ORIGINAL ARTICLE

Development and Characterization of Novel Vesicular System using Purpurin-Phosphatidyl Choline as a Skin Antiaging Agent

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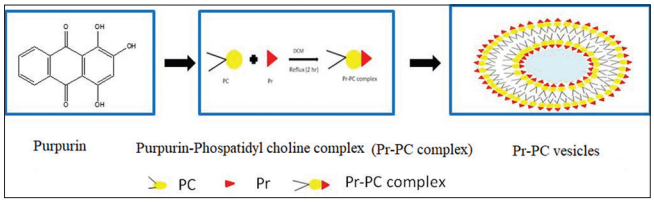
The Purpurin (Pr) loaded vesicles was prepared by the Purpurin- Phosphatidyl choline complex (Pr –PC complex). Pr-PC complex was prepared at a molar ratio of 1:1 (Pr: PC). Pr vesicles showed enhanced anti-aging properties than Pr and Pr-PC.



Abstract

Introduction: Herbal medicines are usually preferred for the treatment of skin ailments in comparison to conventional form of medicines due to their safety, low cost and compatibility. But the poor absorption of herbal drugs limits their use in the treatment of various skin ailments. To challenge this problem, active principles of plants have been encapsulated into several nanocarriers like nanoparticles, liposomes or other phospholipid formulations. **Materials and Methods:** In the present study, vesicles of purpurin-phosphatidyl choline (Pr-PC) complex were prepared and evaluated for the anti-aging activity. For this, initially the complex of Pr with PC was prepared and characterized by thin layer chromatography, differential scanning calorimetry and fourier transform infrared spectroscopy. The complex was converted into vesicles by film cast method. The prepared vesicles were characterized and subjected for *in vivo* studies to evaluate anti-aging activity. Anti-aging activity of Pr-PC vesicles were compared with plain Pr and Pr-PC complex in ultraviolet-induced oxidative stressed mice model. **Results and Discussion:** The results of *in vivo* studies showed the enhanced anti-aging effects of Pr-PC vesicles than other formulations. The increased effectiveness of Pr-PC complex and Pr-PC vesicles may be due to their amphiphilic nature, which enhances the water and lipid miscibility of the Pr. The Pr-PC vesicles were found to be better than Pr-PC complex. This is because of the reduced size of the Pr-PC complex when converted into vesicles. **Conclusion:** Based on the present study, it can be concluded that the Pr-PC vesicles are superior over Pr–PC complex and plain Pr, in terms of providing enhanced anti-aging properties. Thus, Pr-PC vesicles shown a tremendous potential in enhancing topical delivery of Pr.

Key words: Anti-aging, complex, phosphatidyl choline, purpurin, vesicles

INTRODUCTION

Now a day's, the herbal medicines gain a huge popularity in all over the world as a safer, compatible and a less toxic substitute of conventional medicines. As per the World Health Organization (WHO), because of poverty and limited admittance to modern therapeutics, about 60–80% of the global population pivot on herbal drugs forinitial

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health care. WHO set the guidelines for standardization of these drugs and make emphasis on their use for the treatment purposes.

For assuring the efficient use of the herbal medicines, they are converted into suitable dosage form. During their formulation, scientist faces certain challenges i.e., the active principles of the herbal drugs are associated with many harmful compounds and the active herbal principles mostly have less bioavailability.

The active principles are possible to separate from the crude extract by using analytical chemistry[1] but the bioavailability issue still exist. The bioavailability issue with most of the herbal drugs are due to the presence of polyphenolic rings (flavonoids), presence of water soluble constituents like tannins and terpenoidsetc[2] or high molecular weight/size.[3]

The skin is a phenomenal tool for investigation of nanocarriers for dermatological application. Different nanocarriers like liposomes, phytosomes, nanoparticles, nanoemulsion have been developed which promises to transform the treatment of dermatological conditions because of its interaction at the sub-atomic level with the skin tissue.

The technique of the complex formation of herbal drugs with phospholipids has been emerged as a most growing method for enhancing the permeability of many poorly absorbed active plant constituents. The structural similarity of the drug-phospholipid complex with the lipid constituentsof the cell membrane of the mammals enhance the absorption of the drugs.[4-7] In the drug-phospholipid complexes, an active hydrogen atom (-OH) of the drug was esterifiedwith the -OH group of a phosphatidyl choline (PC). The reaction between drug and PC forms an amphiphilic agent whichcan easily cross the cell-membrane.[8,9] This method enhances the absorption of lipid insoluble polar phytoconstituents through oral as well as topical route.

Purpurin (Pr) is chemically an anthraquinone(λ_{max} = 253 nm)

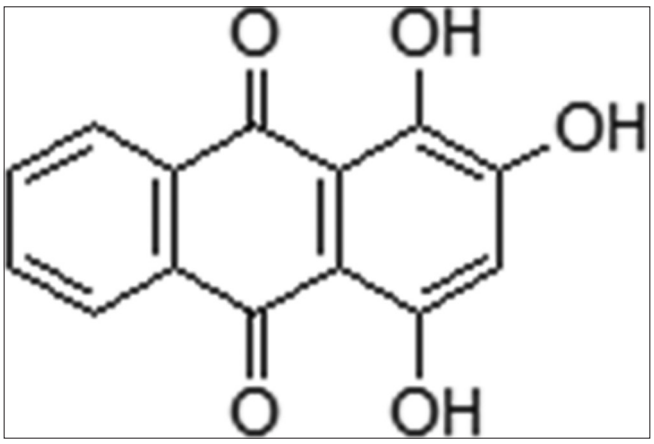

Figure 1: Structure of purpurin

derived from the roots of *Rubia cordifolia* Linn. of the family Rubiaceae [Figure 1].

Pr itself has antioxidant properties.[10] Antioxidants are the scanvangers of the free redicals. Free radicals causes cellular damage and consequent age related disorders like wrinkling etc. So use of Pr will be an effective approach to reduce age related disorders. Apart from the antioxidant properties, Pr also have some other biological and pharmacological activities i.e., anticancer,antimicrobial,antifungal,hypotensive,analgesic, antimalarial,antileukemic, immunomodulatory etc.[11-14]

Pr itself is a lipophilic in nature having partition coefficient value 2.49. Keeping all these facts in consideration in the present work, the Pr-PC complex and their vesicles (Pr-PC vesicles) were prepared and characterized for their skin antiaging effect. The *in vivo* results clearly indicate the effective antiaging effect of the Pr-PC vesicles than pure Pr itself.

MATERIALS AND METHODS

Materials

Pr, cholesterol, PC and Carbopol 934 were purchased from Sigma Chemicals Ltd, USA. All the other chemicals and solvents used were of analytical and high performance liquid chromatography (HPLC) grade.

Preparation of Pr-PC Complex

The Pr-PC complex was prepared by dissolving Pr and PC (1:1 molar ratio) in 20 ml dichloromethane taken in a round bottom flask. The mixture was refluxed for 2 h at room temperature. Then, solvent was completely removed under reduced pressure in rotatory evaporator at 30°C. The resultant Pr–PC complex was washed using n-hexane, dried and stored in amber colored glass bottle.[15]

Characterization of the Pr-PC Complex

Differential scanning calorimetry (DSC)

Mettler Star SW 9.00 DSC instrument (Jade DSC, SI No. 520A8031008, and Software Version: 9.0.1.0174) was used to record DSC thermograms of the Pr, PC and Pr-PC complex.The samples were kept and sealed in the cell of aluminum crimp heated up to 200°C at 10°C/min. Theonset, peak, and end set temperatures of all the three samples (Pr, PC and Pr–PC complex) were recorded and compared.

Thin layer chromatography (TLC)

TLC of the Pr, PC and Pr–PC complex was conducted by using chloroform/methanol (8:2) as the mobile phase. Samples were spotted on the silica gel coated TLC plate (60 F254, E Merk

Ltd, Mumbai). The TLC plate was kept in a mobile phase saturated chamber. The R_f values were calculated by the spots produced in TLC plates at 366 nm in ultraviolet (UV) chamber.

Fourier transform infrared spectroscopy (FTIR)

ThePr-PC complex was scanned using FTIR spectrometer with the wave number 400–4000 cm^{-1} using KBr pellets. IR spectrum of complex was compared with IR spectrum of PC and prto identify the actual site of complex formation.

Preparation of Pr-PC Vesicles

The Pr-PC complex and cholesterol (7:3 ratio) weredissolved in chloroform and methanol (2:1 v/v) mixture. The mixture was taken in a round bottom flask and evaporated under reduced pressure in a rotatory evaporator (Buchi type, York Sci. Co., Bombay) at 45°C until dry, thin film was not formed. The film was exposed to nitrogen gas and kept overnight at room temperature to ensure the complete evaporation of solvents. Then the film was hydrated in phosphate buffer saline (PBS, pH 7.4) for 2 h till resulted vesiclesformation.[15]

Characterization of Pr-PC Vesicles

Vesicle shape

Vesicular system was visualized by a Philips Morgani 268 transmission electron microscope (TEM). A drop of prepared formulation was placed on carbon-coated copper grid and dried to get thin film. Then, the film was negatively stained by placing a single drop of 1% phosphotungustic acid on the film. The excess stain was drained off with a filter paper. The grid was allowed to dry and viewed under the TEM. The photographs were taken at 430 K magnification.

Vesicle size and size distribution

The size and size distribution of the Pr-PC vesicles were determined by Malvern zeta sizer after dilution with PBS (pH 7.4).

% Encapsulated efficiency

The amount of Pr encapsulated in Pr-PC vesicles was determined by dissolving 5 mg vesicles in 10 ml dichloromethane. The mixture was centrifuged at 5000 rpm for 5 min. The amount of Pr was determined in supernatant at 253 nm by HPLC analysis using octadecylsilane (C-18) column and acetonitrile: water (70:30 v/v) solvent system as mobile phase. Flow rate employed for analysis was 0.8 ml/ min.

In vivo Studies

In vivo studies were carried out on swiss albino rats (100–180 g) and mice (20–25 g) of either male or female grouped as shows in Table 1. All the studies were carried out after approval by the Institutional Animal Ethics Committee of the university. All the animals were housed in standard conditions according to committee for the purpose of control and supervision of experiments on animals regulations. Prior the study, approximately 2 cm^2 areas on the dorsal side of each animal wasdemarcated and properly shaved with the help of hair removing cream.

Pr, Pr-PC complex and Pr-PC vesicles were incorporated in 1% carbopol 934 gels to make the formulations feasible for topical applications.

Skin irritation study

The skin irritation activity of Pr gel, Pr-PC complex gel, and Pr-PC vesicles gel were evaluated by Draize patch test.[16] All the gels were applied on the skin and covered with unreactive tape.The entire test site was wrapped with gauze to maintain the tape in position. After 24 h, the gauze and tape were removed and the sites were gently wiped with a wet gauze sponge. The degree of erythema was noted in terms of Grade 0, I, II, III and IV for No, very slight, well defined, moderate and severe erythema respectively at three equal different time intervals for 72 h.[17]

Table 1: Animals and animals grouping[a]

Animal grouping	*In vivo* studies		
	Skin irritation study	Skin permeation study	Anti aging studies (Biochemical estimation and Skin moisture content)
Type of animal used and their weight	Swiss albino rats (male/female) 100–180 g	Swiss albino rats (male/female) 100–180 g	Swiss albino mice (male/female) 20–25 g
Group I	Control group (without formulation treated)	Control group (without formulation treated)	Control group (UV treated/without formulation treated)
Group II	Pr gel	Pr gel	UV treated
Group III	Pr-PC complex gel	Pr-PC complex gel	Pr gel
Group IV	Pr-PC vesiclesgel	Pr-PC vesiclesgel	Pr-PC complex gel
Group V	–	–	Pr-PC vesicles gel

[a]Each group consist of three animals. Pr-PC: Purpurin-Phosphatidyl choline, UV: Ultraviolet

Skin permeation study

Skin permeation study of Pr gel, Pr-PC complex gel, and Pr-PC vesicles gel were evaluated by tape stripping method. 1 g of each gel were applied occlusively on the dorsal side of the rat skin for definite time intervals i.e., 3, 6, 9, 12 and 24 h. After definite time, the excess gel was removed carefully followed by tape stripping. The tape stripping was done (2 times) with adhesive tape. The tape strips of size 1.5–2.0 mm were adhered to the marked skin. After 1–2 min of adhesion, the tape was removed at a 45˚ angle to the skin by using forceps. Then stripped skin was separated by scissors.Both stripped skin andtape strips were cutted into small pieces, then homogenate separately in chloroform at 10,000 rpm for 2 min. The homogenate was filtered via a membrane filter and further centrifuged at 3000 rpm for 10 min. The amount of Prwas finally determined with the help of HPLC analysis as mentioned above.The results are expressed in term of permeation flux (J, µg/cm^2/h).

Anti aging studies

The UV exposure to the animals was done by an UV lamp at 200–400 nm with 3.6 ± 0.4 mW/cm^2 irradiance to achieve the intensity of exposed light radiationof 5 J/cm^2 to produce photo stress on the miceskin, according to the organization for economic co-operation and development guidelines by following formula.[18,19]

$$T\left(\min\right) = \frac{\text{Irradiation dose}\left(\text{J/cm}^2\right)\times 1000}{\text{Irradiance}\left(\text{mW/cm}^2\right)\times 60}$$

The Pr gel, Pr-PC complex geland Pr-PC vesicle gel formulations were applied on the shaved dorsal surface of the mice before irradiation. After 30 days of the study, the mice were sacrificed with the help of lightether anesthesia and their skin was removed surgically. The undesirable adherent tissues were separated via washing with a cooled normal saline solution and thenpiece of skintissue was weighed and placed on a glass plate over icebags followed by homogenizationup to the formation of colloidal solution. The colloidal solution was centrifuged for 10 min at 3000 rpm and supernatant was separated out and stored for estimation of biochemical parameters.Various biochemical parameters, i.e., malondialdehyde (MDA),[20] reduced glutathione (GSH),[21] hydroxyproline,[22] and catalase (CAT)[23] were estimated by well known procedures to determine the UV protection effects of prepared gel formulations.

Histology of skin

For histological studies, the skin tissues were separated and fixed for 24 h in freshly prepared 10% chilled formalin solution. The fixed tissues were rinsed several times with water, followed by the treatment with alcohols and xylene. After this,the tissues were finally embedded in paraffin wax to make tissue blocks. The section of 5 µm thickness was cutted and stained with hematoxylin and eosin dyes, for the determination of structural epidermal and dermal changes.[24]

Statistical Analysis

In order to evaluate the extent of a relationship between two data sets, Pearson's correlation coefficients were used. To analyze statistical differences among groups one-way analysis of variance was used. $P = 0.05$ was considered statistically significant.

RESULTS AND DISCUSSION

The Pr-PC complex vesicles were prepared by using PC and cholesterol. For this, firstly, the Pr-PC complex was prepared and characterized. The synthesis of Pr-PC complex was confirmed by DSC, TLC and FTIR. DSC of PC shows two separate peaks at 113.14°C and 184.16°C [Figure 2]. The two different major peaks may be due to the presence of polar and non polar portions of the PC molecules. Peaks at 113.14°C may be due to the result of hot movement of polar head groups of PC molecules. Peaks at 184.16°C is due to phase changes from gel to liquid crystalline state.The non polarhydrocarbon tail of PC may be melted during this phase and yield a sharp peak. The thermogram of Pr shows a single peak at 261.35°C. In thermogram of Pr-PC complex, single peak at 115.37°C is different from the original peak of Pr and PC. This confirms the formation of Pr-PC complex.

The R_f values of Pr, PC and Pr-PC complex were found to be 0.73, 0.56 and 0.86 respectively by TLC analysisusing chloroform and methanol mixture as a mobile phase. The huge difference in R_f value of Pr, PC and Pr-PC complex indicates the formation of Pr-PC complex.

The Pr-PC complex formation was confirmed by FTIR spectroscopy [Figure 3]. FTIR spectra of Pr-PC complex

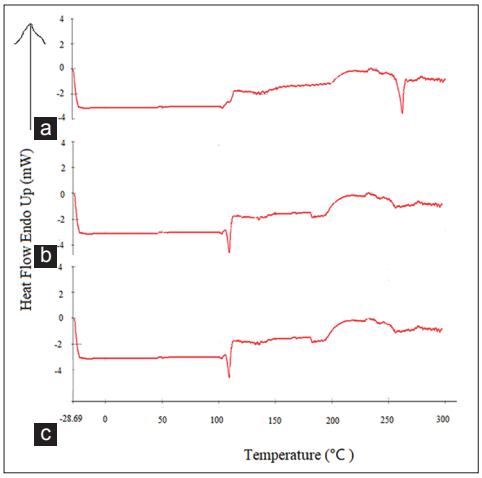

Figure 2: Differential scanning calorimetry thermogram of (a) Purpurin (Pr) (b) Phosphatidyl choline (PC) (c) Pr-PC complex

showsthe changes in the positions of the peaks as compared to Pr and PC. The FTIR spectra of prshowsthe characteristic O-H stretching vibration of phenolic groups at 3350.17 cm^{-1} [Figure 3A]. FTIR spectra of PC shows sharp characteristic C-H stretching vibration peaks (of fatty acid chain) at 2986.11 cm^{-1} and 2815.25 cm^{-1} and C = O stretching vibration at 1280.26 cm^{-1} [Figure 3B]. However, the FTIR spectra of Pr-PC complex shows a sharp peak at 3350.35 cm^{-1} suggesting

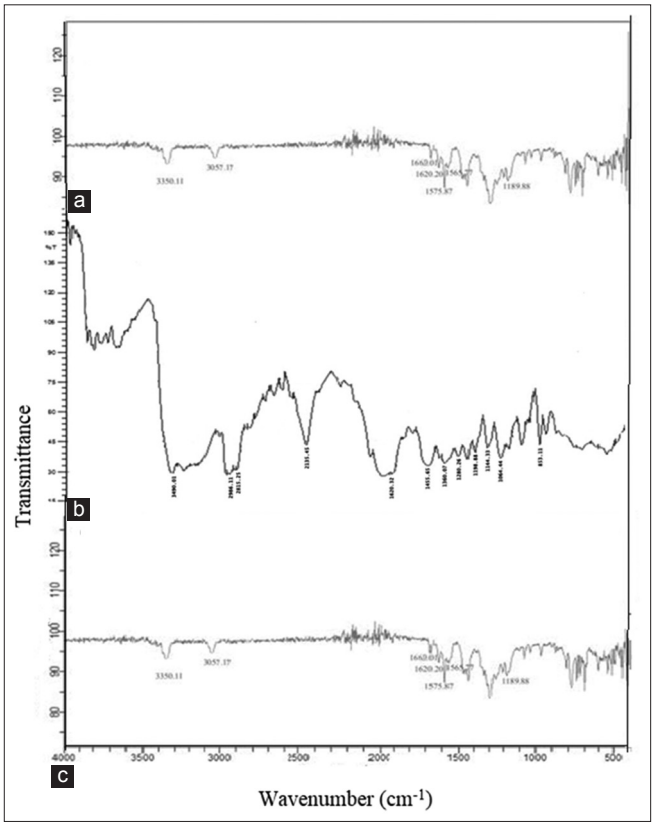

Figure 3: Fourier transform infrared spectroscopy spectrum of (a) purpurin (Pr) (b) phosphatidyl choline (PC) (c) Pr-PC complex

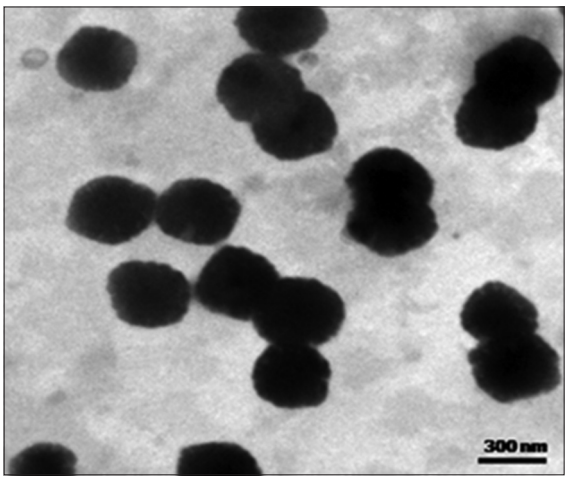

Figure 4: Transmission electron microscope photomicrograph of purpurin-phosphatidyl choline vesicles (magnification of 430 K)

the possible interaction at -OH groups of polyphenols present in the Pr [Figure 3C]. The IR spectrum of Pr–PC complex was found to be significantly different from the IR spectrum of Pr and PC that supports the reaction of –OH group of Pr at choline part of PC during complex formation.

After characterization, the Pr-PC complex was converted into the vesicular form by a film cast method. Figure 4 shows the TEM image of vesicles. The TEM image confirms the formation ofspherical shaped vesicles. The average vesicles size was found to be 345 ± 2.8 nm with polydispersity index less than one.The encapsulation efficiency of Pr in the Pr-PC vesicles was found to be 72 ± 1.8%. The higher percentage of encapsulation efficiency of Pr in vesicles may be due to lipid and water solubility (i.e., amphiphilic) of Pr-PC complex [Table 2].

In vivo studies of the Pr, Pr-PC complex and Pr-PC vesicles were performed by incorporating these formulations in 1% carbopol 934 gels. Draize patch test method was used to evaluate the skin irritation activity of all the formulations on albino rats. The skin was continuously monitoredfor any visible changes, i.e., erythema at 24, 48 and 72 h. The erythemal scores were shown on the basis of severity, ranges from 0 to IV. The Pr gel showed the erythemal effect within 24 h and it scored IV. After 72 h the effect was found to be reduced and it was scored as III. In case of Pr-PC complex gel, the erythemal effect was scored III after 24 h and I after 72 h. Pr-PC vesicles gel showed the better anti irritation effect and scored II and zero after 24 and 72 h, respectively. The better anti irritation effect of Pr-PC complex and vesicles may be due to presence of PC, which is a biolipid of skin.

Skin permeation studies of all formulations were evaluated by tape stripping method. This method is used to observe the distribution of various formulations within the stratum corneum. The concentration of permeated drug was calculated from the concentration of drug recovered in tape strip and in the skin after tape stripping. The results of tape striping methods exhibited that the 2.65 ± 0.15%, 4.65 ± 0.24% and 5.02 ± 0.20% drug were recovered by tape strips in case of Pr, Pr-PC complex and Pr-PC vesicles after 3 h, respectively. While in case of skin after stripping it was found to be 0.010 ± 0.001%, 0.018 ± 0.001% and 0.022 ± 0.001% after 3 h, respectively from Pr, Pr-PC complex and Pr-PC vesicles [Figures 5 and 6].

The significant increased in concentration was observed after 24 h i.e., 28.63 ± 2.05%, 36.65 ± 1.24% and 42.86 ± 2.35% in tape and 29.63 ± 1.89%, 40.86 ± 2.00% and 48.22 ± 2.06% in skin from Pr, Pr-PC complex and Pr-PC vesicles gel formulations, respectively.

The results indicated that the Pr-PC vesicles was permeated and distributed in stratum corneum at higher concentration as compare to plain Pr. This may be due the enhanced water and lipid miscibility of drugs because of amphiphilic nature

of the Pr- PC complex.The values of flux (J, µg/cm²/h) for all three formulationswas calculated by using data in 2^3 factorial design and it was best fitted to firstorder polynomial model. From the ANOVA results of the model relating permeation flux (J, µg/cm²/h) as response, it was observed that all the coefficients of this model equation had statistic significance ($P < 0.05$). The Pr-PC vesicles gel was evaluated to determine the permeation flux (J, µg/cm²/h) through skin. The *in vitr*o permeation flux for the Pr gel, Pr-PC complex gel and Pr-PC vesicles gel was measured 4.95 ± 0.25 µg/cm²/h, 9.39 ± 0.11 µg/cm²/h and 15.25 ± 0.22 µg/cm²/h respectively through skin. The *in vitro* permeation profileevaluated through flux of Pr-PC vesicles gel shows higher permeation rate than Pr and Pr-PC complex.

To study the antiaging activity of the prepared formulations,various biochemical parameters like level of MDA, CAT, GSH and hydroxyproline were determined. MDA level of normal mice was found to be 1.17 ± 0.09 µ moles/g of skin tissue, which was found to be increased up to 2.14 ± 0.15 µ moles/g of tissue after exposure to UV radiations. On treatment with plain Pr gel, it was decreased to 1.54 ± 0.09. On the application of Pr–PC complex and Pr-PC vesicles gel

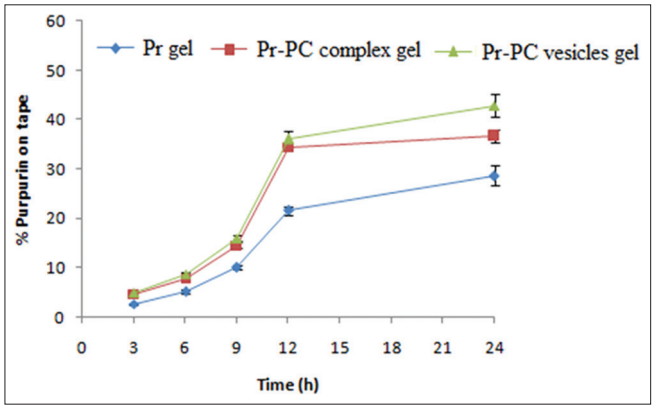

Figure 5: Purpurin recovered in tape stripe

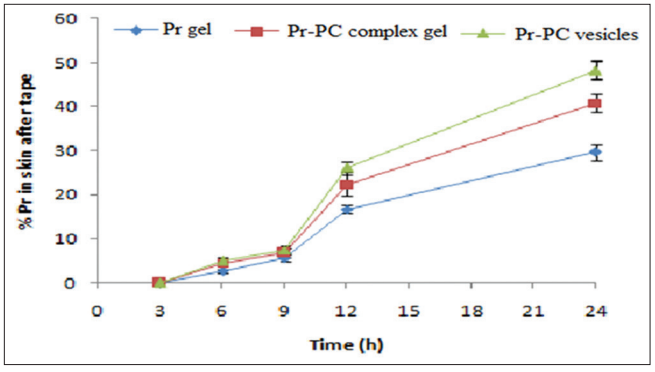

Figure 6: Purpurin recovered in the skin after tape stripe

formulations, the level of MDA was decreased significantly to 1.25 ± 0.07 µ moles/g and 1.09 ± 0.04 µ moles/g of tissues respectively. On the application of Pr-PC vesicles the level of MDA was found to be very less as compared to other formulations, hence vesicle formulations are found to be effective in terms of providing better protection from lipid peroxidation in the skin [Table 3].

The enzyme CAT plays a significant role in the protection of cells from any oxidative damage produced by reactive oxygen species (ROS). The level of CAT in normal mice was found to be 73.49 ± 3.04 U/g tissueswhich were reduced to 35.26 ± 1.65 U/g tissues after exposure to UV radiations. After topical application of gelformulations containing purePr, Pr- PC complex and Pr-PC vesicles, the level of CAT were found to be 63.44 ± 2.56, 79.89 ± 3.36 and 98.12 ± 4.38 U/g tissue, respectively. The high level of CAT i.e., 98.12 ± 4.38 U/g tissues in case of Pr-PC vesicles indicated that the prepared formulations may provide better protection from oxidative damage by ROS.

GSH having the antioxidant property which prevents the damage of cellular components caused due to ROS i.e., free radicals, heavy metals and peroxides. GSH levelin normal mice was found to be 51.23 ± 2.03 mg/g skin tissue, which was decreased to 14.56 ± 0.67 mg/g tissue after exposureto UV radiations which may be because of oxidative stress. On treatment with a gel containing Pr, Pr–PC complex and Pr-PC vesicles, the level of GSH was increased to 36.21 ± 1.72, 53.16 ± 2.16 and 59.39 ± 2.24 mg/g tissue, respectively. On the basis of results of GSH level, it can be concluded that the Pr-PC vsecicles gel were found to much efficient in restoring the level of GSH than other formulations.

The hydroxyproline concentration in normal mice was found to be 55.37 ± 2.32 mg/g. After exposure to UV radiations the level was reduced to 26.14 ± 1.26 mg/g of tissue. Application of gel formulations containing plain Pr was found to be less effective, as the hydroxyproline level was found to be 50.81 ± 2.02 mg/g of tissue. The treatment of skin with a gel formulation containing Pr-PC complex restored the hydroxyproline level to 63.74 ± 2.64 mg/g of tissue, which is much higher than the normal mice. In case of gel formulations containing Pr-PC vesicles,the level of hydroxyproline was found to be 71.88 ± 3.12 mg/g, which was highly significant than control group animals. Thus the results of this study clearly indicate that the Pr-PC vesicles gel formulation restored the hydroxyproline content in mice skin more effectively than others.

The increased effectiveness of Pr-PC complex and Pr-PC vesicles may be due to their amphiphilic nature, which

Table 2: Characterization of vesicular systems[a]

Vesicular system	Vesicles in an mm³	Average vesicle size (nm)	Polydispersity index	% Encapsulation efficiency
Pr-PC vesicles	35	345±2.8	0.16±0.06	72±1.8

[a]Values are Mean±SD, *n*=3 in each group. Pr-PC: Purpurin-phosphatidyl choline

Table 3: Effects of various formulations on, MDA, CAT, GSH and hydroxyproline contents in UV treated mice[a]

Groups	MDA µ moles/g tissues	CAT U/g of tissues	GSH mg/g of tissues	Hydroxy proline content mg/g of tissues
Control group (without UV and or formulation treated)	1.17±0.09	73.49±3.04	51.23±2.03	59.32±2.16
UV Treated	2.14±0.15	35.26±1.65	14.56±0.67	26.14±1.26
Pr gel (UV treated)	1.54±0.09	63.44±2.56	36.21±1.72	50.81±2.02
Pr-PC gel (UV treated)	1.25±0.07	79.89±3.36	53.16±2.16	63.74±2.64
PrP gel (UV treated)	1.09±0.04	98.12±4.38	59.39±2.24	71.88±3.12

[a]Values are Mean±SD, *n*=3 in each group, Pr-PC: Purpurin-phosphatidyl choline, UV: Ultraviolet, MDA: Malondialdehyde, CAT: Catalase, GSH: Glutathione

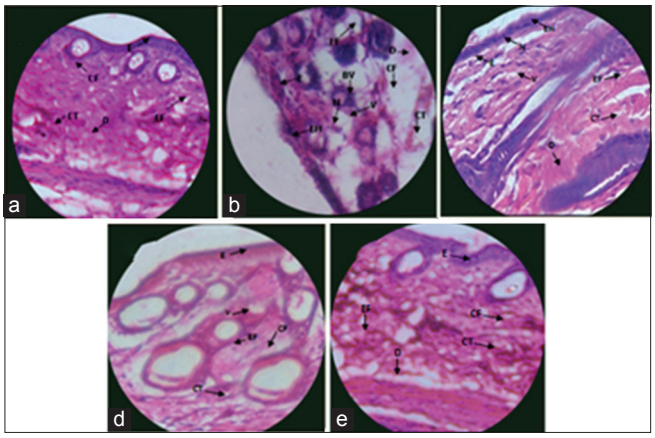

Figure 7: Microphotograph of mice skin (a) Control (b) ultraviolet (UV) treated (c) Purpurin (Pr) (d) Pr-Phosphatidyl choline complex (e) Pr-Phosphatidyl vesicles treated groups after UV exposure (E - Epidermis, D - Dermis, EF - Elastin fiber, EH - Epidermal hyperplasia, CF - Collagen fiber, CT - connective tissues, N - Neutrophils, BV - Blood vessels, V - Vacuoles)

enhances the water and lipid miscibility of the Pr. The Pr-PCvesicles were found to be better than Pr-PC complex. This is because of the reduced size of the Pr-PC complex when converted into vesicles.

Histological arrangement of epidermis, adipose tissues, collagen and elastin connective tissues werefound normal in the skin of the mice which wasnot exposed to UV radiations. Figure 7a and b shows the effect on mice skin after chronic UV exposure. It was found that after chronic exposure to UV radiations, the epidermal hyperplasia, infiltration of the skin layers with neutrofils and the destruction of theintegrity of the connective tissue was found. Severe elastosis, which was spreadonthe upper dermis and denser in deeper dermis layer, discontinuous and fibroblastic collagen bundles were also observed after chronic exposure of UV radiations. The application of gel formulations containing plain Pr provides very less protection against UV exposure and therefore destructive connective tissues, infiltered skin layers with neutrofils and damaged collagen bundles and elastin fibres were found [Figure 7c]. While the mice skin treated with gels formulations containing Pr–PC complex and Pr-PC vesicles [Figure 7d and e] shows prominent regenerative changes in the structural integrity and arrangement of connective tissues, which may be due to the prevention of premature elastic degeneration of collagen and elastin fibres.

CONCLUSION

On the basis of present study, it can be concluded that the Pr-PC vesicles are superior over Pr-PC complex and plain Pr, in terms of providing enhanced anti-aging properties. Thus, Pr-PC vesicles shown a tremendous potential in enhancing topical delivery of Pr. The enhanced topical delivery of Pr open new strategy to explore maximum therapeutic potential of plant substance and in future have extensive opportunity in cosmetology.

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