PSYCHOLOGY AND EDUCATION (2020) 57(4): 522-532
ISSN: 1553-6939

Indian Medicinal Plants on Pro Inflammatory CytokineIL17, IL22, IFNΓ, Lipooxygenase and Hacat Keratinocytes for the Management of Psoriasis

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Abstract: Cubosomes are biocompatible drug delivery system and is a novel approach. The controlled release application of these nanoparticles is of a great significance in cosmeceutical and pharmaceutical fields. Cubosome have become an attractive vehicle for in-vivo drug delivery due to their low cost, versatility and potential for controlled release and fictionalization. Cubosome formulations have been revealed to be safe in brain targeted drug delivery.

Introduction

The cubosomes prepared through top-down approach are always observed to coexist with vesicles (dispersed nanoparticles of lamellar liquid crystalline phase) or vesicle-like structures. It is the most widely used in research area, where by bulk cubic phase is first produced and then dispersed by high energy processing in to cubosomes nanoparticles. Bulk cubicphase is resembling a clear rigid gel farmed by water swollen crossed linked polymer chains; whereas cubic phases are like liquid crystalline structure.

Mono-oleate and surfactant were molted in a water bath and drug is added to this solution. Then the mixture was added drop wise into water at 70°c under mechanical stirring at 1500 rpm for 1hr. Then the dispersion was centrifuge and sonicated after wards the dispersion were maintained in glass vials. Then the formulations were stored at refrigerator at 4°c.

Research work which remains to be done under the project:

- In vitro pharmacokinetic studies
- In vivo studies - Brain targeting studies

Literature Survey

According to the previous studies Psoriasis is a prevalent skin condition that affects many people. Though it is believed to affect 2% of the global population, this figure may range from 0.5 to 4% depending on factors such as location and ethnicity. Conditions favourable to psoriasis, such as those seen in the colder northern latitudes, are less frequent in the warmer tropical climates. Multiple investigations have shown that Caucasians are disproportionately impacted. Although studies find no significant difference in frequency between the sexes, other writers note a higher rate of occurrence in men. Psoriasis may appear at any age, from infancy to old age, while it is most common between the ages of 20 and 30, with another peak frequency between the ages of 50 and 60. Only approximately 10%

of people have the most severe manifestations, whereas 20%-30% have psoriatic arthritis. Seventy to eighty percent of those with psoriasis have mild cases that may be managed with topical treatments alone. Psoriasis incidence may be influenced by factors such as environment and sun exposure in addition to genetics and ethnicity.
In contrast, a recent research by Jacobson et al found only a modest association between latitude and psoriasis incidence. Thus, it seems that additional variables, or more likely combinations of ones, may be at play.

Those who suffer from psoriases are more likely to have a variety of secondary health problems. These include psoriatic arthritis, anxiety, depression, lymphoma, metabolic syndrome, cardiovascular disease, and Crohn's disease.

To trigger psoriasis, all it takes is a little injury, some sun exposure, or contact with an inflammatory chemical. Medication such as NSAIDs, beta-blockers, lithium, and antimalarials may all make the condition worse.

Secondary metabolites found in plants have played a significant role in the search for novel possible psoriasis treatments. Half of psoriasis patients in the United States and Europe utilise some kind of complementary or alternative therapy.

Analyzing the literature makes it clear that there is a growing interest in the use of medications derived from plants in the treatment of psoriasis. The purpose of this article is to describe the most current research on plant extracts and pure chemicals for the treatment of psoriasis and to analyse the mechanisms of action that are associated with each of these treatments. According to what has been documented, there are numerous instances in which it is noticed a stronger activity of plant extracts and/or pure ingredients in contrast to conventional medications such as corticosteroids. Aloe vera, Boswellia serrata, Curcuma longa, Hypericum perforatum, Indigo naturalis, Mahonia aquifolium, and Viola tricolour as well as their primary active constituents boswellic acids, curcumin, hypericin, hyperforin, indirubin, and berberine are thought to be the most promising agents for the future management of psoriasis.

Traditional treatments for mild condition often include the use of topical medicines such glucocorticosteroids and vitamin D derivatives, or a combination of the two. Tactrolimus and pimecrolimus are two topical calcineurin inhibitors used to treat the intertriginous regions and the face, respectively, both of which are notoriously difficult to treat. Combining corticosteroids and vitamin D3 has been shown to be the most effective therapy for the scalp, as shown by Mason et al. However, these topical medications are seldom used because of their inconvenient nature, lengthy treatment times, and unwanted side effects. Treatment for moderate to severe psoriasis often consists of both phototherapy and systemic therapy for the best results. The use of systemic medicines such as acitretin, ciclosporin, and methotrexate has been authorised for the treatment of psoriasis. The use of apremilast has been sanctioned in both the US and EU. UVA light therapy and the drug psoralen are used together in a treatment modality known as photochemotherapy or PUVA (Psoralen Plus Ultraviolet Light Therapy). Unfortunately, PUVA's carcinogenic properties prevent its usage in the long-term.

Psoriasis has been treated with a number of different biologics in recent years, including

TNF- inhibitors (adalimumab, etanercept, and infliximab) and inhibitors of interleukin 12 and 23 (ustekinumab). There is no evidence of cumulative toxicity or safety concerns with the use of biologics. The use of TNF- inhibitors often follows phototherapy and the failure, intolerance, or incompatibility of standard systemic medications. Price is a factor in this case.

Objective of the project

The objectives of this research were:

1. To design and develop cubosomes containing fractional extracts of Convolvulus pluricalusby optimizing various process like Manufacturing and Process variable by using Design expert Software4.0
2. To enhance the bioavailability of the extract by designing into cubosomes
3. To evaluate the prepared cubosomes by
    - Measurement of size and zeta potential of formulation by Malvern Zetasizer.
    - Establishment of HPLC method
    - Assessment of stability of cubosomes
    - Characterization of cubosomes by differential scanning calorimetry (DSC).
    - Characterization ofcubosomes by X-ray diffraction analysis.
    - Characterization of cubosomes by transmission electron microscopy (TEM) and Scanning electron microscopy (SEM).
    - To study the in vitro release characteristics of cubosomes
    - In-vitro pharmacokinetic study of cubosomes
    - In-vivo studiesof cubosomes formulation in wistar mice

Methodology

Plants Material:

Convolvulus pluricaulis (shankhapusphi) is one of the traditional ethanomedicines used in Ayurvedic medicine in India as a controversial source of shankhpushpi for various brain related disorders. The whole plant is used for extraction. The whole plant is dried for few days and grinded using grinding mill. Then the powder is finely sieved for extraction.

The whole herb is used medicinally in the form of decoction with cumin and milk in fever, nervous debility, loss of memory, also in syphilis, and scrofula. Shankhapushpi is used as a brain tonic, febrifuge, in bowel complaints especially dysentery. The plant is reported to be a prominent memory improving drug. It is used as a psycho stimulant and tranquilizer.

Step 1: Extraction Process:

Few grams of powder was taken and packed in the soxhelt apparatus and continuously extracted by ethanol at temperature (70°c) and solvent is removed by reduced distillation and then vaccum dried. The extract was stored.

Step 2: Fractional Extraction (Column Chromatography):

About 30 gm of the extract was loaded on top of activated silica gel which was packed into a glass column (size). The column was eluted with a certain solvent gradient at a flow rate of 1mL/min. The column was then washed with alcohol, Total of all fractions measuring about few ml each were collected and concentrated using rotary evaporator. Each fraction was weighed and stored at 250^{O}C.

Step 3: Isolation of Extraction:

A. Thin Layer Chromatography:

By using the different mobile phases to the extracts were subjected to TLC analysis usingsolvents of different polarity. The Rf values of the resolved components were determined.

B. High Performance Liquid Chromatography:

HPLC was done using mobile phase optimized was methanol: water: acetonitrile (40:45:15) with a flow rate 1 ml/min (detection; λ_{max} – 254 nm).

C. Phytochemical Constituents:

The preliminary phytochemical screening carried out on extracts of *convolvulus pluricaulis* revealed the presence of phytoconstituents such as alkaloids, glycosides, flavonoids, carbohydrates, proteins, sterols, gum and mucilage's compounds. Diverse pharmacological properties and structurally novel compounds have been found for the alkaloids, glycosides, and steroids in *convolvulus pluricaulis* suggesting that these compounds may be the major contributors for the traditional therapeutical effects of *convolvulus pluricaulis*. Other compounds such as proteins, gum and mucilage's have been also reported in *convolvulus pluricaulis*.

D. Chemical Test Of Convolvulus Pluricalulis:

PHYTOCHEMICALS	RESULTS
Test for alkaloids	+(Presence of compound)
Test for glycosides	+(Presence of compound)
Test for carbohydrates	+(Presence of compound)
Test for saponins	-(Absence of compound)
Test for fats and oils	-(Absence of compound)
Test for flavonoids	+(Presence of compound)
Test for tannins and phenolic compounds	-(Absence of compound)
Test for proteins	+(Presence of compound)
Test for gums and mucilage	+(Presence of compound)
Test for steroids	+(Presence of compound)

+ (Presence of compound) ; - (absence of compound)

Step 4: Preparation of cubosomes:

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. Then the dispersion was centrifuge and sonicated after wards the dispersion were maintained in glass vials. Then the formulations were stored at refrigerator at 4°c.

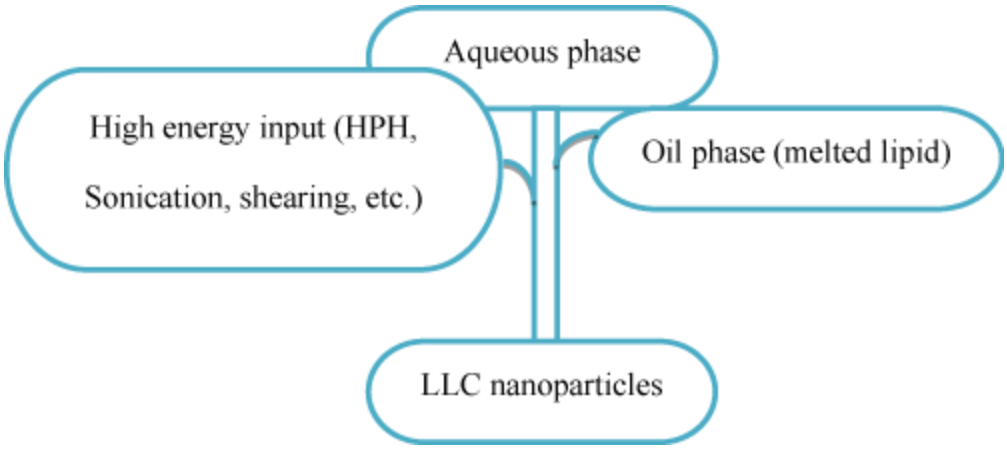

Figure 1. preparation of cubosomes by top-up method

Results and Discussions

Step 5: Evaluation of cubosomes

The cuboidal dispersions can be evaluated by following parameters,

Thermal Analysis:

Polarizing Light Microscopy(SEM)

Transmission Electron Microscopy

X-ray Diffraction Measurements

Drug Content of Dispersions:

Particle Size and polydispersity index (PDI)

The measurement of the mean particle size of cubosomes was done using laser diffraction technique on a Zetasizer (Malvern Instruments Ltd. Malvern, UK). Before the measurement, 0.5 ml sample were diluted with deionized water to a volume of 30 ml to obtain a suitable scattering intensity, the size was measured at 25 ºC, each sample of particle size was determined in triplicate and the mean was considered.

HPLC Procedure:

Construction of HPLC calibration curve The HPLC system was consisted of LC020AB solvent delivery system, SIL-20AHT auto-sampler, CBM-20A communication bus, DGU20A3 vacuum degasser, RF20A fluorescence detector (excitation 320 nm; emission 380 nm), and CTO-20A column oven (all of them by Shimadzu Corporation, Japan). The mobile phase used was consisted of acetonitrile: water: acetic acid (30:70:5). Flow rate was set at 0.5mL/min.

Ofloxacin was used as an internal standard. Ofloxacin stock solution was prepared by dissolving 100 µg of Ofloxacin powder in 200 ml acetonitrile and then this solution was vortex mixed until complete dissolution, the concentration of this stock solution is 0.5 µg/ml. For preparation of stock solution 10 mg of extracted powder was dissolved in 200 ml methanol for a final concentration of 50 µg/ml Using methanol, Extracted stock solution was serial diluted to 10, 5, 2.5, 1, 0.5, 2.5, and 0.1µg/ml. from the dilutions, a calibration curve with different concentration ranges was constructed.

Determination of Zeta potential

The zeta potential ofconvolvulus pluricaulis loadedcubosomes was measured using

Zetasizer Nano (Malvern Instruments, UK). This instrument is laser based multiple angle particle electrophoresis analyzers. It measures the electrophoretic mobility and surface charge. To determine zeta potential, 0.5 ml of the cubosomal nanoparticle was diluted with deionized water to 30 ml; all measurements were done at room temperature (25º C) and repeated three times 18.

Entrapment efficiency %

The entrapment efficiency (EE%) of the drug was determined by centrifugation method. Briefly, 1 mL of the prepared cubosomes were added to 4 ml deionized water and centrifuged at 15000 rpm for 15 minutes till complete precipitation of the cubosomal nanoparticles. One mL of the clear supernatant was added to 4 ml methanol and vortexed for 5 minutes; 100µL sample of the vortexed solution was placed in clean glass tubes, 20 µL of 36% hydrochloric acid was added followed by 2 mL of acetonitrile. Solutions were vortex mixed for 1 min, then centrifuged for 20 min at 5000. After centrifugation, supernatant was transferred to clean glass tubes and evaporated to dryness then reconstituted in 200 µL mobile phase and placed in clean injection vials for HPLC analysis15.

E.E % = X 100 WinitialWfreeWinitial

Where, Winitial drug is the amount of initially used for cubosomes preparation and Wfree drug is the amount of the drug not incorporated in to cubosomes and remain in the supernatant.

Transmission Electron microscope examination

Morphological examination of cubosomes was carried out using a transmission electron microscope (Jeol Company, Japan) equipped with super twin lens. A droplet of cubosomes dispersion was placed on a 200 mesh carbon-coated copper grid, and the excess fluid was removed by an absorbent filter paper. The samples were stained with 1% sodium phosphotungstate solution and were viewed using magnification up to 1,000,000 xs.

Differential scanning calorimetry

This test aims to detect any possible change in the physical state of extract when got entrapped in the cubosomes. DSC was carried out for convolvulus pluricaulis loaded cubosomes, blank cubosomes, convolvulus pluricaulis powder, and GMO using a thermal analysis system (DSC-60, Shimadzu, Japan). The samples (5mg) were heated over a temperature range 30 ºC – 350 ºC at a constant rate of 10ºC/min in an aluminum pan under a nitrogen atmosphere. A similar empty pan was used as the reference.

Infrared Spectroscopy

IR spectra were obtained using Fourier Transformer Infra-Red (Shimadzu, Japan) for convolvulus pluricaulis loaded cubosomes, blank cubosomes. Samples were prepared in KBr discs (about 10 mg sample for 100 mg of dry KBr). The IR spectra were obtained in the spectral region 450–4000 cm-1.

Powder X-ray diffraction

X-ray diffraction patterns of both blank and convolvulus pluricaulisloadedcubosomes as well as pure convolvulus pluricaulis and GMO samples were obtained using the X-ray diffractometer. The samples of the prepared cubosomes were separated from fluids using filter paper and left to dry in air for 12 hours. Diffracotgrams were recorded using Cu as tube anode under the following conditions: voltage was 45 kV, the current was 30 mA, steps were 0.02 º of (º2Th.), and the counting rate was 0.5 s/step at room

temperature. Data were collected from 4 ºC to 40 ºC.

In vivo evaluation

In vivo evaluation of brain targeting potential of P80 coated carboplatin loaded PLGA nanoparticles The concentration of carboplatin (_g/g) in vital organs of wis-tar rats (brain, liver, kidney, spleen and lungs) after intraperitoneal injection of P80 coated NPs, surface unmodified NPs and carbo-platin solution is shown

Stability studies

In Stability studies the formulations were subjected to stability studies as per ICH guidelines and the prepared formulations exhibited a particle size increase from 143.7 nm to 149.9 nm at the end of 3rd month, which is very negligible, indicating that it is suitable for brain targeting.

Table 1: Ash values of *POLYHREBS*

Total ash (%)	Acid Insoluble ash (%)	Water soluble ash(%)
11.23±.115	1.126±0.34	13.340±1.45

Table 2. Moisture and fat content of polyherbs

Parameter	%
1. Moisture content	5.4
2. Fat content	2

Table 3. Biochemical composition of polyherbs

Parameter	Values
Carbohydrate mg/g	0.462±0.012mg/g
Protein mg/g	13.29±0.244 mg/g
Amino Acid mg/g	2.224±0.160 mg/g
Total free Phenols (mg/g)	14.3±0.126 mg/g
Tannins (mg/g)	12.1±0.27mg/g

Total Flavonoids	**91.09± 0.04mg of QEof extract in ethylacetate fraction** **66.08± 0. 33mg/g in methanolic fraction**
Total alkaloids	**66.01± 0.049 mg/g AE/mg of** **91.09± 0.04mg/g**

Table 4: Estimation of metals/Heavy metals in dried material of *Polyherbs*

S.No	Name of the metal/Heavy metal	Amount in mg
1.	**Sodium**	57.34
2.	**Potassium**	49.32
3.	**Phosphorous**	23.6
4.	**Magnesium**	0.89
5.	**Calcium**	67.7
6.	**Copper**	0.12
7.	**Selenium**	0.6
8.	**Zinc**	0.32
9.	**Lead**	Less than 1 PPM

Table 5: Rf values of standard amino acids and sample of *Polyhrebs* by *THIN LAYER CHROMATOGRAPHY*

S.No	Name of the Amino Acid	Rf value	S.No	Name of the Amino Acid	Rf value
1.	L- Proline	0.379	**14.**	L- Histidine	0.102
2.	L-Serine	0.214	**15.**	DL- 2-amino-N-butric acid	0.355

3.	DL-Nor leucine	0.695	**16.**	L.Glycine	0.193
4.	L.Ornithine	0.129	**17.**	L.Arginine	0.163
5.	DL.Threonine	0.238	**18.**	L- Tyrosine	0.373
6.	L-Cysteine	0.121	**19.**	Phenyl alanine	0.587
7.	L-Leucine	0.677	**20.**	Lysine	0.141
8.	DL-Valine	0.468	**21.**	Tryptophan	0.627
9.	3-3,4-dihydroxyphenyl alanine	0.280	**22.**	Methionine	0.156
10.	L.Hydroxy proline	0.218	**23.**	L- Cysteine	0.419
11.	DL- Isoleucine	0.618	**24.**	L – Aspartic acid	0.349
12.	L. Alanine	0.227	**25.**	SAMPLE	0.162,0.236 0.226,0.158 0.162
13.	L- Glutamic acid	0.160			

Table 6: Percentage yield of total extract of plant material

Parameter	**70% Ethanolic extract**
Colour of extract	Dark green
Consistency	Semisolid
Percentage yield (% w/w)	12.50

Flavonoids, alkaloids, terpenoids, tannins, amino acids, carbohydrates, and steroids have all been detected in early phytochemical analyses.

Cassia sophera and Mallotus philippiensis ethyl acetate and methanol fractions were subjected to a preliminary phytochemical screening. Results from a preliminary phytochemical examination showed the existence of many bioactive compounds, including alkaloids, proteins, glucosides, steroids, tannins, and flavonoids.

Conclusion

Cubosomes are a unique medication delivery technique because they are biocompatible. The cosmeceutical and pharmaceutical industries place a premium on the controlled release use of these nanoparticles. With their inexpensive price, adaptability, and possibility for controlled release and fictionalisation, cubosomes have emerged as a promising vehicle for in-vivo drug administration. The use of cubosome formulations for brain-directed medication delivery has been shown to be risk-free. When using a top-down strategy to make cubosomes, vesicles (dispersed nanoparticles of lamellar liquid crystalline phase) or vesicle-like structures are invariably found nearby. Most often, the cubosome nanoparticles are generated in bulk cubic phase and subsequently dispersed using high energy processing. Cubic phases are similar in structure to liquid crystals, whereas bulk cubicphases resemble a transparent, hard gel grown from water-swollen, cross-linked polymer chains.

Acknowledgement

The author Dr. S. Hemalatha thanks the University Grants Commission, Minor Research Project Scheme, Government of India, New Delhi (F.No. 4-4/2015-16 - MRP/UGC-SERO/P.No.2076, Link No.6794 dated 30.06.2017) for its financial support. The authors also thank the Management and Principal of Sri Venkateswara College of Pharmacy for providing facilities towards successful completion of this project.

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