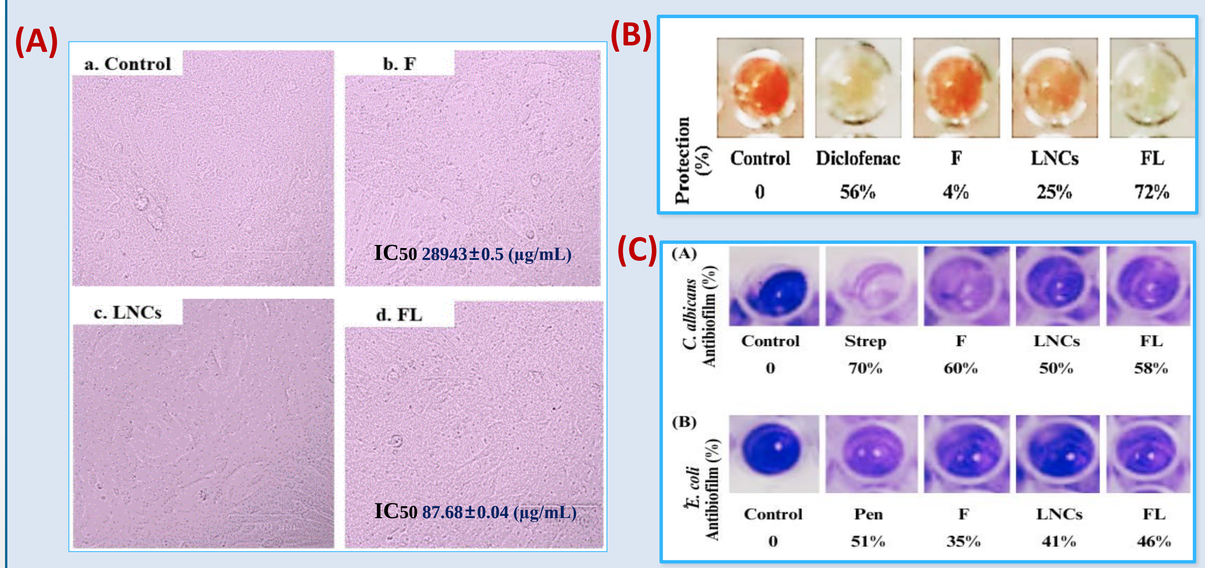

Fig. 4. (A) Effect of safe dose (IC_{50}) concentration on the morphology of human skin fibroblast cells (HSF); (B) *In vitro* anti-inflammatory activity by the HRBCs membrane stabilizing method. (C) Biofilm inhibition assay (%);

4. *In vitro* and *in vivo* wound healing assay

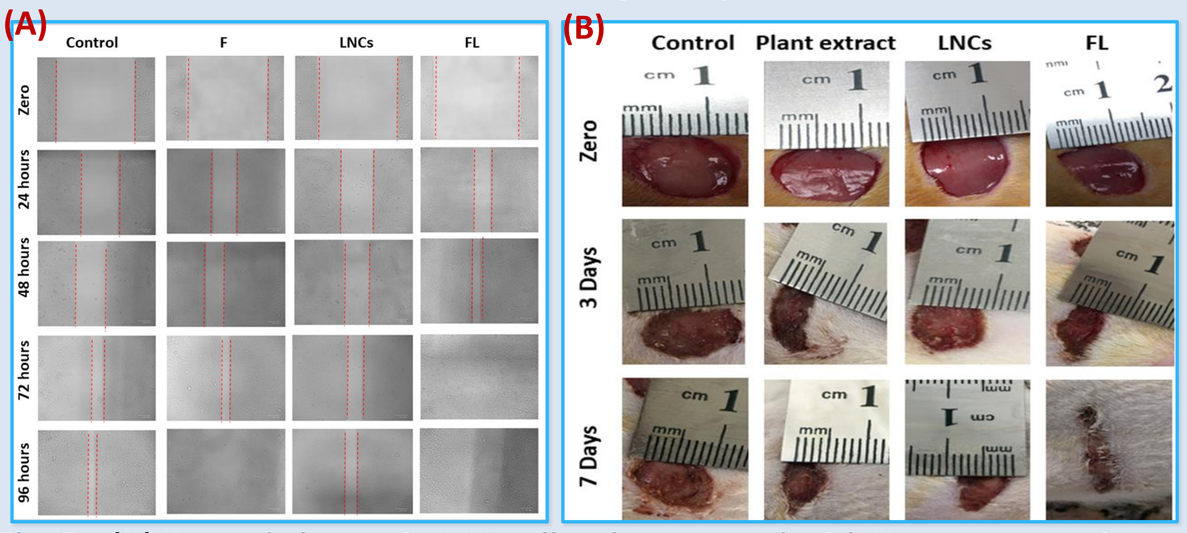

Fig. 5. (A) Wound closure in HSF cells when treated with IC_{50} concentrations of tested compounds. The size of the wound closure was measured in between the red dotted lines in the pictures; (B) *In vivo* full-thickness excision wounds after treatment with tested compounds.

CONCLUSIONS

- Lipid nanocarriers (LNCs) prepared by an eco-friendly method, without using any solvent or non-toxic materials, could be promising as a new drug delivery system for wound healing.
- The prepared lipid nanocarrier exhibited excellent physicochemical properties with a particle size of less than 100 nm, small size distribution , slightly negative surface and a high entrapment efficiency of 98.42%.
- Loaded LNCs demonstrated a promising in vitro anti-inflammatory effect compared to Diclofenac.
- Significant antibiofilm inhibition against *C. albicans and E. coli* was observed.
- *In vitro* and *in vivo* wound healing assays demonstrated great potentials of FL