

AN ALKYL POLYGLUCOSIDE BASED OIL-IN-WATER EMULSION FORMULATION WITH DEPOT WATER CONCEALING LIQUID CRYSTALS FOR DERMAL WOUND REPAIR

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INTRODUCTION

In dynamic biological systems, skin healing is intricately complex and physiological. The layered architectural continuum of skin requires several sequentially coordinated steps to ensure quick restoration of temporal dynamism, to achieve rapid wound closure. Optimal wound moisturization prevents water loss from the site of damage and minimizes hypertrophic scars. It regulates epidermal thickness and maintains dilation of intracellular spaces for rapid restoration of skin functions.

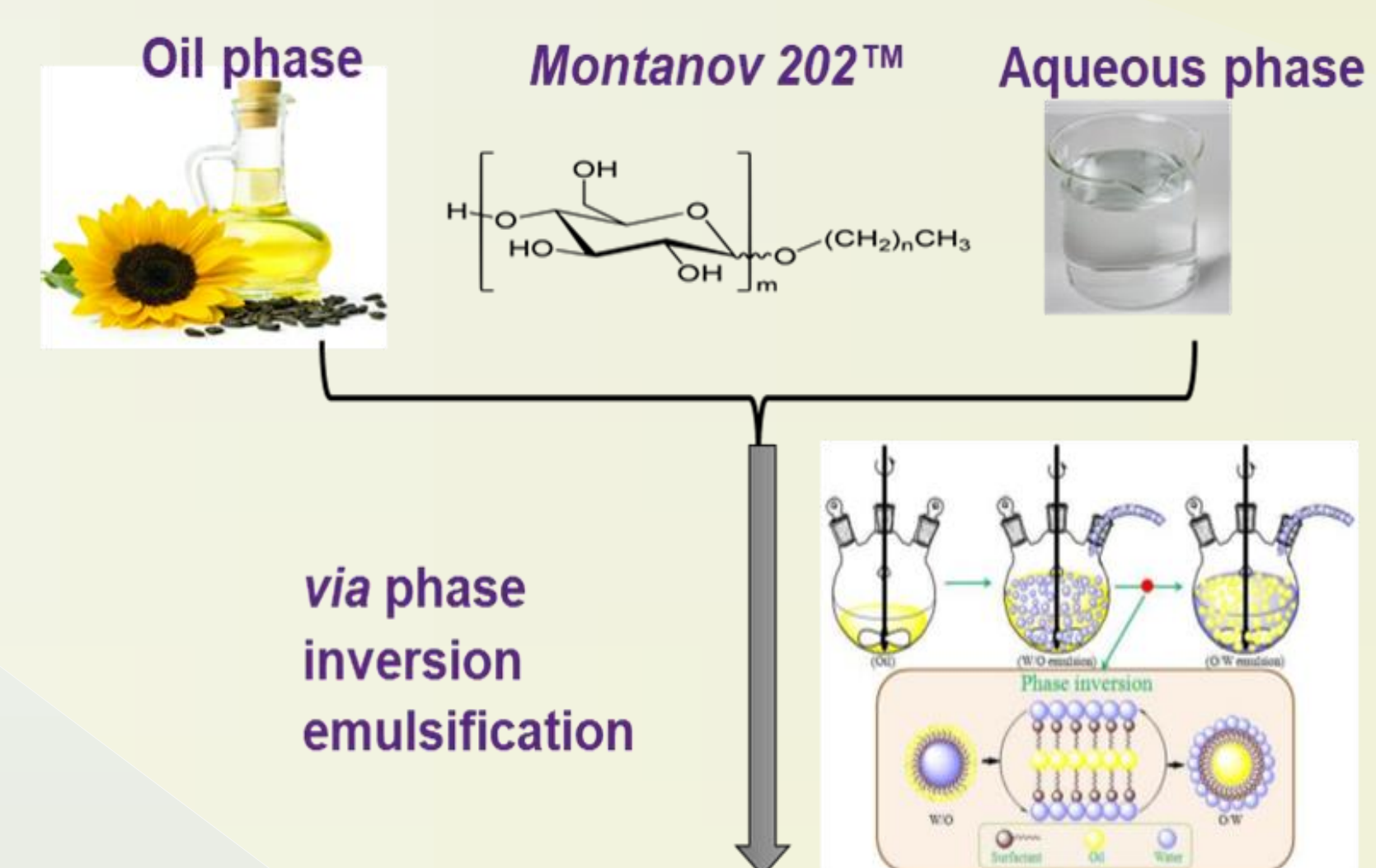
Sunflower oil contributes to skin integrity and firmness and are is an important components of pharmaceutical and cosmeceutical products. Blending the oil with a suitable emulsifier, to form oil-in-water formulations can overcome the limitations of application of crude oil with regard to its spreadability, consistency and ensure dermal compatibility.

Formulation stabilities are essentially controlled by the structural features of their emulsifiers that impart rheological litheness to the constituent systems. Alkyl polyglucoside (APG) emulsifiers derived from natural sources with excellent biocompatibility and safe ecological profiles, serve exceptionally well in this regard. Their long-chain behenyl and arachidyl alcohol, and arachidyl glucose moieties, form liquid crystals that entrap 'interlamellar depot water' within them and bestow a hydrating effect on the constituent systems. Topical application of such formulation systems creates a gel-like protective environ that is most suited for augmented healing of dermal wounds.

AIM

Herein, healing potential of an alkyl polyglucoside-sunflower oil formulation, on incision and excision model induced wounds in female Wistar rats, has been explored. It is thus envisaged that depot water in the liquid crystals of this emulsion synergistically supports bioactivity Helianthus annuus oil to facilitate topical wound healing as effectively as synthetic antibiotics like neomycin, elucidating as a feasible substitute for antibiotic treatment.

METHODS



APG-Sunflower oil-in-water emulsion formulation

Analytical characterizations

Presence of liquid crystals ensconcing depot water in this formulation is attempted to be established by WAXD and OPM and thermal analyses

Initial relevant *in vitro* experiments with m5S fibroblast cells for assessing the oil's concentration and suitability in terms of cell viability, intracellular ROS, apoptosis/necrosis, wound cell migration and extracellular matrix (ECM) gene expression activities for synthesis of the emulsion formulation have been carried out

In vivo healing potential of an alkyl polyglucoside-sunflower oil emulsion on incision and excision model induced wounds in female Wistar rats, has been explored

m5S fibroblasts incubated with concentration ranges of oil to assess for cell viability (LDH) and metabolic activity (MTT), fluorometric intracellular ROS, cell morphology, *in vitro* monolayer wound migration, apoptotic/necrotic, detection (via Hoechst 33342, FITC-Annexin V and EthD-III stains) and Extracellular matrix (ECM) gene expression activities.

For OPM, a thin film imaged with cross polarizers with a wavelength plate. The water evaporation rate recorded with an infrared light heating device and imaged for approximately every 10% of weight loss.

For TGA, 4-5mg of the formulation gradually heated (30-120°C, at 10°C/min ± n=3 and % weight loss (Mean±S.D) plotted against temperature (°C). For WAXD analysis, 2mg of formulation spread over a glass slide, used with CuKα radiation from 10-90°, at a step size of 0.02°. Interlayer positioning calculated from the diffraction angle (θ) and 2θ and represented graphically.

For wound induction studies, six albino female Wistar rats randomly assigned to three groups respectively (Group I: Wound induced without treatment; Group II: Wound induced & topically treated with positive control; Group III: Wound induced & topically treated with emulsion). Results expressed as Mean±SEM (n=6). (Institutional Animal Ethics Committee (Reg. 378/01/ab/CPCSEA; Approval No: KCP/PCOL/09/2017)).

RESULTS

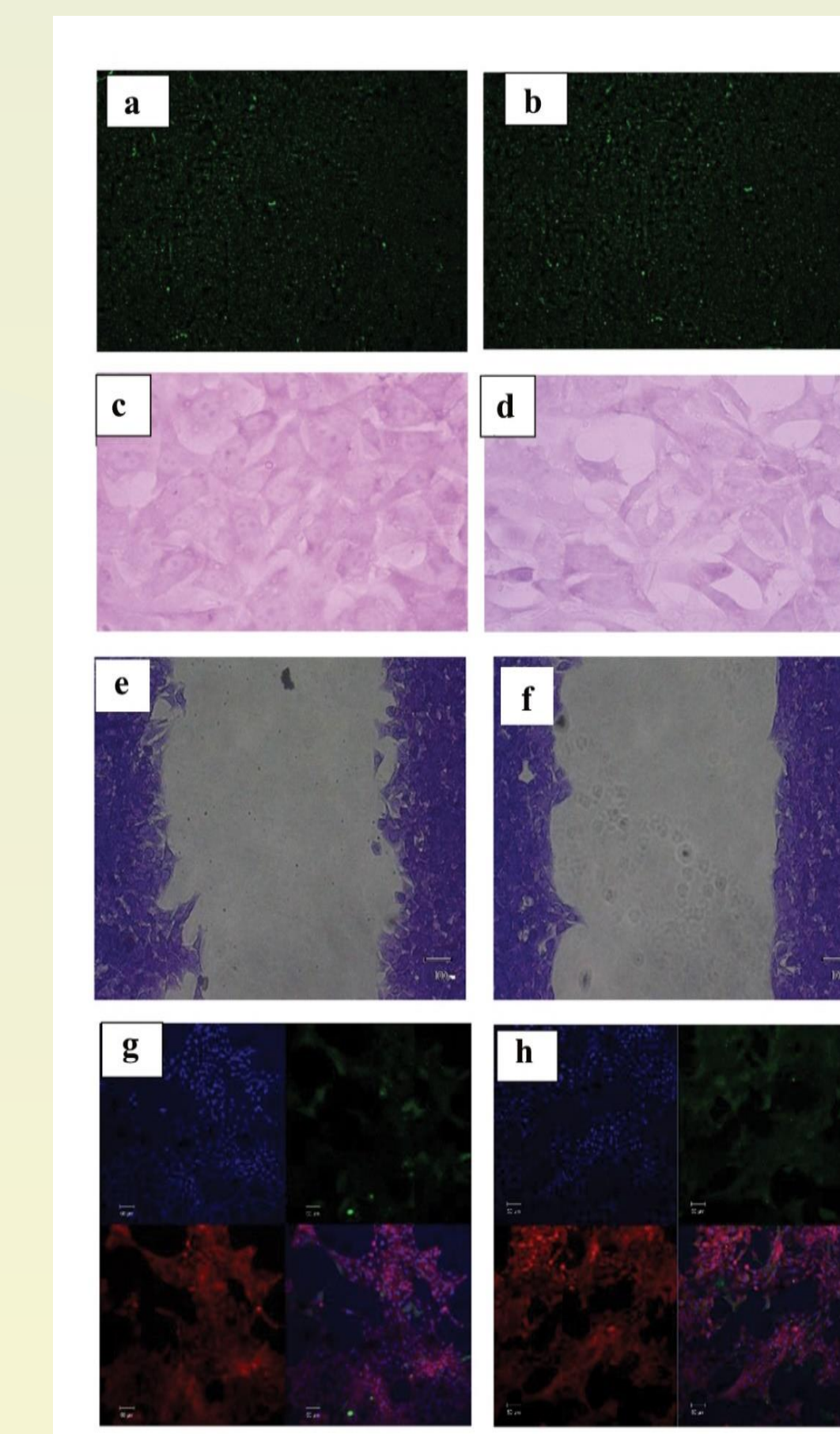


Fig. 1. Microscopic images of untreated m5S fibroblasts and those treated with 15% sunflower oil. 1(a-b) shows no production of intracellular ROS, 1(c-d) shows images (20X) of crystal violet stained cells for morphological analysis, 1(e-f) shows results of *in vitro* monolayer scratch assay (100µm) after 4h, 1(g-h) shows images for observing healthy /apoptotic / necrotic cells after staining (10X. Scale: 50µm)

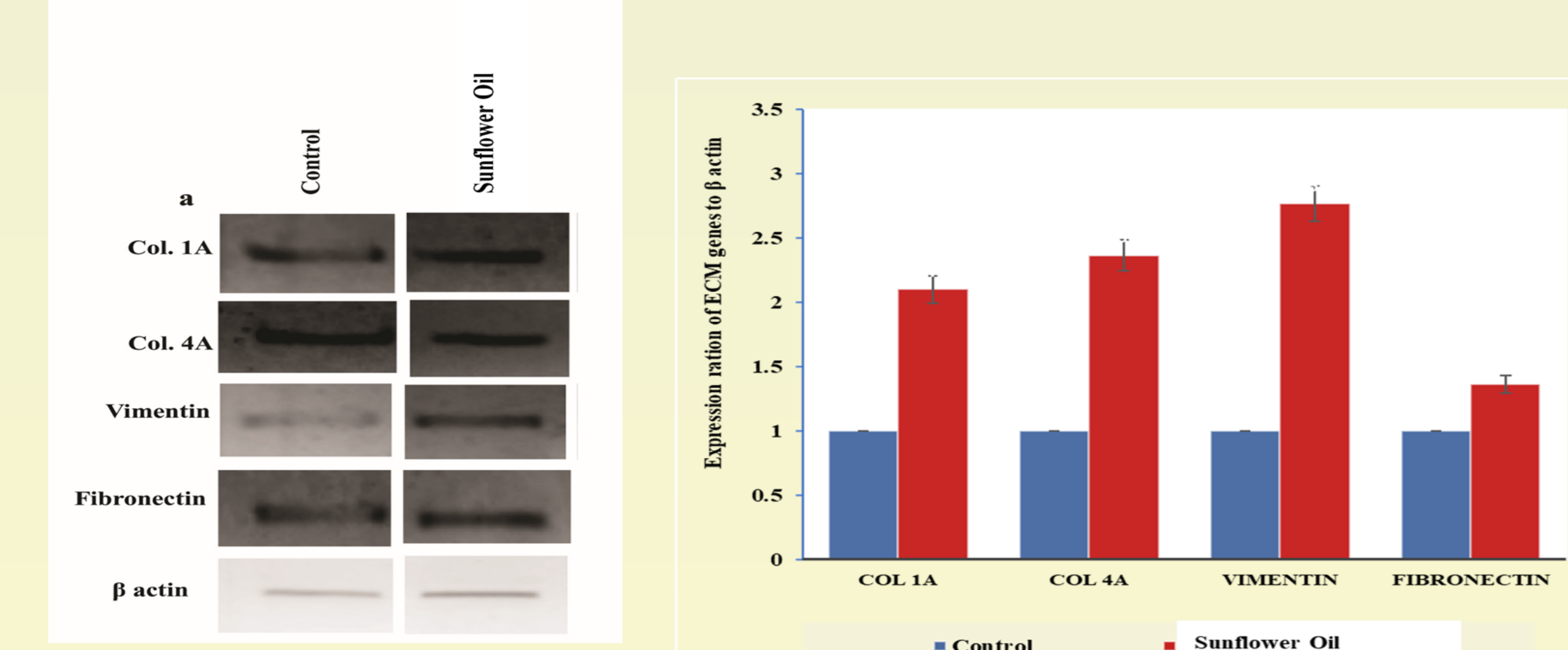


Fig. 2a. ECM gene product expression levels b. ratio of expression levels of ECM genes with β-actin

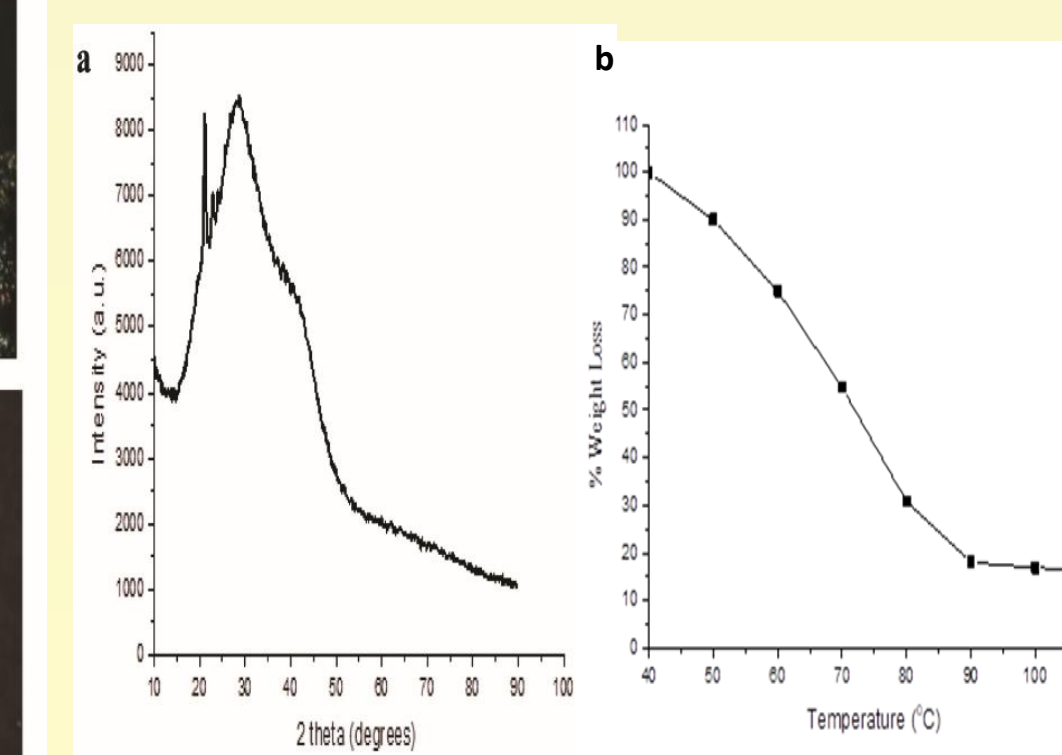
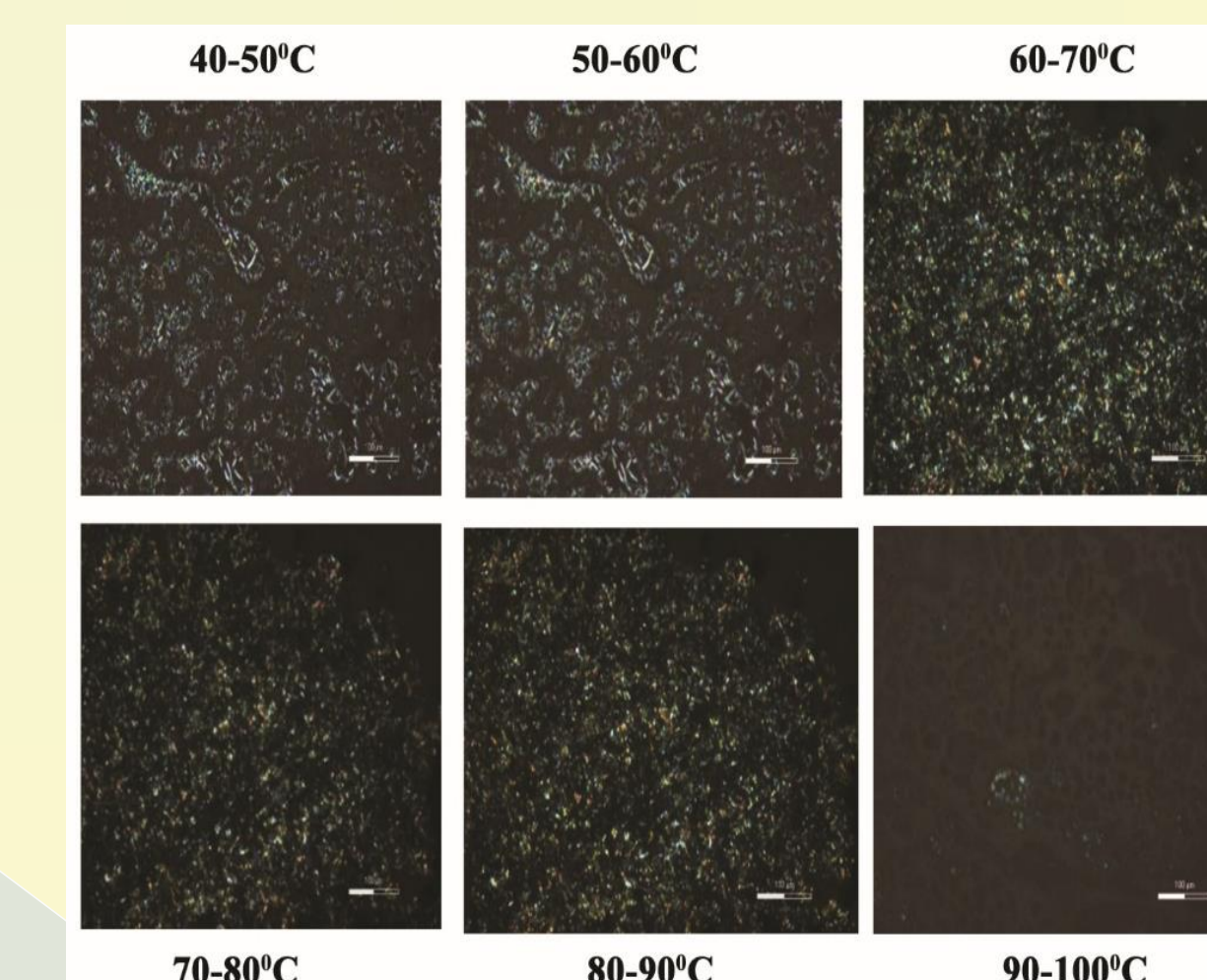


Fig. 3. OPM micrographs (10X,100µm) of formulation showing liquid crystals with increasing temperatures. The last image shows crystal breakdown from 90°C onwards. (a) WAXD profile, (b) Thermogram of formulation

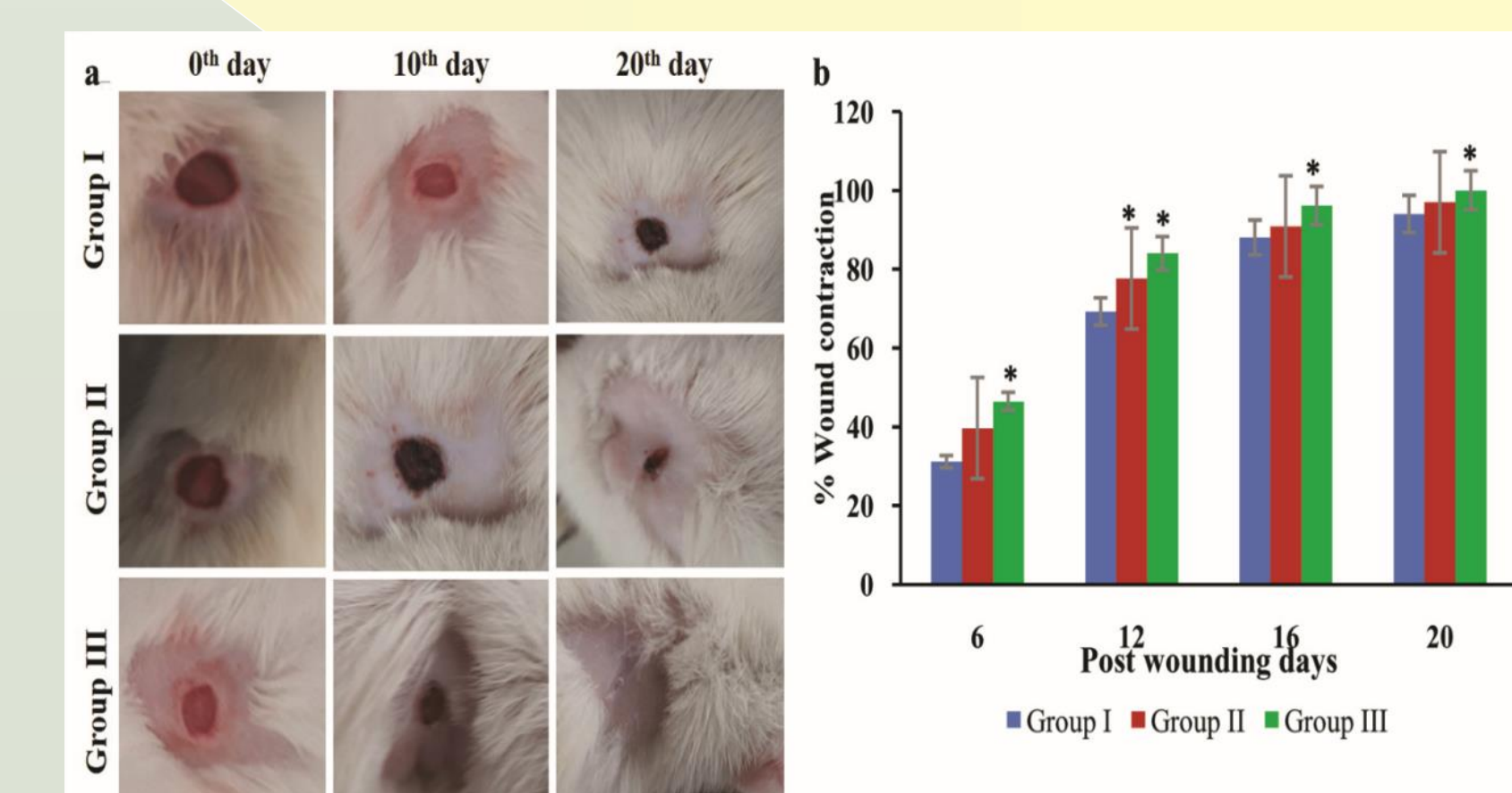


Fig. 4a. Images for induced wound on 0th day and after 10th and 20th post wounding days 4b. Wound contraction (%) in untreated animals (Group I), and animals treated with positive control (Group II) and sunflower oil formulation (Group III) (significant differences (p<0.05) from Group I represented as “**”)

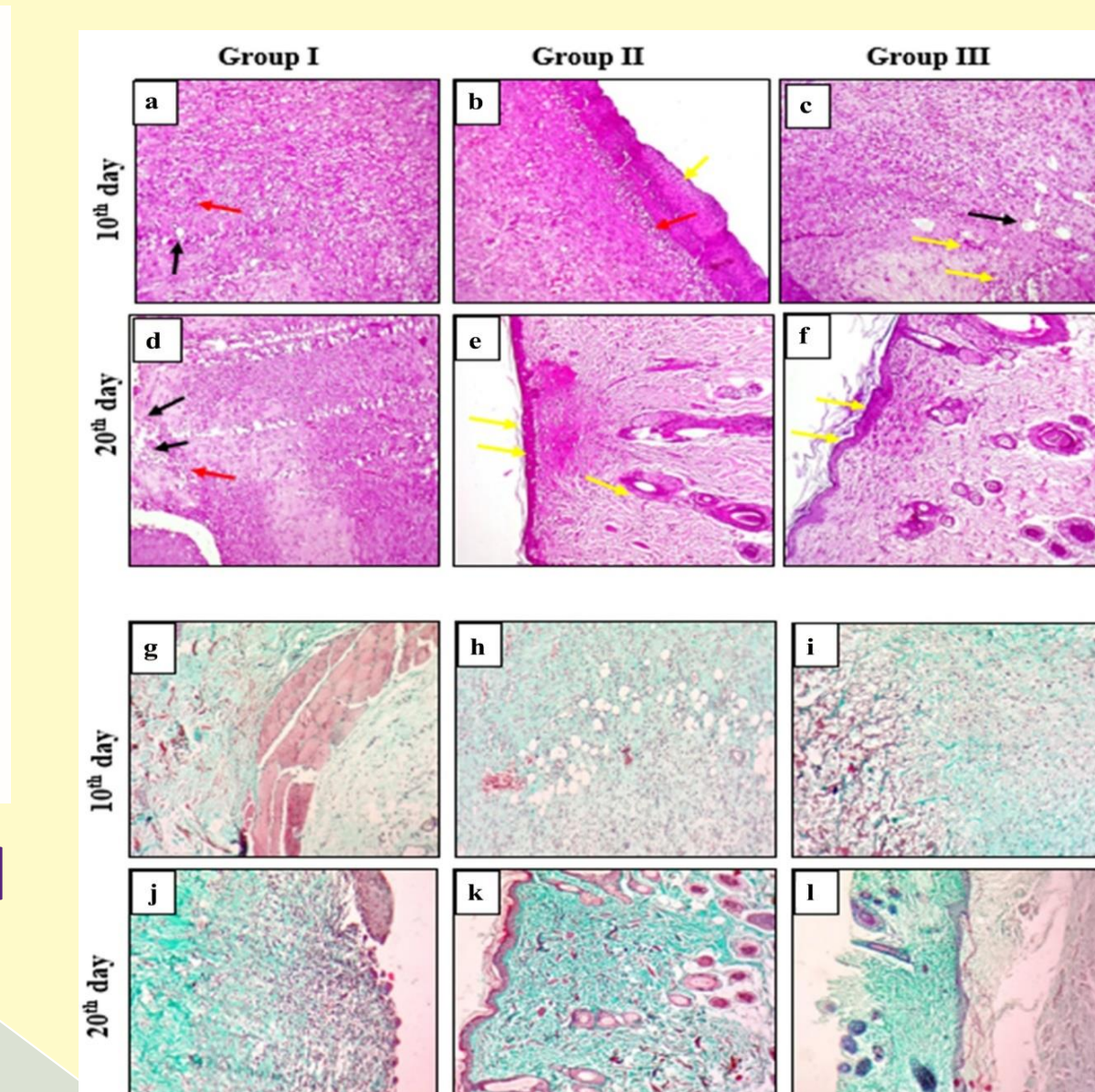


Fig.5 (a-f) H&E stained skin sections* and (g-l) MT stained skin sections of Group I, II and III animals (*black, red and yellow arrows indicate formation of granulation tissue, edema /ulceration and epithelialization respectively)

CONCLUSION

The formulation, hence, could serve as a prospective topical candidate for the accelerated repair of topical wounds and an alternative to many antibiotic creams used for similar applications

REFERENCES

- Chayapong, H. Madhyastha, R. Madhyastha et al., Environ. Sci. and Pollut. Res. 24 (2017) 5316-5325.
- K. Banerjee, H. Madhyastha, V. Rajendra Sandur, N., et al., Colloids Surf. B: Biointerfaces 193 (2020) 111102.

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