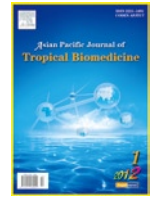




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## Document heading

# Preclinical studies of a novel polyherbal phyto–complex hair growth promoting cream

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## ABSTRACT

**Objective:** The present research is envisaged on the preclinical studies of a polyherbal phyto–complex hair growth promoting cream incorporated with aqueous extracts of *Trichosanthes cucumerina* (*T. cucumerina*) Linn and *Abrus precatorius* (*A. precatoius*) Linn on enhancing the hair growth. **Methods:** Leaves of *T. cucumerina* and *A. precatoius* were separately extracted with distilled water and then subjected to preliminary chemical test. It was then made into a phyto–complex and finally formulated into a hair cream. The various evaluation parameters like organoleptic characters, presence of foreign particles/grittiness, determination of pH, irritancy, partition coefficient, drug content, diffusion studies, stability studies, sterility test, SEM, FT–IR, solubility and hair growth promoting activity were performed. **Results:** Microscopic view of phyto–complex showed micelle structure. The formulated phyto–complex hair growth promoting cream had a smooth consistency with slightly acidic pH. FT–IR studies showed that the phytoconstituents were compatible with the excipients used. SEM studies revealed binding nature of the complex. The cream was found to be stable as the globule size, and was not altered on storage. Sterility testing proved the formulation to be free from microbial attack. The preclinical studies showed that the formulated 2% polyherbal phyto–complex hair growth promoting cream was an effective hair growth promoter as the results were analogous to that of minoxidil 2%. It was observed that percentage of hair follicles in the anagen phase increased considerably which predicts that the formulation can be used in alopecia. **Conclusions:** As the 2% polyherbal phyto–complex hair cream did not show any kind of irritancy or toxicity to the test animals it can be suggested that the prepared cream can be a better alternative for synthetic hair growth promoters.

## 1. Introduction

Hair growth is a common biological process observed in animals and human beings. Hair on scalp grows about 3–4 mm/day or 6 inches per year. The hair fiber is composed of a cuticle that is continuous with the root sheath, an intermediate cortex, and an inner medulla. A number of things like illness or a major surgery, hormonal problems, pregnancy, anticoagulants, medicines used for gout, high blood pressure or heart problems, excess of vitamin A, birth control pills and antidepressants, fungal infections may cause hair fall. Hair loss may occur as part of an underlying disease, such as lupus or diabetes. In terms of healthy hair growth, the most important vitamins and minerals

are iron (and the blood iron carrier ferritin), zinc, copper, selenium, biotin (vitamin H), vitamin B6 (pyridoxine), vitamin B12 (cobalamin)[1,2]. Over the past century, chemical and pharmacologic science established the compositions, biological activities and health giving benefits of numerous plant extracts. But often when individual components were separated from the whole there was loss of activity, the natural ingredient synergy became lost. Standardization was developed to solve this problem. As standardized extracts became established, poor bioavailability often limited their clinical utility. Then it was discovered that complexation with certain other clinically useful nutrients substantially improved the bioavailability of such extracts. The nutrients which are helpful in enhancing the absorption of other nutrients are the phospholipids[3].

Phyto–complex are lipid vesicles incorporated with standardized natural extracts or purified fractions complexed with phospholipids for a better bioavailability and enhanced activities. Their sizes vary between 50 nm to a few hundred  $\mu$ m. The phyto–complex process intensifies

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herbal compounds by improving absorption, increasing bioavailability and enhancing delivery to the tissues as a result produce better results than conventional herbal extracts. They are produced by the individual components of herbal extract by binding to phosphatidylcholine which is an emulsifying compound derived from soy. Phosphatidylcholine is also one of the chief components of the membranes in our cells[4–7].

*Tricosanthes cucumerina* Linn. commonly called as snake gourd is a monoecious annual climbing herb with branched tendrils. It is a rich source of nutrition and is highly constituted with proteins, fat, fibre, carbohydrates, vitamin A and E. The fruit is rich in vitamin C and E, cucurbitacin B, cucurbitacin E, isocucurbitacin B. Leaves of *Tricosanthes cucumerina* is used in the treatment of head ache, alopecia, fever, abdominal tumors, bilious, boils, acute colic, diarrhoea, haematuria and skin allergy[8–10].

*Abrus precatorius* Linn. commonly called as Rosary pea or Jequirity is a perennial climber, with alternately arranged pinnately compound leaves. Seeds are poisonous and contain abrin. It contains abruquinones B,G,D, E, and F, flavonol glycoside, triterpenoid saponins. Leaves, fruits and seeds are used in case of colic, constipation, coughs, venereal disease, cancer and abortifacient. In the Ayurvedic medicine leaves of *Abrus precatorius* are laxative, expectorant and aphrodisiac medicines. Seeds are said to be purgative, emetic, tonic, antiphlogistic, aphrodisiac and anti ophthalmic of epilepsy *Abrus precatorius* is used as a folk-medicine for the treatment of bronchitis, laryngitis, hair fall and hepatitis[9–12].

Hair loss is becoming a major and common problem worldwide and the synthetic hair growth formulations are reported to have lot of side effects. Hence in our current research we decided to investigate a novel phyto-complex hair cream by incorporating aqueous extracts of *Trichosanthes cucumerina* and *Abrus precatorius* leaves which will be able to overlap the side effects of synthetic hair promoters.

## 2. Materials and methods

### 2.1.Plant collection

The plants were collected from in the month of November to February from the surrounding areas of Nalgonda District, Andhra Pradesh, India. The plant material was identified and authenticated by Mr. A. Laxma Reddy Retd. Professor (Botany) Nagarjuna Government College (Affiliated To Osmania University) Nalgonda, Andhra Pradesh, India. The herbarium of the plant specimens were prepared and deposited in the Department of Pharmacognosy, Nalanda College of Pharmacy, Nalgonda, Andhra Pradesh, India, under the voucher No: NCOP–NLG/ph' cog/2009–10/001(*Abrus precatorius*), and NCOPNLG/ph' cog/ 2009–10/002(*Tricosanthes cucumerina*)

### 2.2.Experimental animals, chemicals and reagents

Wistar Albino Rats (150–200 g) of either sex were procured from National Institute of Nutrition, Hyderabad, Andhra Pradesh, India. The experimental protocol was initially approved from the Institutes animal ethics committee under the reference no. NCOP/IAEC/approval/07/2010 and then experimental studies were undertaken as per their rules and regulations. The animals were housed under standard environmental conditions and had free access to standard pellet diet (Goldmohar brand, Lipton India Ltd.) and water *ad libitum*.

Phosphatidylcholine (Lipoid, Germany), 2% Minoxidil (Dr. Reddy's Laboratory, Hyderabad, India), Veet hair removing cream. All the chemicals and reagents used were of analytical grade.

### 2.3.Extraction

Leaf powder weighing 200 g of both the plants were taken and extracted with water for 32 h at 45°C. The thick mass obtained was evaporated with help of rotary vacuum evaporator and then subjected to preliminary chemical tests which revealed the presence of flavonoids, saponins and carbohydrates for both the plant extracts.

### 2.4.Preparation of phyto-complex

About 2 g of aqueous extract of both the plants and phosphatidyl choline were placed in a 100 mL round-bottom flask and dissolved in 30mL of anhydrous ethanol. After ethanol was evaporated off under vacuum at 40°C, the dried residues were gathered and placed in desiccators overnight, then crushed in the mortar and sieved with a 100 mesh. The resultant aqueous extract-phospholipid complex was transferred into a glass bottle, flushed with nitrogen and stored in the room temperature. The complex was suspended in distilled water and a drop was placed on a slide and covered with a cover slip. Microscopic view of the complex was observed at a magnification of 100×[3].

### 2.5.Formulation of 2% polyherbal phyto-complex hair cream (PPHC)

Beeswax(15%), liquid paraffin(20%) and glyceryl monostearate(9%) were taken in one beaker and glycerol(4.5%), water(59%) and cetyl alcohol(15%) in another. Phyto-complex complex(2%) was dissolved in ethanol by sonication and maintained at 40°C. Both the beakers were maintained at 60°C and all the ingredients were melted. Then oily phase was added to aqueous phase along with methyl paraben(0.15%) and propyl paraben(0.15%) and stirred continuously. The phyto-complex was added to the mixture when the temperature dropped to 40°C. As the temperature went down rose oil(q.s) was added and mixed well until required consistency was obtained.

### 2.6.Evaluation of 2% PPHC [13–15]

#### 2.6.1.Organoleptic characters

By visual appearance, colour and odour were noted.

### 2.6.2. Presence of foreign particles/grittiness

A small amount of cream was taken and spread on a glass slide free from grease and was observed against diffused light to check for presence of foreign particles.

### 2.6.3. Determination of pH

The required quantity of cream was solubilized and pH was measured and reported.

### 2.6.4. Irritancy

Marked an area of 1sq.cm on the dorsal surface of the shaved rabbit. The cream was applied on the specified area and time was noted. Irritancy, erythema, edema were checked if any at regular intervals for 24 hrs and were reported.

### 2.6.5. Partition coefficient of cream

The partition coefficient of drug between phosphate buffer solution (pH 7.4) & *n*-hexane was determined at  $(37 \pm 0.2)^\circ\text{C}$ . An excess amount *i.e.*, 50 mg of cream was taken in a separating funnel containing 1:1 ratio of buffer 7.4 & hexane. It was placed on a water bath for 24 h. The solution was shaken occasionally. Then, both of them were separated & filtered through a  $2\ \mu$  filter & the amount solubilized in each phase was determined by measuring the absorbance using UV spectrophotometer. Hexane has polarity zero. Hence it is chosen for the study of partition coefficient.

### 2.6.6. Drug content

1 g of cream was taken and dissolved in methanol completely. The solution was kept aside intact for another half an hour to attain homogenous equilibrium. The solution was withdrawn and the absorbance was estimated UV spectrometrically at 403 nm. Concentration was calculated with the reference of the standard curve.

### 2.6.7. Diffusion studies

Diffusion of topical formulation was performed using biological membrane of pig ear and student diffusion cell. 1 g of cream was placed on membrane and placed in phosphate buffer. The amount of drug diffused into the buffer was calculated by using spectrophotometer at 403 nm.

### 2.6.8. Stability studies

a) Globule size: 1 mL of cream was diluted to 10 mL with glycerin. A few drops of this were transferred onto a glass slide and was focused in a microscope. By using eyepiece micrometer, the diameters of 200 particles were determined randomly.

b) Phase separation: The formulated cream was kept intact in a closed container at  $25\text{--}30^\circ\text{C}$  not exposed to light. Phase separation was observed carefully every 24 hrs for 30 days. Any change in phase separation was checked.

c) Moisture absorption studies: About 50 mg of cream was taken on a watch glass. A beaker was taken with full of water and was kept in a desiccator without adsorbents and allowed to get saturated. Watch glass with cream was introduced into the desiccator. It was left for 24 hrs.

d) Shelf life: The formulated product was stored in different temperature conditions like room temperature,  $45^\circ\text{C}$  and  $55^\circ\text{C}$  to accelerate degradation for 1 month. Samples were withdrawn periodically every week and observed for drug decomposition by taking the absorbance under UV spectrophotometer. From the concentrations, and the temperatures, the shelf life of the product can be estimated.

### 2.6.9. Sterility test

The polyherbal phyto-complex hair growth promoting cream was placed on nutrient agar media and incubated to check the sterility as the complex is made of aqueous extract and phospholipids.

### 2.6.10. Scanning electron microscopy (SEM)

To detect the surface morphology of the prepared polyherbal phyto-complex hair growth promoting cream and phospholipids SEM was performed at various magnifications  $750\times$ ,  $1\ 000\times$ ,  $2\ 000\times$ .

### 2.6.11. Fourier transform infrared spectroscopy (FT-IR)

FTIR spectra for phospholipid, aqueous extracts and PPHC were obtained in the transmission mode with the wave number region  $500\text{--}4\ 000\ \text{cm}^{-1}$ . KBr pellets were prepared by gently mixing 1 mg sample powder with 100 mg KBr.

### 2.6.12. Solubility

To determine the change in solubility due to complexation, solubility of drug and the complex was determined in buffer/water and *n*-hexane by shake flask method. 50 mg of drug was taken in a 100 mL conical flask. 50 mL of distilled water was added and then stirred for 15 min. The suspension was then transferred to 250 mL separating funnel with 50 mL of *n*-hexane and was shaken well for 2 h. Then the separating funnel was allowed to stand for about 30 min. Concentration of the drug was determined from the aqueous layer spectrophotometrically.

### 2.6.13. Evaluation of hair growth promoting activity of the polyherbal phyto-complex hair growth promoting cream<sup>[16–23]</sup>

Wistar albino rats of either sex weighing 150–250 g were taken. They were maintained in condition below room temperature ( $21 \pm 0.5^\circ\text{C}$ ). They were caged and provided with food and water *ad libitum*. The hair on dorsal portion was clipped with scissors and removed with cream in an area of 3 cm diameter. The animals were divided into 3 groups with 6 animals in each group. Group I was applied with water (control), Group II was applied with reference drug 2% minoxidil (standard) and Group III was applied with polyherbal phyto-complex hair growth promoting cream (PPHC) (Test). The drug was applied twice daily for 30 days on denuded area. The animals were observed for hair growth.

Time taken to initiate hair growth and time taken to complete hair growth was observed and reported.

The hair follicles were observed for the respective stage of hair growth. Anagen and telogen phases were considered and the percentages of follicles in both stages were reported. Average length of hair was calculated using 25 hairs. The length of hair was noted for every ten days. The completion

of hair growth was considered based on the average length of hair.

The hair growth in the denuded area was observed and the time taken to cover the bald patch was noted.

The animals were anaesthetized and blood samples were collected and were diagnosed for concentration of iron, zinc, total protein which aid hair growth.

### 3. Results

The consistency of the formulated 2% PPHC was smooth and no gritty nature was present. Color was cream to pale yellow and odor was rose fragrance. The pH of the cream was found to be 5.9 which denoted that the cream was slightly acidic in nature. The drug content in the formulation was found to be 95.33%. Partition coefficient of cream was determined by finding out the drug concentration in *n*-hexane and buffer and was found to be 74.37% and 8.75%, respectively.

**Table 1.**  
Diffusion studies.

| Time (hrs) | Amount of drug diffused (mg) | Percentage of drug release |
|------------|------------------------------|----------------------------|
| 0.5        | 0.414                        | 2.07                       |
| 1          | 3.315                        | 16.57                      |
| 1.5        | 4.414                        | 20.72                      |
| 2          | 4.835                        | 24.17                      |
| 2.5        | 5.250                        | 26.25                      |
| 3          | 6.078                        | 30.39                      |
| 3.5        | 7.184                        | 35.92                      |
| 4          | 7.875                        | 39.37                      |
| 4.5        | 8.289                        | 41.44                      |
| 5          | 9.118                        | 45.09                      |
| 5.5        | 9.809                        | 49.04                      |
| 6          | 10.638                       | 53.19                      |
| 6.5        | 11.881                       | 59.40                      |
| 7          | 12.848                       | 64.24                      |

A uniform % of drug release was observed till 7 hours. By the end above 64% of drug was released which shows the high diffusion rate.

**Table 2.**  
The drug retained (in mg) at different temperatures.

| Week | 55°C  | 45°C  | 27°C  |
|------|-------|-------|-------|
| 0    | 19.85 | 19.85 | 19.85 |
| 1    | 18.97 | 19.10 | 19.81 |
| 2    | 18.24 | 18.83 | 19.80 |
| 3    | 17.86 | 18.69 | 19.80 |
| 4    | 17.14 | 18.20 | 19.79 |

At 27°C the drug was retained at highest concentration when compared to other two temperature exposures. Hence storage at this temperature would be optimum as less deterioration is observed.

**Table 5.**  
Quantitative observation of 2% PPHC on hair growth.

| Treatment group | Dose | Percentage of hair |            |            |            |            |            |
|-----------------|------|--------------------|------------|------------|------------|------------|------------|
|                 |      | Day 10             |            | Day 20     |            | Day 30     |            |
|                 |      | Anagen             | Telogen    | Anagen     | Telogen    | Anagen     | Telogen    |
| Group I         | 2 mL | 30.00±1.27         | 70.00±1.37 | 51.00±1.37 | 49.00±1.55 | 55.00±0.75 | 45.00±0.52 |
| Group II        | 2%   | 48.00±0.75         | 52.00±0.82 | 68.00±0.63 | 32.00±1.51 | 69.00±0.75 | 31.00±1.23 |
| Group III       | 2%   | 37.00±2.04         | 63.00±1.03 | 64.00±0.84 | 41.00±1.51 | 68.00±0.84 | 36.00±0.82 |

The test formulation has exhibited a comparable result as that of standard formulation by the end of 20th and 30th day.

### 3.1. Microscopic view of complex

Once hydrated, the phyto-complex complex and phospholipids was seen under microscope as dull spherical vesicles shown in Figure 1 and Figure 2.

### 3.2. Diffusion studies

The amount of drug diffused for unit time was calculated using student diffusion cell and spectrophotometer (Table 1).

**Table 3.**  
Qualitative observation of hair growth.

| Treatment group | Dose | Average number of days taken for hair growth initiation | Average number of days taken for hair growth completion |
|-----------------|------|---|---|
| Group I         | 2 mL | 3.00±0.26   | 23.33±0.42  |
| Group II        | 2%   | 1.83±0.31*  | 16.00±0.26*   |
| Group III       | 2%   | 1.83±0.31*  | 17.00±0.45*   |

The difference between experimental groups was compared by One-way Analysis of Variance (ANOVA) followed by Dunnet Multiple comparison test (control *vs.* test). The differences were considered to be statistically significant when \**P*<0.05.

**Table 4.**  
Effect of 2% PPHC on hair length of albino rats.

| Treatment group | Dose | Length of hair (mm) |             |             |
|-----------------|------|---------------------|-------------|-------------|
|                 |      | Day 10              | Day 20      | Day 30      |
| Group I         | 2 mL | 7.83±0.05*          | 16.68±0.06* | 20.51±0.06* |
| Group II        | 2%   | 9.48±0.06*          | 18.97±0.04* | 22.45±0.02* |
| Group III       | 2%   | 9.40±0.03*          | 18.81±0.03* | 22.21±0.02* |

The difference between experimental groups was compared by One-way Analysis of Variance (ANOVA) followed by Dunnet Multiple comparison test (control *vs.* test). The differences were considered to be statistically significant when \**P*<0.05.

### 3.3. Stability studies

Globule size determination, phase separation and moisture absorption were conducted as part of stability study.

### 3.4. Globule size determination

Globule size was determined under microscope using eye piece micrometer and the dimensions are shown in Figure 3 and Figure 4. No phase separation was observed after 30 days and the cream appeared to be stable. The weights of the formulation before and after moisture absorption studies were almost same.

### 3.5. Shelf life



**Table 6.**

Effects of PPHC on hair growth.

| Treatment | Concentration | Diameter of shaved area (cm) |            |            |            |            |            |            |            |            |
|-----------|---------------|------------------------------|------------|------------|------------|------------|------------|------------|------------|------------|
|           |               | Day 0                        | Day 1      | Day 2      | Day 3      | Day 4      | Day 5      | Day 6      | Day 7      | Day 8      |
| Group I   | –             | 3.00±0.00                    | 3.00±0.00  | 3.00±0.00  | 3.00±0.00  | 2.70±0.10* | 2.10±0.10  | 1.60±0.14* | 1.00±0.00* | 0.60±0.16* |
| Group II  | 2%            | 3.00±0.00                    | 2.80±0.12* | 2.70±0.16* | 2.50±0.15* | 1.90±0.11* | 1.30±0.13* | 1.00±0.11  | 0.30±0.11* | 0.00±0.00  |
| Group III | 2%            | 3.00±0.00                    | 2.90±0.13* | 2.70±0.18* | 2.30±0.09* | 2.00±0.00* | 1.30±0.00* | 0.80±0.16* | 0.30±0.11* | 0.00±0.00  |

The difference between experimental groups was compared by One-way Analysis of Variance (ANOVA) followed by Dunnet Multiple comparison test (control vs. test). The differences were considered to be statistically significant when  $P < 0.05$ . The test formulation has shown a better activity than the standard from the day 6 onwards as the area covered was reasonably more.

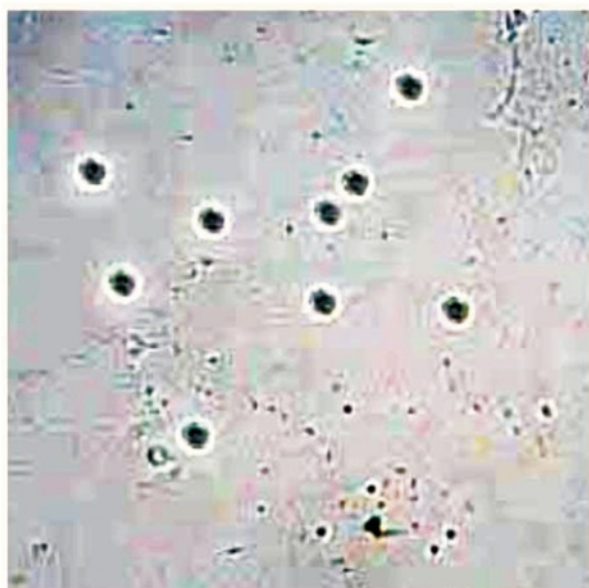
**Table 7.**

Effect of 2% PPHC on blood minerals.

| Treatment group | Concentration (mg/mL) | Minerals             | Mineral concentration in blood |        |        |
|-----------------|-----------------------|----------------------|--------------------------------|--------|--------|
|                 |                       |                      | Day 0                          | Day 15 | Day 30 |
| Group I         | –                     | Iron ( $\pm$ g/dL)   | 63.0                           | 63.5   | 63.9   |
| Group II        | –                     | Zinc ( $\pm$ mol/L)  | 84.4                           | 84.9   | 85.3   |
|                 |                       | Total protein (g/dL) | 6.2                            | 6.4    | 6.7    |
| Group I         | 2%                    | Iron ( $\pm$ g/dL)   | 58.1                           | 58.9   | 59.1   |
|                 |                       | Zinc ( $\pm$ mol/L)  | 84.3                           | 84.6   | 84.8   |
|                 |                       | Total protein (g/dL) | 6.0                            | 6.6    | 6.9    |
| Group II        | 2%                    | Iron ( $\pm$ g/dL)   | 62.9                           | 63.6   | 63.8   |
|                 |                       | Zinc ( $\pm$ mol/L)  | 83.3                           | 83.7   | 83.7   |
|                 |                       | Total protein (g/dL) | 6.0                            | 6.3    | 6.4    |

No much deviations in the mineral concentrations were observed in the test when compared to the control treated groups which shows that the formulated cream do not have any effect on altering the mineral content.

The formulated product was stored at different temperature conditions like room temperature, 45°C and 55°C to accelerate degradation rate for 1 month. Samples were withdrawn periodically every week and were observed for drug decomposition taking the absorbance under UV spectrophotometer. From the concentrations, and the temperatures, the shelf life of the product was estimated.

**Figure 1.** Microscopic view of phyto-complex.

From the above values, log% retained was calculated and a graph was plot between log% retained and time in hours. As it was straight line, it followed first order reaction. From the

graph the k values were calculated for 55°C and 45°C (Table 2). A graph is plotted between the 1/T values and log k values and the k value obtained by extrapolating the curve at 27°C is 0.006/months (Figure 5, Figure 6, Figure 7).

$$\text{First order reaction: } \log c = \frac{\log C_0 - k t}{2.303}$$

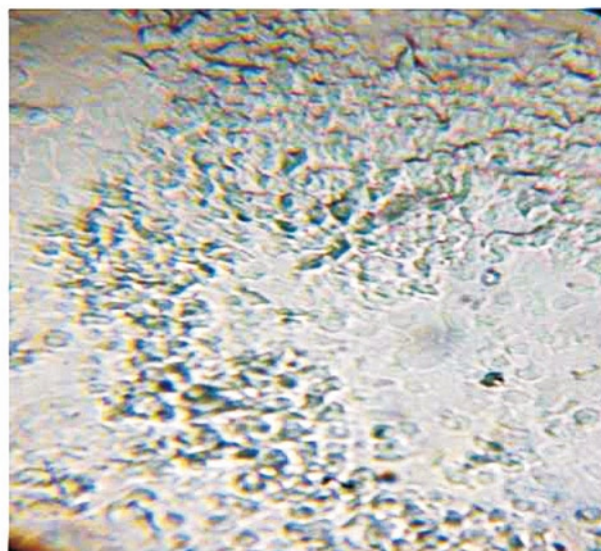
$$C = \text{potency at time } t.$$

$$C_0 = \text{potency at time zero.}$$

$$t_{90\%} = \frac{\log 100 - \log 90}{k} \times 2.303$$

$$= \frac{0.046}{0.006} \times 2.303$$

$$= 17.57 \text{ months.}$$

**Figure 2.** Microscopic view of phospholipids.

Hence the shelf life of the prepared drug dispersion complex emulsion system was found to be 17. 57 months if stored at 27°C.

3.8. SEM of 2%PPHC

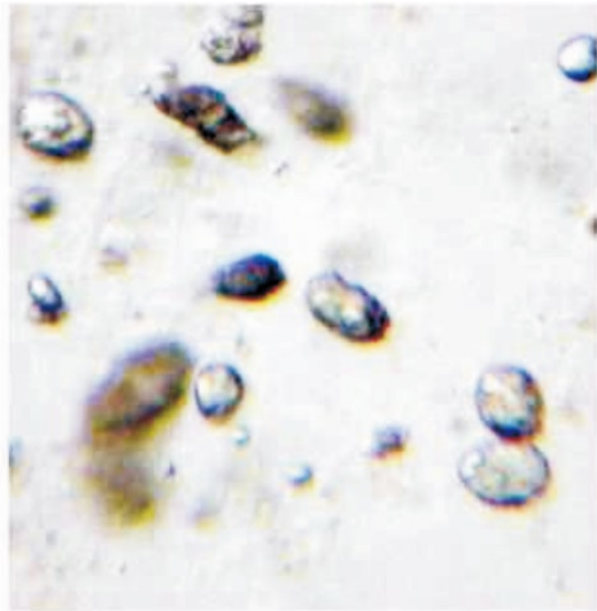


Figure 3. Globule size in 1st week 39.12 μ –67.80 μ .

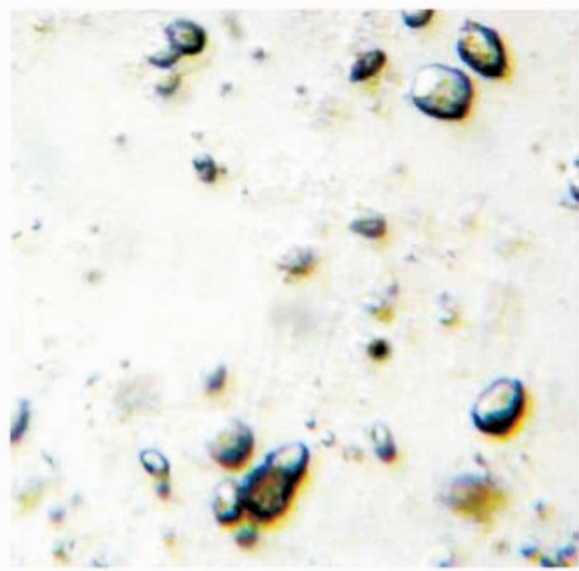


Figure 4. Globule size in 4th week 39.98 μ –69.41 μ .

3.6.Irritancy

There were no symptoms of irritancy, erythema and edema when applied on to skin of wistar albino rats. The irritancy was checked for 24 hrs at regular intervals.

3.7.Sterility test

The cream was found to be free of microbial attack (Figure 8).

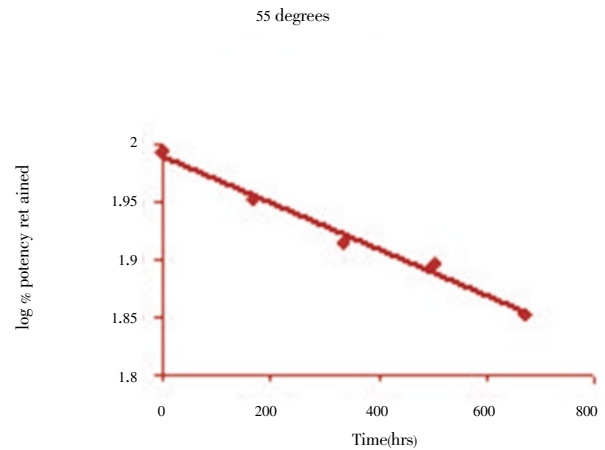


Figure 5. Graphical representation of log % retained at 55 h .

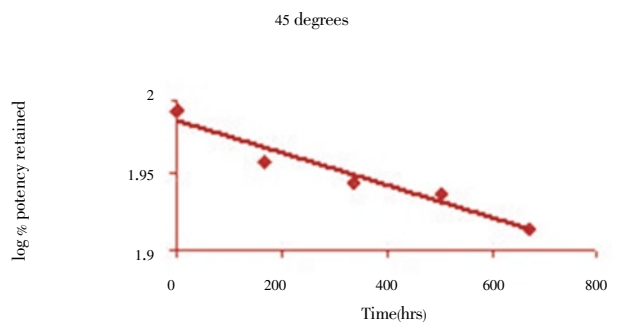


Figure 6. Graphical representation of log % retained at 45°C.

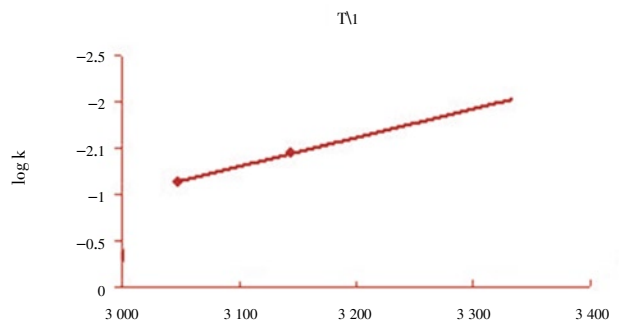


Figure 7. Graphical representation of k value.

The SEM of the cream showed good binding property (Figure 9, Figure 10).

3.9. FT-IR report

As no much changes in the peaks were observed in th FT-IR spectra of the 2% PPHC and excipients, it can be concluded that no drug excipient interactions were present (Figure 11, Figure 12).

3.10.Hair growth promoting activity

### 3.10.1. Effect of 2%PPHC on qualitative observation of hair growth

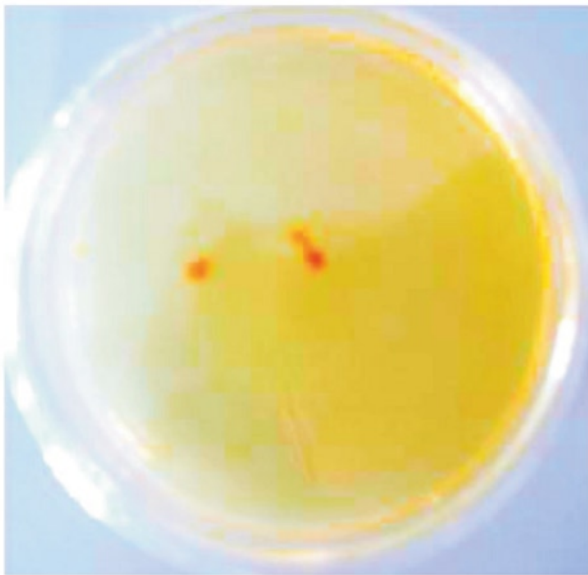


Figure 8. Sterility test.

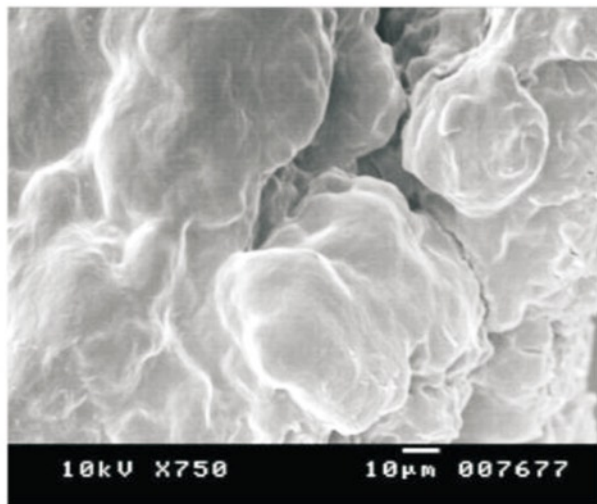


Figure 9. SEM of phyto-complex hair promoting cream.

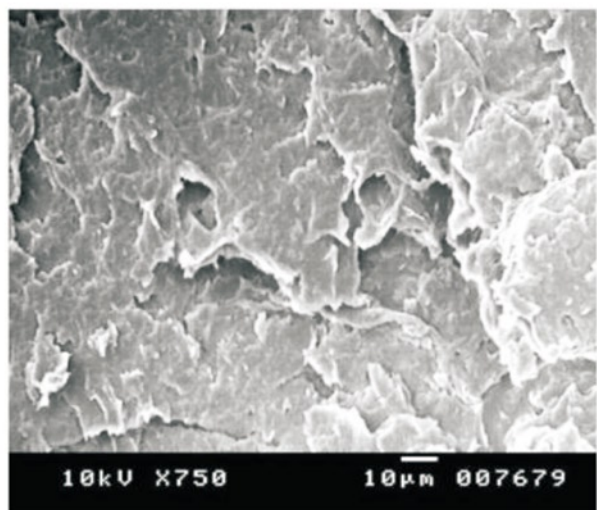


Figure 10. SEM of phospholipids in dry state.

The initiation of hair growth time reduced considerably for 2% PPHC treated group of animals and 2% standard minoxidil. The time taken for hair growth initiation was found to be same for both 2% PPHC and 2% minoxidil. The hair growth completion time for 2% PPHC treated group of animals was comparable to that of 2%minoxidil which was found to be  $17.00 \pm 0.45$  and  $16.00 \pm 0.26$  days, respectively. The control treated group of animals took  $23.33 \pm 0.42$  days for hair growth completion (Table 3).

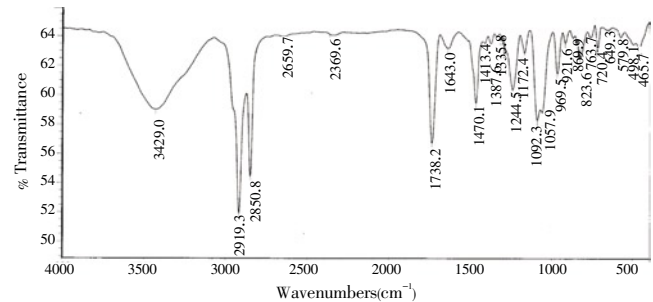


Figure 11. FT-IR of phosphatidyl choline.

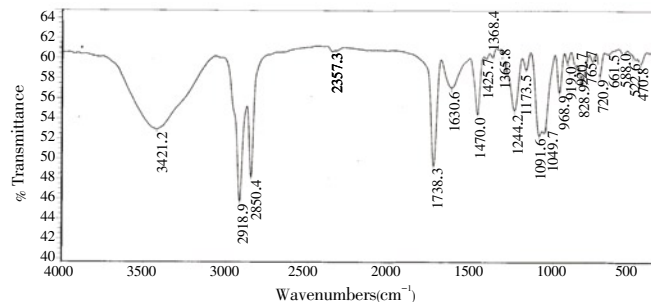


Figure 12. FT-IR of PPHC.

### 3.10.2. Effect of 2%PPHC on hair length of albino rats

Length of hair exhibited by 2% PPHC and 2% minoxidil treated group of animals were found to be almost same for the observations made on 10th, 20th and 30th days. The control treated group of animals showed comparatively less hair length on all these days (Table 4).

### 3.10.3. Quantitative observation of hair growth

The various phases of hair growth was examined to determine the efficiency of the formulation. It was observed that on the day 10 the percentage of telogen phase was found to be more than anagen phase for 2% PPHC, 2% minoxidil and control treated group of animals. But the entire scenario changed on 20th day of observation. A significant difference in cyclic phase of hair growth was observed for 2% PPHC and 2% minoxidil treated group of animals. A prominent increase in percentage of anagenic phase was observed where as percentage of hair follicles in telogenic phase reduced considerably on the 20th day and 30th days of observation. The control treated group of animals showed mild difference in the cyclic phases of hair follicles (Table 5).

### 3.10.4 Effects of PPHC on hair growth

The rate of hair growth on shaved area was determined and it was observed that by day 5 more than 50% of the bald patch of denuded area were covered by hair. On 8th day the



diameter of the shaved area turned zero for 2% PPHC and 2% minoxidil treated group of animals. The control treated group of animals took almost 13 days to cover the bald patch (Table 6).

### 3.10.5 Change in concentration of minerals

The content of minerals like iron, zinc and total proteins were determined and no substantial change in these constituents were observed which shows that 2% PPHC does not alter the mineral contents in the blood. The concentration of mineral levels in blood observed on day 0, 15 and 30 were found to be almost same (Table 7).

## 4. Discussion

As discussed earlier in this article *Trichosanthes cucumerina* and *Abrus precatorius* are well known plants used in the treatment of hair fall by the folklore. But since the active pharmaceutical ingredient (API) is predominantly hydrophilic and skin being lipoprotein in nature, specialized carrier systems gain significance in transporting the drug across the stratum corneum and into the dermal layers of the skin where the roots of follicular appendages are embedded. Taking advantage of this situation we proposed the role of lipid vesicular structures incorporated with the polyherbal ingredients, thus termed as phyto-complex. The preliminary chemical screening revealed the presence of flavonoids and saponins as major phytochemicals in the aqueous extracts of both the plants. It is hypothetically expected that presence of saponins make the vesicular walls more flexible rendering a smooth vesicular transport through the pores (paracellular) by which targeting the deep dermis. It is important at this point to understand how the vesicles are formed.

Molecules greater than 500 Da normally do not cross the skin. This prevents epicutaneous delivery of the high molecular weight therapeutics as well as non-invasive trans-cutaneous immunization. Molecules on the surface feels curvature when the size is small and molecules experience stress. The stress gets significant role when the size is below 100 nm. For bilayer formation, the molecules must be amphiphilic. Typical geometry for a vesicle formation is,

$$P = V / a_0 I_c \dots\dots\dots (1)$$

Where P = critical packing parameter; I<sub>c</sub> = hydrophilic chain length; V = volume of hydrophilic part; a<sub>0</sub> = optimum surface area/molecule at interface.

The API of these polyherbal extracts lend themselves quite well for the direct binding to phosphatidylcholine. Specifically, the choline head of the phosphatidylcholine molecule binds to these compounds while the fat-soluble phosphatidyl portion comprising the body and tail then envelopes the choline-bound material. The result is a little micro sphere or cell is produced. Here phytoconstituent is an integral part of the vesicle wall, rather than lodged in the core. The complex is obtained by reaction of stoichiometric amounts of phospholipid and the substrate in an appropriate

solvent. By spectroscopic data it has shown that the main phospholipid-substrate interaction was due to the formation of hydrogen bonds between the polar head of phospholipids and the polar functionalities of the substrate. These phyto-phospholipid complexes are often freely soluble in aprotic solvents, moderately soluble in fats, insoluble in water and relatively unstable in alcohol. When treated with water, phyto-complexes assume a micellar shape forming liposome-like structures. The active in phyto-complexes is anchored to the polar head of phospholipids, becoming an integral part of the membrane. Phyto-complex was added at lower temperature to avoid degradation at elevated temperature.

pH 5.9 of the final formulation was matching with that of the skin assuring non irritancy. Further it was confirmed by skin irritation test. Smooth consistency of the formulation increases the patient compliance. The globule size was unaltered and no phase separation occurred suggesting the formulation storage temperature is further reduced to less than 10°C and shelf life can further be extended.

Diffusion studies were performed to check the permeation nature of the API. Pork skin was used as a semi permeable membrane because of the resemblance with human skin in terms of lipoprotein contents. By preparing phyto-complexes, the hydrophilic drug was able to penetrate through the stratum corneum and get into the deeper layers of the skin where the hair roots are available. The diffusion studies correlated with the hair growth activity confirm the findings.

A complex was formed by mixing aqueous extracts of plants with phospholipids in equal ratio. The microscopic view of the complex showed the presence of micelle (vesicles). The complex binding nature is clearly seen in the SEM pictures of pure phosphatidylcholine and that of the complex. The presence of extra peaks at 412, 470, 522, 588, 920, 1 368, 1 425, 1 630, 2 357  $\bar{u}$  in the graph of phyto-complex indicated presence of new functional groups. The presence of peaks at 1 050, 1 380  $\bar{u}$  indicated presence of primary alcohols, phenols. The change in peak intensity and the slope of peak indicated bonding of chemical entities.

The dissolution study and solubility studies showed the total amount of drug content in phyto-complex. The more amount of the drug in the buffer (64.24%) when compared with the membrane concentration (12.848%) indicates phyto-complexes are capable of penetrating the rigid stratum corneum.

The group of animals treated with 2% PPHC exhibited a pronounced and significant hair growth promoting activity as compared to 2% minoxidil. The time taken for hair growth initiation and completion in the denuded area of the albino rats was found reduced drastically for 2% PPHC, which shows its quick onset of action. The length of hair was doubled on the 20th day of observation which indicates that 2% formulation promotes the faster growth in length. The quality of the hair produced was found to be impressive, as it was found to be silky, shiny and soft texture. A substantial increase in percentage of hair follicles in the anagenic phase was observed on the 20th day for 2% PPHC and 2% minoxidil and considerable decrease in telogenic phase was observed. This shows that the formulated 2% PPHC has potent follicle stimulating property and can be very well



considered as an effective medicament for treatment of alopecia. As the diameter of the shaved area was covered by hair on 8th day of treatment by 2% PPHC and 2% minoxidil, it can be determined that the formulated cream is effective as that of standard drug. As 2% PPHC did not alter the mineral concentrations in the blood this formulation can be considered to be a safe one.

To conclude we found that the 2% PPHC was non irritant, non toxic, easily absorbable, with quick onset of action and potent hair growth promoting activity as that of standard 2% minoxidil. As most of the synthetic hair growth promoters are reported to have side effects we suggest that the formulated novel hair cream would be an effective alternative.

### Conflict of interest statement

We declare that we have no conflict of interest.

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