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Formulation of liposome incorporated polyherbal gel for management of gout

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Abstract

Trigonella foenum-graecum, Zingiber officinale and *Piper nigrum* were extracted using ethanol and incorporated in to liposomes. These liposomes were formulated as gel formulation for topical application for management of gout pain. The extraction yield for TF, ZO and PN were found to be 28.7, 37.4 and 21.1% respectively respectively. The total phenolic content in TF, ZO and PN extracts were found to be 28.3 ± 1.301, 13.2 ± 1.196 and 9.8 ± 0.894 GAE mg/100g whereas it was found to be 38.6 ± 0.735, 51.4 ± 0.721 and 63.08 ± 0.823 GAE mg/100g for 1:1:1, 1:2:1 and 2:1:1 mixture ratio of TF, ZO and PN respectively. The particle size of the liposomes was found to decrease with an increase in sonication time but the sonication time did not affect the total phenolic content of the liposomes. The particle size of the liposome formulated using 5 min sonication time was considered optimum and was found to be 4.27 ± 0.033µm. The liposomes were stable at both the temperature conditions and the particle size of the liposomes did not change considerably over a period of three months. ELF3 loaded gel formulations were prepared with different ratio of ELF3 using carbopol 934. The pH of all the formulations was in the range of the pH of the skin (5.0-5.5) and they exhibited optimum viscosity. The pH, viscosity and spreadability of PHLG3 was 5.3, 1694 cp & 62 mm respectively.

Keywords: Liposome, polyherbal, carbopol, gout, pain, extract, phenolic

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Introduction

Gout is a painful condition usually involving the joints similar to arthritis caused due to high levels of uric acid in serum. Progressive or chronic gout can lead to bone destruction and deformity. Recent studies have revealed the advantages and benefits of using natural products in human health issues. Indeed, the use of plants, parts of plants and isolated phytochemicals for the prevention and treatment of various diseases have been practiced since ancient times. It is estimated that about 25% of the drugs prescribed worldwide ultimately originate from plants.

The use of herbal remedies carries along with it number of problems, like low solubility and associated limited absorption and bioavailability. Such biologically active compounds are also prone to *in vivo* hydrolysis, oxidation, and photolysis, urging the need for stabilization platforms (Ehrlich, 2011; Ayurveda, 2008). Several novel techniques like formulation of liquid crystal systems, polymeric and solid lipid nanoparticles, precursors systems for liquid crystals, liposomes, and microemulsions have been reported that have the capability to overcome such limitations (Oreagba et al., 2011). These drug delivery systems also improve compatibility, allowing substances with different physicochemical characters to be used within the same formulation. Some even make it possible to change the drug's obvious characters and hence its behavior in the biological environment (Kamnoj, 2000).

It is a well-known fact that many Indian herbs are capable of a wide range of medicinal effects. From the time immemorial, mainly based on the practical experiences, these medicinal practices were developed and followed.

Trigonella foenum-graecum has been known to contain flavonoids and the extracts are reported to possess potential against acute and chronic inflammatory conditions (Goyal et al., 2016).

Zingiber officinale has also been reported to produce xanthine oxidase inhibitory potential and can be utilized for the management of gout (Wikipedia, 2019).

Piper nigrum extracts rich in piperine have been reported to have efficacy against inflammatory mediators. It inhibits the expression of IL6 and MMP13 and reduced the production of PGE2 in a dose dependant manner (Bang et al., 2009).

It was therefore decided upon to prepare a polyherbal liposomal formulation containing the extracts of *Trigonella foenum-graecum, Piper nigrum* and *Zingiber officinale* in order to improve the absorption and bioavailability of the phytoconstituents for the management of gout and incorporating the liposome into gel for topical application.

Material and Methods

Trigonella foenum (TF), *Zingiber officinale* (ZO) and *Piper nigrum* (PN) plants were procured from Shubham Nursery Bhopal. High purity Soy Lecithin and cholesterol were procured from Merck Life Sciences,

Mumbai. Ethanol, acetone, ortho phosphoric acid, triethanolamine, and chloroform were purchased from SD Fine Chemicals, Mumbai. Sodium hydroxide, sodium chloride, sodium carbonate, magnesium carbonate, and calcium carbonate were purchased from Oxford Fine Chemical LLP, Mumbai. Purified water was prepared using Millipore DQ3 water purifier.

Extraction of plant material (Sahira Banu, 2015)

The shade dried, powdered plant material was used for the extraction process. 100 g of plant powder (of individual plants) was evenly packed in the extractor of the soxhlet apparatus and extracted with ethanol by hot continuous extraction process for about 13 h. The extracts were filtered while hot through Whatman filter paper to remove any impurity. The extract were allowed to dry in air and then transferred to lyophillizer for complete drying of the extracts. The dried extracts were stored in air tight containers until further processing.

Total Phenolic content in the extracts (Chanthasri et al., 2018)

The extracts of *Trigonella foenum* (TF), *Zingiber officinale* (ZO) & *Piper nigrum* (PN) were mixed in 1:1:1, 1:2:1 and 2:1:1 ratio respectively and each mixture were dissolved in ethanol to obtain a stock solution (50 mg/mL) for analyses. For total phenolic content determination, 200µL of each sample was mixed with 1.4mL purified water and 100µL of Folin-Ciocalteu reagent. After at least 30 s (but not exceeding 8 min), 300µL of 20% Na_2CO_3 aqueous solution was added and the mixture was allowed to stand for 2 h. The absorbance was measured at 765 nm with a UV-Vis spectrophotometer. Standard solutions of gallic acid (10-60 ppm) were similarly treated to plot the analytical curve. The control solution contained 200µL of ethanol and suitable reagents, and it was prepared and incubated under the same conditions as the rest of the samples. Results were expressed as milligrams of gallic acid equivalent (GAE) per 100 g of the dry sample.

Preparation of Phosphate buffer Saline (pH 7.4) (Indian Pharmacopoeia, 1996)

2.38 g Na_2HPO_4, 0.19 g KH_2PO_4 and 8 g NaCl were dissolved in 1000 ml distilled water to prepare the desired buffer solution

*Preparation of Liposomes (*Armann et al., 1990)

Multilamellar vesicles (MLVs) were prepared by physical dispersion method from a lipid mixture of lecithin: cholesterol (Table 1). Briefly, 240 mg of lecithin and 60 mg of Cholesterol were dissolved in 100 ml of a ethanol:chloroform (1:2, v/v) solution in a round bottomed flask. Extract (200 mg) dissolved in 5 ml of ethanol was added to the lipid mixture. The organic solvent was evaporated to dryness in a rotary evaporator that was rotated at 180 rpm in a 40ºC water bath. When a thin film of lipid was deposited on the inner wall of the flask, phosphate buffered saline (PBS, pH 7.4, 5 ml) was added and the preparation was rotated for a further 30 min until a white homogenous dispersion of liposomes was obtained. The dispersion was then incubated in a shaker bath for 2 h at 37ºC to complete the swelling

process. Smaller MLVS were produced from the larger MLVs by probe sonication. Sonication was performed intermittently, with each cycle comprising of sonication at 40 W for 1 min followed by 1 min of rest, and 10 - 100 of such cycles were applied to a batch of liposomes. The alternating cycle was to ensure that the liposomes were maintained at the ambient temperature of film hydration. The liposomes were incubated for another 2 h at 37ºC to allow for the completion of the annealing process. Control (drug-free) liposomes were prepared in a similar manner except that the extract was not added to the mixture. All liposome dispersions were characterized immediately after preparation.

Table 1 Composition of liposome formulations

S. No	Formulation code	Lecithin	Cholesterol	Extract Ratio (TF:ZO:PN)
		Parts		
1	ELF1	8	2	1:1:1
2	ELF2	8	2	1:2:1
3	ELF3	8	2	2:1:1

Characterization of Liposomes (Jain et al., 2018)

The characterization of the liposomes was carried out for determination of particle size, total phenol content and stability of the liposomes.

Size and size distribution

The particle size of the microspheres was determined by using an MKOW optic microscope, employing the calibrated eye piece and stage micrometer method. Size of liposomal vesicles was measured at different location on slide by taking a small drop of liposomal dispersion on it and average size of liposomal vesicles was determined.

Total phenolic content in liposomes

A standard solution was prepared with ethanol, acetic acid and distilled water at 50:8:42 ratios, respectively. One milliliter liposome sample was diluted at a volume ratio of 1:4 with the standard solution and filtered with 0.45 mm microfilter. After dilution the liposome sample was agitated by vortexing for 1 min. 2.5 mL Folin solution was added to 500 µL diluted liposome sample. Following vortexing, it was left to stand 5 min in the dark. Then, 2 mL of sodium carbonate solution was added to the mixture it was left to stand in the dark for another 60 min. For the blank, 2 mL sodium carbonate solution was added to 2.5 mL Folin solution and the mixture was left in dark for 60 min. The absorbance was measured at 765 nm with a UV-Vis spectrophotometer.

Stability of Liposomes

The stability of the liposomal preparations was evaluated as a function of storage time. In the preliminary experiments, liposomal samples were stored in a refrigerator at 4º and 40°C for 3 months immediately after preparation. Once every 2 weeks, the size and size distribution of the samples were determined using the method described in previous section.

Formulation and evaluation of polyherbal gel

The liposomes loaded with different ratio of the extracts were formulated as gel. To a 1.0 % w/v dispersion of carbopol 934 in distilled water was added the weighed amounts of the liposomal formulation. The dispersion was allowed to hydrate overnight to complete the swelling of the gelling agent. Glycerine was added to the hydrated dispersion and mixed with stirring. To the above mixture, SLS was added slowly introduced with continuous stirring. The gelation was finally accomplished by the addition of the required quantity of TEA (Singh et al., 2017).

Table 2 Composition of liposomal gel

S.No.	Ingredient	Formulation Code		
		PHG1	PHG2	PHG3
1	ELF (g)	0.5	1	0.5
2	Carbopol 934 (% w/v)	1.0	1.0	1.0
3	Sodium lauryl sulfate (% w/v)	1.5	1.5	1.5
4	Methyl Paraben (g)	0.15	0.15	0.15
5	Propyl Paraben (g)	0.05	0.05	0.05
6	Glycerin (% w/v)	4	4	4
7	Distilled Water (mL)	100	100	100
8	TEA	QS	QS	QS

QS- Quantity sufficient

Characterization of Gel formulations

The optimization of gel base was done for viscosity and spreadability whereas for face wash formulations foaming is an important aspect. The optimization of the surfactant concentration was done for obtaining good foaming. The gel base, surfactant containing gel base and the face wash formulations were characterized for the various parameters according to the reported procedures.

Physical appearance

The physical appearance of the polyherbal liposomal gels (PHLG) was inspected for color, homogeneity (non occurrence of lumps) and odor.

Measurement of pH

The PHLG formulations (0.1 g) were dissolved in 10 mL of water and allowed to stand for 2 h. The pH of these solutions was then recorded using a digital pH meter.

Viscosity

Viscosity of PHLG was measured using Brookfield viscometer at 20 rpm applying spindle no. 64. The reading on the display after 2 min of shear was recorded as the viscosity of the formulation.

Spreadability

Spreadability of PHLG was evaluated using Arvouet-Grand Method.[44] The method involved placing 1 g of the formulation between two glass plates of 20 X 20 cm and placing a weight of 125 g on the upper plate. The diameter of the formulation was recorded as an index of spreadability.

Washing off ability

A small amount of PHLG was rubbed on to the skin and was washed off with warm water in absence of soap.

Grittiness

The PHLG formulations were smeared on glass slide and observed under light microscope for occurrence of uneven particles, if any.

Results and Discussion

Extraction Yield

The ability of ethanol to extract phytoconstituents was determined by weighing the dried extract and calculating the percentage of extract obtained in relation to the weight of the plant powder used for extraction. The extraction yield for TF, ZO and PN were found to be 28.7, 37.4 and 21.1% respectively. The dried extracts were found to be blackish brown in color and were stored in air tight containers.

Total Phenolic Content

The extracts of TF, ZO and PN were evaluated for quantification of the total phenolic content in them. The total phenolic content is said to be responsible for the neutralization of the free radicals and other mediators of several diseases including gout. The results of the total phenolic content of the extracts examined, using Folin-Ciocalteu method, are depicted in Table 3.

Table 3 Total Phenolic content of TF, ZO, PN and extract mixtures

Extract	Total phenolic content (GAE mg/100g)
Trigonella foenum-graecum	28.3 ± 1.301
Zingiber officinale	13.2 ± 1.196
Piper nigrum	9.8 ± 0.894
Extract mixture 1:1:1	38.6 ± 0.735
Extract mixture 1:2:1	51.4 ± 0.721
Extract mixture 2:1:1	63.08 ± 0.823

Data expressed as gallic acid equivalent (GAE) mg per gm of the extract, Values are mean ± SEM of triplicate determinations

The mixture of the extracts demonstrated an additive increase in the total phenolic content revealing the highest phenolic content in the mixture containing 2:1:1 ratio of TF, ZO and PN respectively.

Liposomal Formulation

The formulation parameters are known to be affected by the method of formulation of liposomes which in turn affects the characteristics of drug-loaded liposomes. The parameters used to characterize the liposomes in the preliminary experiments included particle size and morphology determination. Stability studies using particle size as an indicator of stability were also conducted over a 3-month period.

Table 4 presents the average size of multilamellar blank and extract-loaded liposomes prepared using different sonication times. All readings were taken as average ± standard deviation.

Table 4 Particle size of liposomes

Formulation Code	Sonication time (min)	Particle Size (µm)
		Extract loaded liposome
ELF1	5	6.18 ± 1.033
	15	4.61 ± 0.666
	30	2.63 ± 0.666
ELF2	5	5.17 ± 1.033
	15	4.11 ± 0.869
	30	2.46 ± 1.066
ELF3	5	5.21 ± 0.333
	15	4.14 ± 1.033
	30	2.01 ± 0.066
Blank liposome	5	4.27 ± 0.033
	15	3.43 ± 0.033
	30	1.96 ± 0.033

Total phenolic content in the liposomes

The total phenolic content of all the formulated liposomes was determined. The results reveal the phenolic content in the liposomes was in tandem with the extract mixture incorporated into the liposome and the sonication time had no significant effect on the total phenolic content indicating that the encapsulation of the extract in the liposome shell was unabated by the sonication time. The results are presented in Table 5.

Stability of liposomes

The change in particle size over a period of three months was considered to ascertain the stability of the liposomal formulation. The stability was assessed for the optimized liposomal formulation (formed by 5 min of sonication) by storing them in three different batches for stability monitoring.

The results reveal that the liposomes were stable at both the temperature conditions and the particle size of the liposomes did not change considerably over a period of three months.

Table 5 Total phenolic content in liposomes

Formulation Code	Sonication time (min)	TPC
ELF1	5	38.68 ± 1.295
	15	38.42 ± 1.066
	30	38.75 ± 1.524
ELF2	5	50.64 ± 1.033
	15	50.08 ± 1.899
	30	50.93 ± 1.066
ELF3	5	62.81 ± 1.629
	15	62.89 ± 1.033
	30	62.75 ± 1.066

Evaluation of Polyherbal liposomal gel

As ELF3 exhibited the highest phenolic content of all the three formulations, it was incorporated into gel formulation in different ratios, using carbopol 934 as the gelling agent. The gel was characterized for its properties and the results obtained were used to decide-upon the best formulation.

Methyl paraben and propyl paraben were used for preventing microbial contamination in PHLG. Glycerin was incorporated in to PHLG to provide humectants and moisturizing properties to the gel.

All the gel formulations were non-gritty, dark yellow in color, homogenous and had a slight odor.

Viscosity, pH and spreadability

All the PHLGs exhibited optimum viscosity. The pH of all the formulations was in the range of the pH of the skin (5.0-5.5) (table 6) and were semi-fluid.

Table 6 Viscosity, pH, and spreadability of PHLGs

Formulation Code	pH	Viscosity (cp)	Spreadability (mm)
PHG1	5.3	1684	62
PHG2	5.4	1670	65
PHG3	5.3	1694	62

Accelerated Stability Study

Indeed all the PHLGs exhibited similar physical properties, pH and viscosity but PHLG3 had the highest TPC. Hence PHLG3 was subjected for accelerated stability study testing for 2 months. The purpose of stability study was to determine whether or not the PHLG could retain its physical properties at temperature conditions exceeding the normal room temperature. The pH, viscosity and liquefaction of the formulation were observed after the end of 2 months of the study.

No change in physical parameters of PHLG3 occurred and also no liquefaction was observed in the formulation. Hence it could be inferred that the formulation PHLG3 was stable and could be used as a good anti inflammatory formulation for topical application.

Conclusion

The present work was undertaken with an aim to develop polyherbal formulation for the management of gout. Ethanolic extracts of *Trigonella foenum-graecum, Zingiber officinale* and *Piper nigrum* were mixed in various ratios and developed as liposomes which were incorporated into gel formulation for topical application. The approach was found to be quite promising as the total phenolic content in the liposomes was found to be significantly at par with that of the extract mixtures indicating a good incorporation of the extracts in the liposomal shell. The liposomes were found to be stable at the temperature conditions examined thereby presenting a good approach for incorporation into gel form and topical application.

In future the *in vitro* assessment of the anti gout potential using enzymatic models would be undertaken to prove the efficacy of the liposomal gel.

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