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Formulation and evaluation of polyherbal capsules for the management of gout

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Abstract

Trigonella foenum-graecum (TF), Zingiber officinale (ZO) and *Piper nigrum (PN)* were extracted using ethanol and incorporated in to liposomes. These liposomes were formulated as capsule dosage form for the management of gout. The extraction yield for TF, ZO and PN were found to be 38.9 41.6 and 21.6% respectively respectively. The total phenolic content in TF, ZO and PN extracts were found to be 31.3 ± 1.367, 11.7 ± 2.896 and 8.7 ± 0.894 GAE mg/100g whereas it was found to be 49.8 ± 0.735, 58.1 ± 1.721 and 69.06 ± 1.823 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 2.38 ± 0.0929 µm. The phenolic content in the liposomes was in accordance with the extract mixture incorporated into the liposome with LF3 exhibiting the highest phenolics. The liposomes were evaluated for stability for a period of 3 months at storage temperature of 4° and 40°C. 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. The weight variation of polyherbal liposome filled capsules was between 3.5-4.1% whereas the disintegration time was found to be between 3.2 to 3.7 min.

Keywords: Polyherbal, liposomes, gout, stability, phenolics, prefill parameters

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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.[1] 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.[2] 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.[3,4] 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.[5] 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.[6]

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. *Cissus quadrangularis* is a medicinal plant which belongs to the Vitaceae family usually cultivated in India and Ceylon. In Ayurvedic system of medicine, *Cissus quadrangularis* is used for the treatment of sexually contracted diseases, gout, piles, leucorrhoea and syphilis.[7] In Siddha traditional medicine this plant is believed to heal broken bones, as also act as an analgesic and a tonic.[8]

Trigonella foenum-graecum has been known to contain flavonoids and the extracts are reported to possess potential against acute and chronic inflammatory conditions.[9]

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

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.[10]

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.

Material and Methods

Trigonella foenum-gradecum (TF) seeds, *Zingiber officinale* (ZO) rhizomes and Piper nigrum (PN) seeds were procured from local market. 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[11]

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[12]

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 Liposomes[13]

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	LF1	8	2	1:1:1
2	LF2	8	2	1:2:1
3	LF3	8	2	2:1:1

Characterization of Liposomes[14]

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.

Formulation and evaluation of polyherbal capsules

The liposomes loaded with different ratio of the extracts were formulated in capsule dosage form using method reported by Shanmuga *et al*[15]. The liposomes (6g) were weighed accurately and mixed with 2g each of magnesium carbonate and calcium carbonate using trituration method. The blend was sifted through sieve no. 26 to obtain fine powder. The fine powder was subjected to characterization of powder properties and hand filled in capsules (500mg per capsule). The capsules were evaluated for weight variation and disintegration time.

Angle of Repose[16]

The powder mixture was allowed to flow through the funnel fixed to a stand at definite height (h). The angle of repose (θ) was then calculated by measuring the height and radius (r) of the heap of powder formed using the following formula

$$\tan\theta = \frac{h}{r}$$

Bulk and Tapped Density[17]

A weighed quantity of blend (10g) was taken into a graduated cylinder (50 mL) and measuring the volume of this weight. The bulk density (ϱ bulk) was calculated by the formula

ϱ bulk = weight of the powder/initial volume

The above cylinder containing the powder blend was tapped until no further volume change occurs. The tapped density (ϱ tap) was calculated by the formula

ϱ tap = weight of the powder/finial volume

Hausner's ratio and Carr's Index[17]

Hausner's ratio is the ratio of tapped density to bulk density and is calculated by the following formula

HR = ϱ tap/ ϱ bulk

The Compressibility index is also known as Carr's Index and is calculated using the values of bulk and tapped density using the formula

$$\text{Carr's Index} = \frac{\varrho\,\text{tap} - \varrho\,\text{bulk}}{\varrho\,\text{tap}} \text{ X } 100$$

Results and Discussion

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 calibration curve of gallic acid was constructed using distilled water for obtaining the absorption data. The results of the total phenolic content of the extracts examined, using Folin-Ciocalteu method, are depicted in Table 2. The total phenolic content in extracts, expressed as gallic acid equivalents. The total phenolic content in TF, ZO and PN extracts were

found to be 31.3 ± 1.367, 11.7 ± 2.896 and 8.7 ± 0.894 GAE mg/100g.

Table 2 Total Phenolic content of TF, ZO, PN and extract mixtures

Extract	Total phenolic content (GAE mg/100g)
Trigonella foenum-graecum	31.3 ± 1.367
Zingiber officinale	11.7 ± 2.896
Piper nigrum	8.7 ± 0.894
Extract mixture 1:1:1	49.8 ± 0.735
Extract mixture 1:2:1	58.1 ± 1.721
Extract mixture 2:1:1	69.06 ± 1.823

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.

Characterization of Liposomes

Particle size

Table 3 presents the average size of multilamellar blank and extract-loaded liposomes prepared using different sonication times.When the sonication time was kept over 30 min, it was found that liposomes of size range 1.65 – 2.06 μm were obtained (blank). Decreasing the sonication time caused an increase in the size of the liposomes. The size range of blank liposomes obtained on sonicating for 15 and 5 min was found to be 3.17-3.3 μm and 2.28 – 2.47 μm respectively.

Annealing was performed on the prepared liposomes to prevent physical degradation and drug loss (extract loss). The process of annealing involves incubating the liposome dispersion for 2 h at 35ºC thereby equilibrating the opposite sides of the lipid bilayer by trans-membrane flip-flop.

Table 3 Particle size of liposomes

Formulation Code	Sonication time (min)	Particle Size (μm)
		Extract loaded liposome
LF1	5	5.24 ± 0.1353
	15	4.03 ± 0.1966
	30	2.27 ± 0.1904
LF2	5	4.97 ± 0.1242
	15	4.20 ± 0.1609
	30	2.24 ± 0.2577
LF3	5	5.23 ± 0.2369
	15	4.20 ± 0.1201
	30	2.08 ± 0.0776
Blank liposome	5	2.38 ± 0.0929
	15	3.24 ± 0.0602
	30	1.86 ± 0.2095

Results are mean ± SD for 3 replicate readings

Indeed the smaller vesicle size is preferred over the larger vesicles but the fact that the efficiency of the liposomes is related to the lipid content and not to the particle size provides significant evidence to use lower sonication times for stability study and further preparation of the capsule dosage form.

Total phenolic content in the liposomes

The total phenolic content of all the formulated liposomes was determined using the method reported by Dag.[18] 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 4.

Table 4 Total phenolic content in liposomes

Formulation Code	Sonication time (min)	TPC
LF1	5	39.68 ± 1.066
	15	39.42 ± 1.295
	30	39.75 ± 1.524
LF2	5	46.64 ± 1.033
	15	46.08 ± 1.899
	30	46.93 ± 1.066
LF3	5	55.81 ± 1.629
	15	55.89 ± 1.033
	30	55.75 ± 1.066

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. Figure 1 presents a comparative representation of the change in particle size of the extract loaded liposomes stored for stability monitoring.

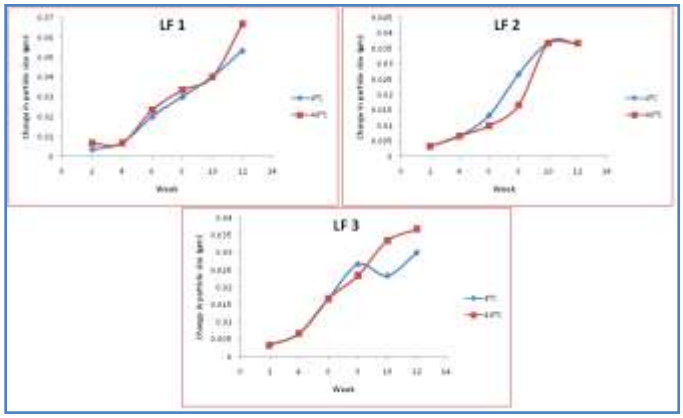

Figure 1 Change in particle size on storage

Evaluation of blends

The results of prefill evaluation of the formulation blends are presented in Table 5. From the results it is evident that all the blends possessed the capability to flow freely and may present no hindrance in capsule filling process. The values of Hausner's ratio and Carr's Index are found to be within the specifications of good flow property of powders.

Table 5 Prefill Parameters of the blends

Formulation Code	Bulk density (g/cm^3)	Tap density (g/cm^3)	Angle of repose (degree)	Carr's Index (%)	Hausner's Ratio
BLF 1	0.321	0.408	27°89'	21.32	1.27

BLF 2	0.341	0.418	29°03'	18.42	1.23
BLF 3	0.338	0.435	30°01'	22.30	1.29

Evaluation of capsules

The capsules were evaluated for weight variation by weighing individual capsules and determining the deviation of each capsule from the average weight of 20 capsules and for disintegration time using tablet disintegration test apparatus (Table 6).

Though disintegration test is usually not required for capsules owing to the rapidity of solublization of the gelatin shell of the capsule in stomach yet the test was performed for proving the quality of the developed product.

The weight variation of capsules was between 3.5-4.1% and complied the specifications of Indian pharmacopoeia.

Table 6 Evaluation parameters of polyherbal capsules

Formulation Code	**Average Weight variation (%)**	**Disintegration time (min)**
CLF1	3.8	3.7 ± 0.0590
CLF2	4.1	3.2 ± 0.0806
CLF3	3.5	3.5 ± 0.0413

Conclusion

The use of liposomes in oral drug delivery has been an area of investigation in the recent times. Herbal drugs have been used since ages for the treatment of otherwise unmanageable ailments like arthritis and gout. 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 capsule dosage forms for oral delivery. 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 capsules and ultimately oral drug delivery.

In future the *in vitro* assessment of the anti gout potential using enzymatic models would be undertaken to prove the efficacy of the liposome filled capsules. Standardization of the capsules would also be undertaken according the guidelines for standardization of polyherbal formulations.

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