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Development and Evaluation of Topical Hyalurosomes Nanogel of *Calotropis procera* for the management of Vitiligo

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Abstract
Objective: *The analysis and pharmacological assessment of topical hyalurosome nanogel of Calotropis procera for the treatment of vitiligo is the aim of this paper.*
Material and methods: *Using the B16F10 cell line, cell viability, melanin content, tyrosinase activity assay, and Fontana-Masson silver staining, the anti-vitiligo activity of the nanogel was assessed and carried out. Fresh milky white latex of Calotropis procera was obtained by cutting stem twigs and identified by FT-IR for the presence of active phytoconstituents. Hyalurosomes as nanogel formulation was made using the solvent evaporation method.*
Result: *The large peak at 3253 cm-1 to the OH-str. frequencies of b-amyrin, lubeol, calotropin, and uscharin was shown by the FT-IR study. Calotropis procera petroleum ether latex contains beta-amyrin, alpha calotropeol, beta-calotropeol, and lupeol. These substances were used as drugs for topical nanogel delivery. In contrast to CP-Carbopol Gel (51.20±9.63%) at 50μM concentration, CPHGF demonstrated 64.57±2.76% cytotoxicity, according to the cell viability assay. The MTT assay reported that at 50 μM concentration, the cytotoxicity of CPHGF was 81.93±11.51% while that of the CP-Carbopol gel was 53.12±11.52%. Tyrosinase activity and melanin revealed that the petroleum latex of Calotropis procera solution increased the amount of melanin by 1.20 times after the first treatment ($p<0.05$) and again after the second treatment ($p<0.05$). In the first treatment, CPHGF increased melanin by 2.25 fold ($p<0.0001$), and in the second treatment, it increased melanin by 2.89 fold ($p<0.0001$). To see the melanin pigment in cells exposed to petroleum latex of Calotropis procer a CPHGF formulation, Fontana-Masson silver staining is used. In petroleum latex of Calotropis procera based nanogel, the quantity of melanin granules was markedly elevated and stained in comparison to the control.*
Discussion: *The unique deformability of CP and hyalurosomes Additionally, carbopol gel helps improve the skin penetration of latex that contains active ingredients. Higher penetration of the encapsulated moiety through produced CPHGF was confirmed by cell uptake experiments.*
Keywords: *Hyalurosomes, Calotropis procera, HPLC, B16F10 cell line, cell uptake*

INTRODUCTION

A common skin pigmentation illness known as vitiligo (leukoderma) is caused by the progressive, selective death of epidermal melanocytes that function as pigmentation cells. An acquired idiopathic dermatological condition called vitiligo is typified by well-defined, milky white macules that lack distinguishable melanocytes. [1] Some people may even try suicide as a result of these asymptomatic white macules, which can have a very negative psychological impact.3-4% of Indians are affected, and about one percent of people worldwide are. The most often affected areas are the scalp (11.2%), neck (18.8%), and face (24.5%).The vitiligo results in obvious flat white lesions in normally pigmented skin, which commonly appear on face, arms, hands, feet, and lips. Patches may be progressive and arise at any age.[2]

Melanocytes and other epidermal cells are typically impacted by exposure to environmental stressors such ultraviolet (UV) radiation and other chemicals, which increases the generation of reactive oxygen species (ROS). Melanocytes in vitiligo sufferers have inherent flaws that impair their capacity to react effectively to external stimuli. Consequently, it has been discovered that there are higher levels of epidermal H2O2 and lower levels of catalase, which shield cells from oxidative damage. By reducing oxidative stress and producing healthy melanocytes in place of damaged ones, the treatment of vitiligo aims to regulate immunoreactions and restore the normal skin color in the affected area.. [3]

to data no curative therapy for vitiligo. Vitiligo treatment has historically relied on systemic therapy, topical medications, phototherapy, and surgery to slow the disease's progression and promote skin repigmentation. For vitiligo patients, the chronic nature of the condition and the absence of consistent treatment over an extended period of time are extremely discouraging. Hypericin, khellin, and berberine are regarded as useful therapeutic agents for the treatment of vitiligo, according to natural treatment techniques..[4] Recently, Nano-dermatology science applies nanotechnology approaches in the field of dermatology and have involved sunscreens and maintenance of skin health as well as providing a tool for the diagnosis and management of skin disease and transport bioactive compounds at effective concentrations over a predetermined period. Such tactic has been directed toward enhancement of the activity problems of many biologically active compounds via improving drug solubility, skin deposition, skin permeability as well as minimize toxicity.[5]

The sodium salt form of hyaluronic acid, sodium hyaluronate (hyaluronan), is found in many connective tissues, the lungs, synovial fluid, and muscle tissues in human organs. It is regarded as a dual-purpose ingredient that has been utilized to repair dry skin by refilling the hyaluronic acid content and to increase the viscosity of skin care products.[6] Hyalurosomes are a modified nanovesicles that possess the intrinsic characteristics of phospholipid nanovesicles potentiated with hyaluronan penetration enhancer and gelling capabilities. Consequently, hyalurosomes combine the privileges of both elastic features of deformable liposomes and the stability of gel-core vesicles. Hyalurosomes would provide many benefits in local skin delivery owing to hyaluronan such as; longer residence time at the application site, penetration enhancing ability, hence facilitating skin permeation and drug deposition.[7] Therefore, loading of drug in hyalurosomes are expected to yield an outstanding outcome by enhancing the low skin permeability of *Calotropis procera* latex providing promising antioxidant and anti-inflammatory effects. Consequently, the current study is the first work to represent topical *Calotropis procera* latex-hyalurosomes nanogel as a targeted nano- therapy to enhance the skin permeability and deposition of drug for treatment of vitiligo.[8]

Vitiligo patients' management and treatment plans are taken into consideration by the natural product made from plant sources. The primary methods of the study are to concentrate on the petroleum latex of Calotropis procera, a herbal-based nano-formulation (hyalurosomes) to improve skin penetration and therapeutic action, and to assess their anti-vitiligo properties. The topical approach may reduce toxicity while increasing the bioavailability and effectiveness of vitiligo treatment.

MATERIALS AND METHODS

Lipoid AG (Ludwigshafen, Germany) provided the gift sample, Lipoid® S100 (l-α-phosphatidylcholine). I received sodium hyaluronate (also known as hyaluronan) from Euromedex in France. Calotropis procera petroleum ether latex was utilized as a medication to be encapsulated in a polymeric nanocarrier. Acridine orange (AO), nuclear fast red, hydroquinone, carbopol-940, sodium deoxycholate (SDC), bovine serum albumin, cellulose dialysis tubing with a molecular weight cutoff of 12000 Da, chloroform, methanol, and acetonitrile were all utilized. Every additional chemical and reagent that was used was of analytical quality.

Extraction and Identification of *Calotropis procera* plant

Fresh milky white latex of *Calotropis procera* was collected by cutting of the stem twigs. Moist Lax was dried under open air during the day.[9] The dried Lax was divided in two parts; the first sample was evaluated for its solubility in various solvents such as hexane, ethyl ether, chloroform, ethyl acetate, butanol, methanol, acetone, acetonitrile, and water. The maximum solubility was observed in petroleum ether and methanol. The presence of active constituents was defined by the IR analysis.

Preparation and characterization of Topical hyalurosomes nanogel (CPHGF)

Solvent evaporation method was used for the preparation of hyalurosomes nanogel with some modification.[10] Briefly L- α-phosphatidylcholine (5% w/v) and sodium deoxy cholate (SDC; 3.25%w/w) was dissolved in 2 ml of absolute ethanol. Then, the resulting ethanolic solution was injected dropwise through a 23-G syringe into a 10-ml hydrating medium solution containing hyaluronan (2.5%w/v) and petroleum ether latex of *Calotropis procera* as drug (0.25%w/w, 5 mg) in water: ethanol (80:20) under constant magnetic stirring at 1500 rpm and room temperature for 90 min. Hyaluronan was used as a self-gelling agent at a concentration of 2.5%(w/v). The placebo- hyalurosomes (CPPHGF) was prepared by same procedure without using the latex (drug).

Preparation of conventional Carbopol gel formulation loaded with Petroleum ether latex of *Calotropis procera*(CP-Carbopol Gel)

For comparison with hyalurosomes, a typical Carbopol hydrogel loaded with petroleum ether latex of Calotropis procera was prepared as a control gel. [11] Gel was prepared by dispersing 5 mg of petroleum ether latex of *Calotropis procera*(0.25% w/v) in 20 ml of distilled water. One gram of carbopol-940 was added portion-wise under magnetic stirring until a gel was obtained at room temperature.

PHARMACOLOGICAL EVALUATION

Evaluation studies on B16F10 cell line

Melanocyte cells (B16F10) used in the study were procured from the National Center for Cell Science, Pune and were cultured in Dulbecco's Modified Eagle's Medium (DMEM) containing 10% heat inactivated fetal bovine serum, 1.5g/L $NaHCO_3$, 2mM L-glutamine, 10,000 units penicillin, 10μg/mL streptomycin, and 25μg/mL amphotericin B, incubated at 37°C with 5% CO_2 in a humidified atmosphere. [12]

Cell Viability Assay

Cells were seeded in 96-well plates at a density of 1×10^4 cells per well and incubated for 24h. Initially cells were treated with *Petroleum latex of Calotropis procera* as drug (0.25%w/w, 5 mg) solutions at 10 to 250μM equivalent drug concentrations to obtain 50% cell growth inhibitory concentration (IC_{50}) for the respective drugs. After 24h incubation period, fresh DMEM containing 500μg/ml of 3-(4,5-Dimethylthiazol-2-yl)]-2,5-diphenyltetrazolium bromide (MTT) was added to replace the formulations and incubated for 3h. [13]

After aspirating the MTT solution, the formazan crystals were dissolved by adding dimethyl sulphoxide (DMSO). A microplate reader (Spectramax, Molecular Devices LLC, USA) was used to measure absorbance at 570 nm. [14] Cells without MTT were utilized as a blank, while untreated cells with 100% vitality were used as a control. Based on the above testing results, cells were treated with CG-Carbopol gel and petroleum latex of Calotropis procera loaded hyalurosomes (CPHGF) at concentrations ranging from 1 to 50μM. The impact of the various formulations on cell viability was assessed after the cells were exposed to them for 24 hours.

Melanin Content Assay

B16F10 cells were seeded in two 12 well plates at a density of 1×10^5 cells/well and incubated for 24 hrs. For evaluating the effect of first treatment (cells were treated once), the media was replaced by test formulations equivalent to 5 μM concentration of each drug followed by UV exposure for groups. After 48 hrs. of exposure to formulations, cells were processed for further steps. For another 12-well plate, second treatment was also given (cells were treated twice) and similar procedure was followed as per first treatment. Media from the first treatment was replaced by fresh media containing formulations at 48 hrs. (followed by UV exposure for PSR groups) and incubated for additional 48 hrs. After 48 hrs. exposure (for first treatment) and 96 h exposure (for second treatment), cells were washed twice with PBS and lysed by incubation in lysis buffer (PBS with 1% Triton X-100) at 4 °C for 20 min. [15]

The lysates from both the plates were centrifuged separately at 14000 rpm for 15 min to collect the pellet. Supernatants were processed for tyrosinase activity determination whereas the cell pellet is dissolved using 1N sodium hydroxide (NaOH) containing 10% DMSO for 1h at 80°C to solubilize the melanin. The protein estimation was performed using Bradford's assay and bovine serum albumin was taken as a standard. Aliquots containing same amount of protein were taken and volume was made up to 100μL with NaOH solution. Absorbance was measured for samples at 366 nm and melanin content was calculated from a standard curve using synthetic melanin.

Tyrosinase Activity Assay

Bradford's evaluate was applied to determine the protein content of the cell supernatant that was obtained from the aforementioned process. Samples with identical protein contents were collected, 100 μL of lysis buffer was added, and then 100 μL of a 0.1% L-DOPA solution produced in PBS was added. The absorbance at 475 nm was used to measure the dopachrome after the plate was incubated for one hour at 37°C. [16]

Cell Free Tyrosinase Assay

Tyrosinase activity was tested for direct effects of CPHGF using a cell-free assay method. 20 μl of mushroom tyrosinase (1000 units) and 100 μl of L-DOPA solution (2 mg/ml) were combined with about 130 μl of sample dilutions made from test formulations at concentrations ranging from 1 to 50 μM. The assay mixtures were incubated for 20 minutes at 37 °C, and a microplate reader was used to measure the dopachrome absorbance at 475 nm. Tyrosinase activity in mushrooms was measured and contrasted with control.[17]

Fontana-Masson Silver Staining

After being planted in the appropriate conditions at a density of 5×104 cells/well in a 12-well plate, the cells were incubated for 24 hours. After that, CPHFG formulations (5 µM equivalent drug concentration) were added to the media, exposed to UV light, and incubated for 24 hours. Following a wash and fixation in 4% v/v formalin, the cells were stained with 2.5% w/v ammonical silver nitrate while being kept at 56°C. After an hour of incubation, the plate was cleaned with distilled water. After five minutes of incubation, a 5% w/v sodium thiosulfate solution was added, and distilled water was used for washing. After five minutes of staining with nuclear quicker red solution, the cells were once more cleaned.Finally, after dehydration with ethanol the wells were washed with xylene twice. Then the plate was observed under phase contrast microscope for visualization of melanin pigment in wells. [18]

RESULTS AND DISCUSSION

FTIR analysis

The functionality of the Latex compounds was characterized by FTIR spectroscopy (Figure 1). The FTIR spectrum of pure Latex (A), Petroleum ether portion of latex (B), and methanol portion of latex (C) was determined. A broad peak at 3253 cm^{-1} was noted, this is response to the OH stretching frequencies of uscharin, calotropin, lubeol, and b-amyrin. High intense broad peaks were appeared at 1628 cm^{-1} of di-ketonic bonds. The peaks at 1065 cm^{-1} corresponded to the C-N stretching bonds of aliphatic amines. The Petroleum ether soluble lax was identified to determine the characteristics of the compounds based on FTIR spectrum (Figure 1B) and compared with FTIR spectrum of pure latex (Figure 1A). The OH stretching frequency peak was not present, confirming that polar compounds were not present in the Petroleum ether portion. Other characteristic peaks indicated a COOH stretching vibration at 2975 cm^{-1} and bending vibration at 1320 cm^{-1}. Polar solvent soluble compounds showed a broad peak at 3375 cm^{-1} because of the OH group of the polar molecules (Figure 1C).

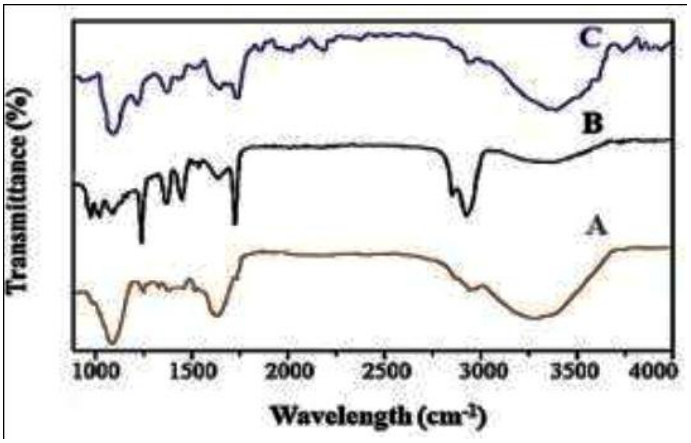

Figure 1: FTIR spectra of samples: (A) pure latex; (B) Petroleum ether latex; (C) methanol latex

Fluorescent studies

The Fluorescent studies of latex of *Calotropis procera* by UV spectrophotometry stated that at visible-light the latex has shown the Yellowish white, at Short UV light (252 nm) Light yellow colour is shown and at Long UV light (366 nm) Alice blue colour is seen. The Petroleum ether latex at Visible light has shown the light cream, at Short UV light (252 nm) Yellowish Green colour is shown and at Long UV light (366 nm) cream colour is seen. The methanol latex at Visible light has shown the light yellow, at Short UV light (252 nm) Yellowish Green colour is shown and at Long UV light (366 nm) brown colour is seen.

The *Petroleum ether latex of Calotropis procera* contain the lupeol, beta-amyrin (Water fraction of latex), alpha-calotropeol, and beta-calotropeol used as drug for the Topical Nano delivery of therapeutics and used as drug for treatment of vitiligo by topical nanogel delivery system.

Development of Hyalurosomes nanogel formulation

Hyaluronan was chosen as a major component of the hyalurosome formulation due to its self-gelling properties,

deep skin penetration, and inherent non-irritating properties. Furthermore, as hyaluronan's concentration and viscosity are directly correlated, liquified-HS could be successfully transformed into a more viscous hyalurosomes nanogel formulation by increasing its content from 0.2% to 3%. The pharmacological tests were conducted using the improved CPHGF formulation.

PHARMACOLOGICAL EVALUATION

Evaluation studies on B16F10 cell line

The study constraints in developing animal model for vitiligo, *in vitro* assessment was performed to evaluate the efficacy of CPHGF. Spontaneous and induced animal models developed to evaluate new treatments of vitiligo have their distinct advantages and disadvantages. Induced models may not demonstrate initiating events of vitiligo while spontaneous models which develop in a more physiologic manner can be time-consuming and costly due to low incidence of disease. Hence, mouse melanoma cells (B16F10) which address the experimental questions of present study were chosen to evaluate CPHGF formulations.

Cell viability assay

This study was performed to decide on the concentration that exhibits maximum stimulatory effect on pigmentation parameters with minimal cytotoxic effect. Initially, this assay was done using Petroleum latex of *Calotropis procera* as drug (0.25%w/w, 5 mg) to determine IC50, the concentration which inhibits 50% cell viability and in-turn ascertain working concentration range. From this assay, CPHGF showed 64.57±2.76% cytotoxicity, Petroleum latex of *Calotropis procera* solution showed 21.30±9.63% cytotoxicity and CP-Carbopol Gel showed 51.20±9.63% cytotoxicity at 50µM concentration (Figure 2A). Further, MTT assay was performed to evaluate the cytotoxic effect of different test formulations i.e., Latex solution, test formulations (CP-Carbopol Gel) and Petroleum latex of *Calotropis procera* loaded-hyalurosomes (CPHGF) on B16F10 cells. All the formulations were tested at respective drug concentration ranging from 1 to 50 µM (this concentration range was selected based on the results of initial cell viability assay). Dose dependent increase in cytotoxicity was observed with all the formulations and the cytotoxicity observed with drug loaded

CPHGF was slightly higher compared to respective drug solution (Figure 2B). A concentration which shows therapeutic efficacy with minimal toxicity has to be selected for further studies.

Figure 2 (A): Cell Viability study of Petroleum latex of *Calotropis procera,* CPHGF and CP-Carbopol gel

Figure 2(B): Effect of different nano-formulation on cell viability, B16F10 cells were exposed to different formulation i.e., Petroleum latex of *Calotropis procera* CPHGF and CP-Carbopol gel in concentration range 1-50 µM. All dare

represent mean±SD (n=3).

The Latex solution has shown the CP-Carbopol gel showed 53.12±11.52% cytotoxicity at 50 µM concentration of Latex while showed CPHGF 81.93±11.51% cytotoxicity at 50 µM concentration of latex. Low drug concentrations i.e., 1 µM and 5 µM showed minimal cytotoxicity with all the formulations. Latex solution showed about 11.13±5.37% and CPHGF showed 31.52.±0.43% cytotoxicity at 5 µM concentration. CG-Carbopol gel showed 21.6±1.25% cytotoxicity at 5 µM concentration (Figure 2B). As above 60% of cells are viable in all the formulations at 5 µM equivalent drug concentration, this was selected as working concentration for further studies. The *Petroleum latex of Calotropis procera* showed dose dependent therapeutic effect by stimulation of melanogenesis and tyrosinase activity at a concentration ranging from 1 to 10 µM.

Melanin And Tyrosinase Activity Assay

Petroleum latex of *Calotropis procera* may acts in vitiligo by stimulating melanisation, increased synthesis of tyrosinase via cAMP activity and by photo-polymerization of melanogenic precursors. In this study, the effect of Latex solution, test formulations (CP-Carbopol Gel) and Petroleum latex of *Calotropis procera* loaded hyalurosomes (CPHGF) on melanin content and tyrosinase activity was determined. These parameters were evaluated in terms of first treatment (cells were treated once) and second treatment (cells were treated twice).

Melanin is a natural pigment that plays a vital role in skin pigmentation and destruction of melanocytes leads to depigmentation which is the pathological hallmark of vitiligo. Compared to control (85 relative melanin content), Petroleum latex of *Calotropis procera* solution showed an increase in melanin content by 1.20 fold upon first treatment ($p<0.05$) and 1.20 fold upon second treatment ($p<0.05$). CPHGF is showed a 2.25 fold increase in melanin with first treatment ($p<0.0001$) and a 2.89 fold increase with second treatment ($p<0.0001$). This showed that effect of CPHGF showed enhanced stimulation of melanin levels compared to Petroleum latex of *Calotropis procera*. CPHGF showed a significant difference in melanin proportion between first and second

treatment levels ($p<0.05$) which indicates dose dependent elevation of melanin content.
For Petroleum latex of *Calotropis procera* solution and CP-Carbopol Gel, significant increase in melanin content was observed in comparison with control. This confirmed that has either inhibitory or stimulatory effects on melanin content at 5 µM working concentration. For CP-Carbopol Gel, there was 2.05 fold increase in melanin content with first treatment ($p<0.01$) while 2.64 fold increase was observed with second treatment ($p<0.01$) (Figure 3A).
Figure 3(A): Effect of different Nano-formulation on relative melanin content in B16F10 cells.
Tyrosinase is considered as the key determinant of pigmentation and as rate limiting enzyme for melanin synthesis. CPHGF showed a significant increase in tyrosinase activity by 2.64 fold with first treatment ($p<0.001$) and 2.72 fold increase with second treatment ($p<0.0001$) compared to control while a very slight increase in tyrosinase activity was observed with Petroleum latex of *Calotropis procera* solution. (Figure 3B) This ensured enhanced therapeutic effect of PUVA with CPHGF compared to Petroleum latex of *Calotropis procera* solution.
Figure 3(B): Effect of different Nano-formulation on relative tyrosinase activity in B16F10 cells.
Figure 3(C): Cell free tyrosinase assay to determine the direct effect of different formulation on tyrosinase activity.
In a cell free system, tyrosinase assay was performed with mushroom tyrosinase and LDOPA to determine the direct effect of Petroleum latex of *Calotropis procera* formulations on tyrosinase activity. No significant difference was observed in tyrosinase activity with any of the formulation groups compared to control (Figure 3C). This showed that Petroleum latex of *Calotropis procera* did not have direct effects on tyrosinase activity. This is in support with a previous report which stated indirect effect of PSR on tyrosinase synthesis via cAMP that further increases tyrosinase enzymatic activity.
Fontana-Masson Silver Staining
This staining is performed to visualize melanin pigment in cells exposed to *Petroleum latex of Calotropis procera* CPHGF formulations.

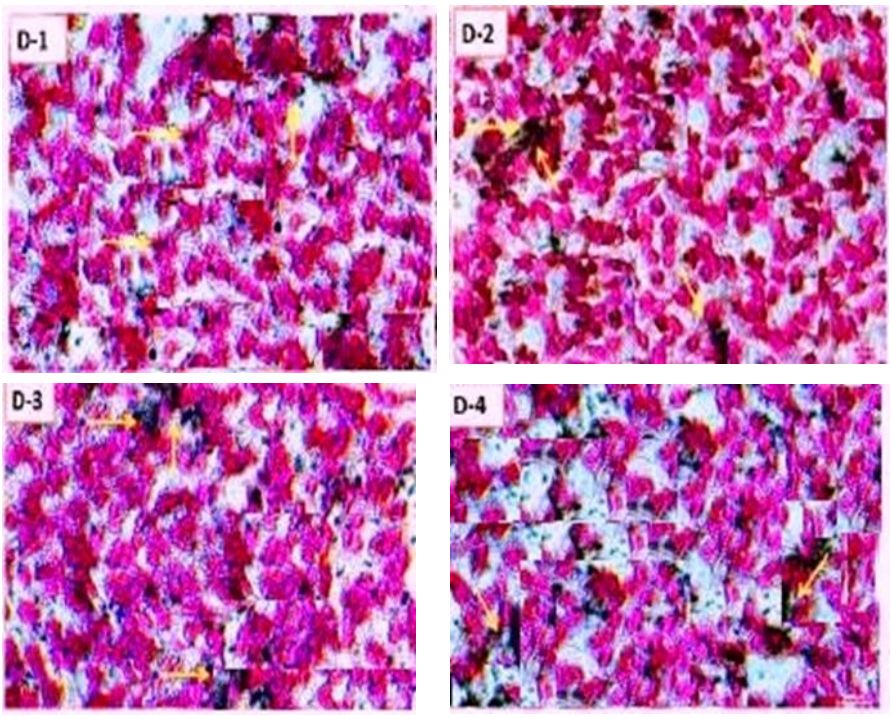

Figure 3D: Fontana-Masson silver staining to visualize melanin pigment in B16F10 cell line where melanin is stained by dark black (indicated by arrows). D-1= Control, D-2= Petroleum latex of *Calotropis procera* solution, D-3= CG- Carbopol Gel and D-4= CGHGF.
It is a histochemical technique based on oxidation of melanin and reduction of silver resulting in a black stain that can be visualized under microscope. Compared to the control (Figure 3D-1), the amount of melanin granules was significantly increased and stained in Petroleum latex of *Calotropis procera* solution (Figure 3D-2), CP-Carbopol Gel (Figure 3D- 3) and CPHGF (Figure 3D-4) groups (indicated by yellow arrows in figure). The obtained results were in agreement with the results obtained with melanin content and tyrosinase assay.

CONCLUSION

In view of the poor efficacy of the currently available treatments, there is a significant need for novel vitiligo treatments. By permitting its distribution into human skin with a high deposition level, the topical petroleum latex of *Calotropis procera* loaded-hyalurosomes (CPHGF) examined in this work shown exceptional qualities for the successful treatment of vitiligo. In summary, the study's investigation of petroleum latex of *Calotropis procera* loaded hyalurosomes (CPHGF) revealed encouraging skin penetration and deposition characteristics that should be very helpful for the clinical treatment of a variety of skin conditions.

REFERENCES

1. Elhalmoushy PM, Elsheikh MA, Matar NA, El-Hadidy WF, Kamel MA, Omran GA, Elnaggar YSR. Novel berberine-loaded hyalurosomes as a promising nanodermatological treatment for vitiligo: Biochemical, biological and gene expression studies. International Journal of Pharmaceutics 2022; 615: 121-130.
2. Alafnan A, Sridharagatta S, Saleem H, Khurshid U, Alamri A, Ansari SY, Zainal Abidin SA, Ansari SA, Alamri AS, Ahemad N and Anwar S. Evaluation of the Phytochemical, Antioxidant, Enzyme Inhibition, and Wound Healing Potential of Calotropis procera(L.) Dryand: A Source of a Bioactive Medicinal Product. Front Pharmacol 2021; 12: 70136
3. Sun MC, Xu XL, Lou XF, Du YZ. Recent Progress and Future Directions: The Nano-Drug Delivery System for the Treatment of Vitiligo. Int J Nanomed 2021; 15: 32-47.
4. Ahmad W. Preliminary phytochemical, antimicrobial and photochemical study of Calotropis proceraleaf extract. Cur Chem Lett 2020; 9: 105- 112.
5. Hoopes GM, Hamilton JP, Kim J, Zhao D, Wiegert-Rininger K, Crisovan E, Buell CR. Genome assembly and annotation of the medicinal plant Calotropis gigantea, a producer of anticancer and antimalarial Cardenolides. G3 Bethesda 2018; 8(2): 385–91.
6. Obese E, Ameyaw E, Biney R, Henneh I, Edzeamey F, Woode E. Phytochemical screening and anti-inflammatory properties of the hydroethanolic leaf extract of Calotropis Procera (ait). R. Br. (Apocynaceae) J Pharm Res Int 2018; 23(1): 1–11.
7. Sharma M, Tandon S, Nayak UA, Kappadi D, Rathore AS, Goyal A. Calotropis proceraas a potential anticariogenic extract agents against Streptococcus mutans: An in-vivo comparative evaluation. J Conserv Dent 2017; 20(3): 174–179.
8. Sanyal S, Maity P, Pradhan A. Sub-acute toxicity study of Calotropis proceralatex extracts in male Swiss albino mice. Toxicol Forensic Med Open J 2016; 1(2): 54-64.
9. Sharma N, Shankar R, Gupta N, Prakash P. A preliminary phyto pharmacognostical evaluation of Calotropis procera(L.) R. Br. (Alarka or Mandara) Root. Int J Ayur Med 2016; 7(1):44–48.
10. Singh M, Javed K; Comparative study of chemical composition of Calotropis proceraflower, leaf and fruit essential oil. Eur Chem Bull 2015; 4(10):577–480.
11. Srivastava S, Singh A, Rawat A. Comparative Botanical and Phytochemical Evaluation of Calotropis Procera Linn and Calotropis proceraLinn. Root. J App Pharm Sci 2015; 5: 41-47
12. Alluri N, Majumdar M. Phytochemical analysis and in vitro antimicrobial activity of Calotropis Gigantea, Lawsonia inermis and Trigonella foecum Graecum. Int J Pharm Pharm Sci 2014; 6(4): 524-527.
13. Rajamohan S, Kalaivanan P, Sivangnanam H, Rajamanickam M. Antioxidant, Antimicrobial activities and GC-MS analysis of Calotropis procerawhite flowers. J Phytopharmacol 2014; 3(6): 405-409.
14. Habib MR, Karim MR. Effect of anhydrosophoradiol-3-acetate of Calotropis procera(Linn.) flower as antitumoric agent against Ehrlich's ascites carcinoma in mice. Pharmacol Rep 2013; 65: 761-767
15. Nenaah G. Antimicrobial activity of Calotropis Procera Ait. (Asclepiadaceae) and isolation of four flavonoid glycosides as the active constituents. World J Micro Biotech 2013; 29(7): 1255–1262.
16. Chandrabhan S, Sumint ST. Antibacterial efficacy and phytochemical analysis of organic solvent extracts of Calotropis Gigantea. J Chem Pharm Res 2011; 3(6): 330-336
17. Hemalatha M, Arirudran B. Antimicrobial effect of separate extract of acetone, ethyl acetate, methanol and aqueous from leaf of milkweed (Calotropis proceraL. Asian J Pharm Res 2011; 1(4): 102-107.
18. Jain V, Nath B, Gupta GK, Shah PP, Siddiqui MA, Pant AB, Mishra PR. Galactose grafted chylomicron-mimicking emulsion: evaluation of specificity against HepG-2 and MCF-7 cell lines. J Pharm Pharmacol 2009;61(3): 303–310