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Research Article

FORMULATION AND EVALUATION OF PIPERINE CREAM-A NEW HERBAL DIMENSIONAL APPROACH FOR VITILIGO PATIENTS

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ABSTRACT: Vitiligo also known as leukoderma is a pigmentation disorder in which melanocytes (the cells that make pigment) in the skin are destroyed. As a result, white patches appear on the skin on different parts of the body which affects even the psychology and social status of the patient. In recent years, it has been proved that Piperine, an alkaloid from black pepper has the repigmenting capacity. Use of Piperine in Vitiligo not only reduces UV Radiation but also prevents side effects. The present work is about the extraction of Piperine from Black Pepper and its evaluation followed by formulation and evaluation of cream.

Keywords: Vitiligo, Leukoderma, Pigmentation, Melanocytes, UV Radiation.

INTRODUCTION

Vitiligo is a pigmentation disorder in which melanocytes (the cells that make pigment) in the skin are destroyed.[1] As a result, white patches appear on the skin on different parts of the body. Similar patches also appear on both the mucous membranes (tissues that line the inside of the mouth and nose), and the retina (inner layer of the eyeball). The hair that grows on areas affected by Vitiligo sometimes turns white. Vitiligo is not a fatal disease, but it is chronic and progressive. The most important consequence of the disease is probably social and psychological, as people may feel devastated by their changed appearance. [2]

There are many treatments available for Vitiligo but has some side effects like Cushing's syndrome, Skin Cancer , GI disorders etc. Piperine is used as anti Vitiligo agent by reducing the effects of UV radiation and also in avoiding side effects.[3- 5]

Piperine is extracted and isolated from Black pepper by two different methods.[6, 7] They are soxhlation and reflux method in which highest %yield is obtained for soxhlation (89.432%).The following evaluation works has been carried out for Piperine .pH, Solubility, thin layer chromatography, X-Ray diffraction studies, IR spectral analysis, chemical tests, Melting point, Partition coefficient, Particle size and UV Spectral analysis

A 1%Piperine cream was prepared using bees wax as base and it's evaluation was carried out. Thin layer chromatography, Globule size, Partition coefficient, Scanning electron microscopic studies, pH, Moisture absorption studies, consistency, Irritancy test, Drug content uniformity, phase separation, Organoleptic characters etc

MATERIALS AND METHODS

A comparative extraction of Piperine and its recrystallization

The piperine was extracted by both Reflux method and Soxhlation method by using 95% ethanol as solvent. The solution was filtered and concentrated under vacuum in a water bath at 60°C. 50ml alcoholic potassium hydroxide was added to the concentrate and the solution was stirred continuously for 30min. The obtained solution was heated and water was added drop wise until yellow precipitate was formed. Water was added until no more precipitate appeared to form and this was allowed to settle overnight. Needles of Piperine were observed to be separated out. The solid was collected and washed with cold ether 2-3 times. It was recrystallized by using acetone. For this, dissolve solid in acetone and filter it to remove extraneous matter and keep the filtrate aside for 24hrs so that crystals of Piperine are formed. Yellow coloured rod shaped crystals were recrystallized after 24 hrs.

Analytical works on Piperine

UV spectral analysis

5µg/ml of the drug in ethanol was used for complete scan between 190-900nm and the maximum absorption was obtained at 344nm as shown in the fig.1 whereas the λ max for standard Piperine is 342nm.[8]

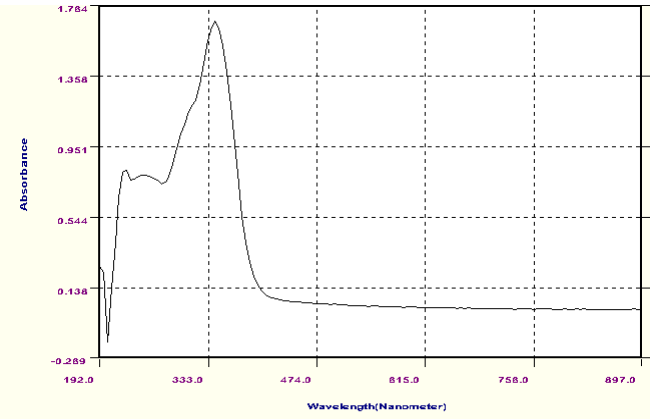

Fig. 1: Absorption maximum of Piperine

Partition coefficient of Piperine [9]

Procedure: The partition coefficient of drug between phosphate buffer solution (P^H 6.6) & n-hexane was determined at 37° ± 0.2°. An excess amount i.e. 50 mg of Piperine was taken in a separating funnel containing 1:1 ratio of buffer 6.6 & n-hexane & placed in a water bath for 24h. The solution was shaken at regular intervals. Then, both of them were separated & filtered through a 2 µ filter & the drug concentration in each phase was determined by measuring the absorbance using UV spectrophotometer at 344nm.

Particle size of Piperine using microscope

A small amount of powdered drug is placed on the slide & mounted using glycerin. By using eye piece micrometer, the diameters of 200 particles are determined randomly. Particle size distribution was expressed as histogram (Fig 2) [10-12].

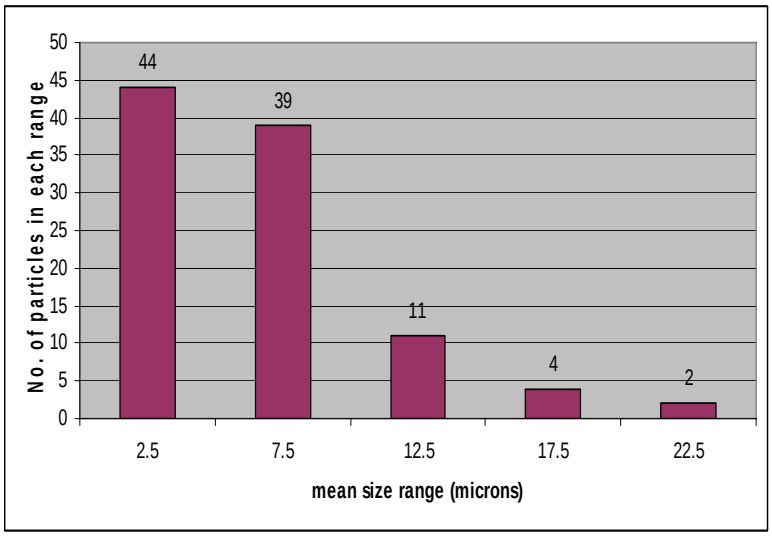

Fig. 2: Histogram of Particle size distribution of Piperine

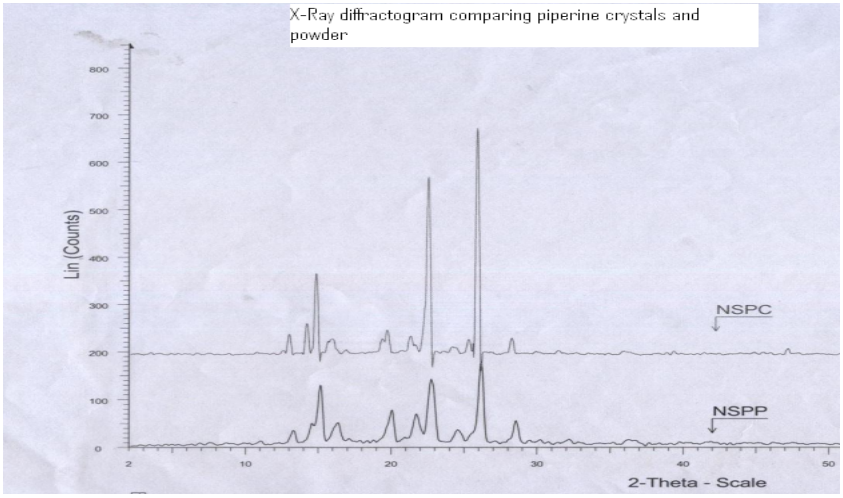

Fig. 3: X-ray Diffractograms of Piperine crystals and powder

Solubility of Piperine was determined in water, ethyl alcohol, chloroform and Acetone. Temperature was maintained at 37±0.2[13]. Melting point and TLC studies of piperine were also conducted. The chromatogram was observed under UV lamp (365nm). The alkaloid shows violet colored zone. R_f value was found to be 0.26 (value of standard piperine~0.25)[14]

X-Ray diffraction studies

Powdered X-Ray diffraction studies of Piperine crystals and powdered Piperine by sonication were carried out to study crystalline nature of the drug .D8 Advanced Bruker AXS, instrument was used for this purpose .Type of radiation used was copper radiation. The diffractograms are shown in fig 3. It shows that the crystallinity reduces for Piperine powdered using ultrasonicator i.e., solubility has increased.

Determination of pH [11, 13]

A small quantity of Piperine was dissolved in ethanol and it's pH was checked by using pH meter and it is found to be 7.9.

Chemical tests

10 mg of Piperine crystals were dissolved in 10ml ethanol and this solution is used as sample for chemical tests[15, 16].

Test	Observation
Dragendroff's test	Orange brown ppt is formed
Mayer's test	Cream coloured ppt was observed
Hager's test	Yellow ppt was observed
Wagner's test	Reddish brown ppt was formed

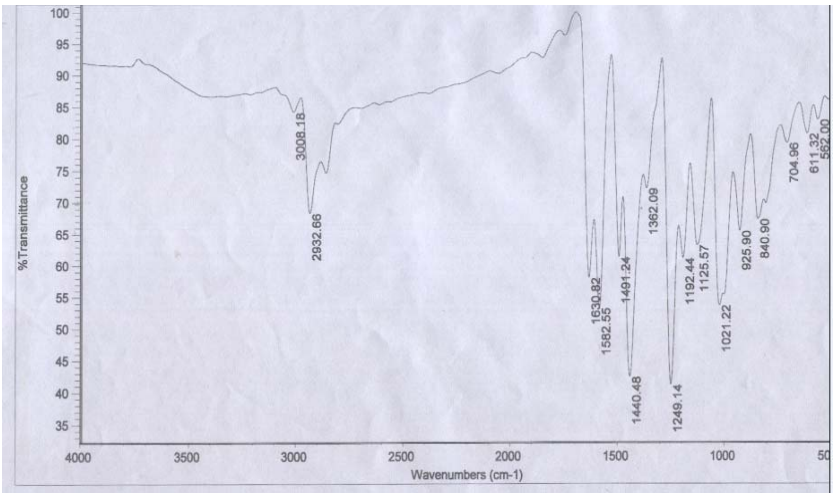

Fig. 4: IR Spectra of Piperine and its structure [16-20]

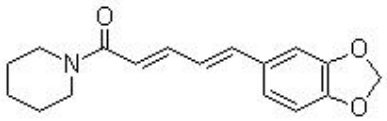

Table 1

3008.18cm-1	Alkenes	Present
2932.66cm-1	CH2-CH2 -CH3	Present
1630.82cm-1	Amines	Present
1582.55	Ketonic group	Present

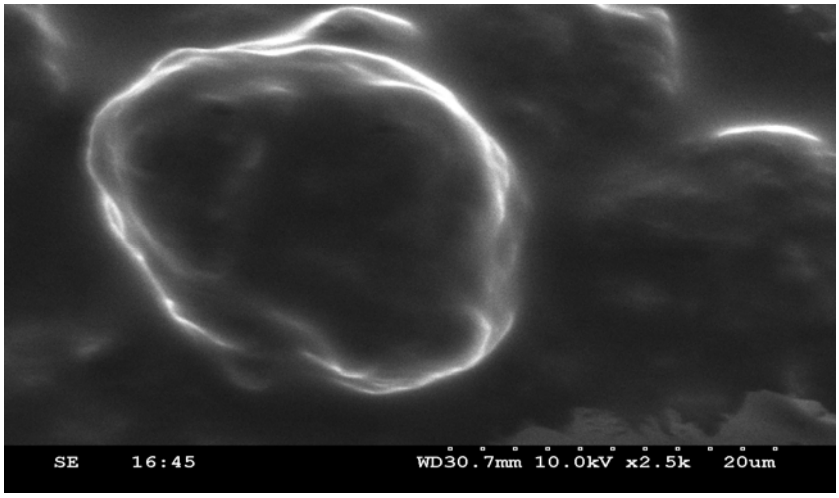

Fig. 5: SEM picture of cream diluted with glycerin

Formulation of cream [21, 22]

Procedure: Beeswax, Lanolin and Stearic were taken in one beaker. In another beaker, Piperine was dissolved in ethanol by sonication and introduced into glycerine, water, triethanolamine. Both the beakers were maintained at 60°C and all the ingredients were melted. Then oily phase is added to aqueous phase and stirred continuously. As the temperature goes down peppermint oil was added and mixed well until required consistency was obtained.

Evaluation of cream

Organoleptic characters were studied by visual appearance, colour and odour[23].

By using eyepiece micrometer, the diameters of 200 particles are determined randomly[10, 11, 12]. Topology studies were carried out for cream by using scanning electron microscopy

Presence of foreign particles/grittiness was observed against diffused light to check for foreign particles[13]. Drug content uniformity of the cream was also carried out[24,25].

Partition coefficient of cream

The partition coefficient of drug between phosphate buffer solution (P^H 6.6) & n-hexane was determined at $37^o \pm 0.2^o$. An excess amount i.e. 50 mg of cream was taken in a separating funnel containing 1:1 ratio of buffer 6.6 & hexane & placed in a water bath for 24h. The solution was shaken occasionally. Then, both of them were separated & filtered through a 2 µ filter & the amount solubilized in each phase was determined by measuring the absorbance using UV spectrophotometer at 344nm[9].

The formulated cream was kept intact in a closed container at 25-30°C not exposed to light. Any change in phase separation was checked every 24 hrs for one month.

Irritancy test[26]

Mark an area (1sq.cm) on the left hand dorsal surface. The cream was applied to the specified area and time was noted. Irritancy, erythma, edema, was checked if any for regular intervals up to 24 hrs and reported.

Rheological studies [27]

The formulated cream was found to be non - newtonian. Take a fixed quantity 10gms of cream in a 10ml beaker. Keep it impact for 1 hr. The beaker was inclined to one side see whether the cream is liquefied or not. beaker is shaken to and fro for continuous 5mns and checked whether consistency has changed or not. The beaker was again tilted and checked for pourability of the cream. The formulation showed no thixotropic (shear thinning) characteristics.

Diffusion studies of Piperine incorporated cream using goat's skin as semi-permeable membrane

Fresh goat's skin was shaved well to remove all the hair and cleaned thoroughly in water and rinsed in isotonic solution and later with 6.6 buffer solution. Fresh skin is used for the study. A student diffusion cell was fabricated and the goat's skin was used as semi permeable membrane. A 200mg of 1% Piperine cream was placed on the outer layer of the skin which was fixed as to face unit I from inside.

At predetermined intervals, samples 2ml from recipient chamber were withdrawn and transferred to amber coloured ampoules. The samples were suitably replaced. The samples were estimated for drug content, analyzed at maximum absorbance 344nm using UV-Spectrophotometer (Elico SL 196).The above work was repeated 3 times and average was calculated .From this, concentration of cream in buffer can be obtained.

After the completion of diffusion studies the skin was taken and dissolved in ethanol and left for 24hrs.Then the absorbance was calculated at 344nm.From this, concentration of cream in skin layer was calculated.

RESULTS AND DISCUSSION

Black pepper which was obtained from the local source was subjected to standardization and the results were found to comply with the standard values. Piperine was extracted from black pepper by using Soxhlet and Reflux method and the % yield was found to be maximum in Soxhlet method i.e., 89.632% whereas with reflux method it is 85%.Yellow coloured rod shaped crystals were found. Melting point determination of Piperine was performed thrice by melting point apparatus and the average was found to be 129°C which was compared with the standard value 130 °C. The alkaloid shows violet coloured zone under UV Radiation in TLC studies. The R_f value of test (extracted Piperine) is found to be 0.26 which corresponds to the standard value of Piperine (0.25) and only one spot was obtained indicating that it is pure. From UV Spectral analysis absorption maximum was found to be 344nm which almost matched with the standard. Infra red spectra of Piperine were compared with structure and the corresponding bonds were found to present.

Test for alkaloids was done by Dragendroff's test, Hager's test, Wagner's test and Mayer's test and it has given positive reaction for all the reagents confirming the presence of alkaloids. Assay of Piperine was performed and % purity of Piperine extract was 98.67%. Solubility of piperine was found to be in the order-chloroform>ethanol>Acetone but insoluble in water.

X-Ray diffractograms revealed peak heights was reduced in powder after sonication indicating that crystallinity has reduced by ultrasonication and solubility has increased.

Piperine was formulated into % cream and was subjected to various evaluation works Thin layer chromatographic studies were performed. Clear single spots were obtained and R_f value of Piperine and the formulated cream were found to be correlating with each other. Melting point and R_f values of test(cream) and standard(Piperine) were comparable which indicates there is no change in the physical and chemical nature of the Piperine.This also reflects that the drug is compatable with other excepients like bees wax, lanolin etc. Arithmetic mean particle size of Piperine and globule size was found to 21.6 µ and 28.67µ respectively. Minimum globule size and maximum globule size of cream was found to be 4.72µ and 52.72µ respectively.

Expectation of the topical formulation was that the drug should penetrate the stratum cornium, get into the dermis but should not get into the systemic circulation. The % of Piperine for cream retained in the skin, the site of action was found to be was found to be 69.06% drug released into the buffer was found to 18.62%. it could be postulated that in addition to transcellular permeation, paracellular permeation also is significant through tight junctions. The final preparation was found to be smooth texture and consistency and free from gritty nature with light yellow colour and peppermint odour. The globules retained its size and no coalescence was found. Accelerated stability studies were done which shows no significant change in the concentration of drug which shows stability of the formulation. Moisture absorption studies showed no significant absorption of moisture. pH of the cream was found to be 6.5. Topology studies by SEM revealed almost smooth globules. In addition irritancy test was also performed on rabbits and found no redness, edema, Inflammation and irritation.

CONCLUSION

The formulation of Piperine cream intended for Vitiligo was successfully done and evaluated. Drug targeting at the skin were the melanocytic proliferation is intended was achieved 69.06%. The topical formulation was physically stable throughout the shelf life. Further novel drug delivery formulations are highly recommended to increase the percentage drug targeting.

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