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Study of lycopene and allopurinol blended transfersomes for the treatment of gout

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ABSTRACT

This study aimed to enhance and assess the efficacy of film hydration-prepared allopurinol and lycopene blended transferosomes (ALT). It was found that there were no changes in the FTIR spectra, i.e. no chemical interactions between the components. The average particle size of the ALT was 165 nm. The study concluded that lycopene (natural phytoconstituent), can be used with allopurinol to enhance the anti-gout potential of later.

Keywords: Allopurinol, ALT, FTIR, gout, lycopene, polymer, phytoconstituents, transferosomes

INTRODUCTION

In recent decades, various drug delivery methods have been developed, attracting pharmaceutical industry interest (6). Applying transfersomes to skin reduces the chance of complete vesicle rupture, since their membranes are ultra-deformable and may follow the typical water gradient across the epidermis (7). The addition of surfactant increases the transferosomes' elasticity than conventional liposomes. This makes their better penetration through small pores and less likely to disrupt the bilayer than hard liposomes (11). Allopurinol is a potent inhibitor of the enzyme xanthine oxidase, which is used to reduce blood urate levels and thereby mitigate the frequency of gout attacks (4). The metabolite of allopurinol, oxipurinol, plays main role in hypouricaemic effects of allopurinol (12). Hence, the limited accessibility after administration in some dosage forms may be due to inadequate solubility of allopurinol in the dosage form.

Lycopene (acyclic carotenoids family) is natural carotenoid (red pigment) found in tomatoes, watermelon, pink grapefruit and other red fruits (5). It has antioxidant and anti-inflammatory properties, but it is not a standard treatment for gout. Allopurinol is a xanthine oxidase inhibitor commonly used in the long-term management of gout, due to higher uric acid levels, leading to deposition of urate crystals in joints and soft tissues. The vesicular carriers, such as transferosomes, penetrates the stratum corneum has garnered much interest in its study. Hence, hyperlipidemia, cancer and other cardiovascular problems can be avoided. Thermal processing enhances the bioavailability by rupturing cellular membranes, which release lycopene from the tissues. Lycopene is lipid-soluble; thus, fat increases its bioavailability (2). Gout patients have high purine consumption medicines or high cell turnover (such as malignancy), due to the development of uric acid stones. In US 65 % individuals with hypertension have developed gout (8,9). This study aimed to enhance the transdermal penetration of a transferosomal gel formulation containing allopurinol and lycopene through optimization via a Box-Behnken design. The optimized transferosome-

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loaded gel was evaluated for its ability to facilitate transdermal delivery of allopurinol as an alternative to oral administration. To ensure safety and compatibility, non-toxic and biocompatible surfactants were used in the formulation and characterization of the transferosomal gels.

MATERIALS AND METHODS

I. Experimental Design

A commercially accessible software program (Design Expert, Version 7.0.2, Stat-Ease Inc., Minneapolis, MN) was used. We used Response Surface, 3-factor, 3-level factorial experimental design and 17-formulations were prepared (Table 1). The thin-film hydration approach was used to generate a transferosomal formulation containing Allopurinol and lycopene. The organic solvent was used to dissolve allopurinol, lycopene, soya lecithin and sodium deoxycolate. The solvent evaporating at 60 rpm and 40 ℃ developed a thin coating in the rotary evaporator. The excess solvent was removed by vacuum-drying overnight and then dried it for 10 min at 50 ℃. Hydrogenated lipid films were deposited by spinning at 60 rpm/min for 1 h at 50 ℃. with a buffer solution (pH 6.5). In 2.0 h, the resulting vesicles expanded when left at room temperature. The dispersion was sonicated for 30 min after 4-h hydration interval at room temperature and the sample was then examined (10). The generated ALT was kept at 4 °C overnight for further testing.

II. Particle Size

The transfersomes were analysed using a Zetasizer Nano (Malvern, United Kingdom, by Malvern Instruments Ltd.), a dynamic light scattering device to determine their zeta potential, particle size and polydispersity index (PDI). The Malvern zeta potential cuvette (1.0 mL formulation), was put into the analyzer for the purpose of estimation.

III. FTIR study

The chemical interactions in the materials were examined by using Shimadzu Fourier Transform Infra-Red Spectrophotometer, (manufactured by IR Affinity in Kyoto, Japan) to analyse the FT-IR spectra. Potassium bromide (KBr) pellets were used to generate the samples. Aashli *et al.* (1) generated each graph by calculating the average of 45 scans and subsequently plotted the resulting spectra as transmittance (%) cm.

IV. *In-vitro* drug release study

In-vitro drug release from the transferosomal formulation was investigated on 0.75 cm^2 Franz diffusion cells. An egg membrane separated the donor and receptor sections. The conditions were mimicked by constantly swirling a magnetic bead in the receptor compartment and maintaining a temperature of 37±1 ℃. The pH of the phosphate-containing buffer was 7.4. The donor compartment was filled with 1.0 mL transferosomal formulation (3). Two mL portions of buffer were taken and replaced with a new equivalent volume at 0, 2, 4, 6, 12, 18 and 24 h intervals. To determine the content of allopurinol and lycopene, ultraviolet spectroscopy was used respectively at 251 and 471 nm. The tests were repeated thrice.

IN-VIVO STUDY

I. Experimental animals

We used *in-vivo* research procedures approved by the Ethical Committee of Institutional Animals (1273/PO/Re/S/09/CPCSEA). The guidelines followed by the lab were followed precisely. The experiment involved acclimatizing 9- albino rabbits, ranging in weight from 1.5 to 2.5 kg, to temperature of 25±2 °C and relative humidity of 50±5 %.

II. Induction of gout

The evaluation of the transferosomal gel's aggravation was conducted through the assessment of knee width, estimation of uric acid in synovial fluid and analysis of X-ray pictures of joints to observe changes in the interspaces between them. The onset of gout was induced through the administration of MSU crystals on day '0', followed by the transdermal application of a combined transfersomal (6.6 mg/kg) treatment on day '21', after the initiation of gout in joints exhibiting signs of excitement and swelling. After the investigation, namely on the 45^{th} day, a significant alteration in the knee measurement of rabbits and a reduction in aggravation were noted.

III. Histopathological study

In order to conduct histological investigations, the areas around the ankles and back paws were cut off and preserved in 10 % formalin for the night. The bones were then decalcified in 14 % EDTA until they were soft enough to be handled, embedded in paraffin and sectioned at 4-5 µm. In a blinded way, tissue sections were stained with H&E stain and photographed under a light microscope (Olympus BX60, Tokyo, Japan). Following the method outlined by Pettit et al., we evaluated the extent of inflammation, synovial proliferation, bone erosions and cartilage destruction. It has summarized the scoring system as follows: 0 for normal, 1 for minimal, 2 for mild, 3 for moderate, 4 for marked and 5 for severe. Using the International Osteoarthritis Research Society (OARSI) grading system, the extent of cartilage degradation was assessed and Safranin O-Fast Green Staining was utilised to identify cartilage loss.

RESULTS AND DISCUSSION

I. Characterization of prepared formulation

The transferosomes of lycopene and allopurinol were prepared to treat gout. The results are shown in Table 1. Table 1 presents a factorial design exploring the influence of 3-independent variables: Factor A: Polymer concentration, Factor B: Surfactant concentration (Factor B) and Factor C: Drug concentration on key formulation responses for lycopene and allopurinol blended transfersomes. The dependent variables assessed include Particle size (Y1), Polydispersity index (Y2), and Entrapment efficiency (Y3), which collectively reflected the physicochemical integrity and drug-loading capacity of the vesicular system. This structured dataset enables systematic optimization of formulation parameters to enhance stability, uniformity and therapeutic potential for gout treatment.

Table 1. Factors and responses to all formulations

RUNS	Independent Variables			Dependent Variable		
	Factor A	Factor B	Factor C	Y1	Y 2	Y3
1	90	20	75	187	25.2	78.90 %
2	85	15	75	267	26.2	83.40 %
3	80	10	75	238	30.0	88.10 %
4	80	20	75	151	25.7	89.50 %
5	80	15	100	174	27.9	87.50 %
6	90	10	75	132	25.7	89.5 %
7	85	10	100	159	30.1	78.40 %
8	85	20	50	180	29.8	87.50 %
9	85	15	75	366	22.9	74.60 %
10	90	15	50	185	27.3	68.20 %
11	80	15	50	238	30.7	68.10 %
12	85	15	75	241	23.6	70.40 %
13	85	10	50	250	22.2	81.10 %
14	85	20	100	178	28.2	85.40 %
15	85	15	75	265	25.1	80.30 %
16	90	15	100	161	29.5	71.50 %
17	85	15	75	260	25.9	80.30 %

Factor A: Polymer concentration, Factor B: Surfactant concentration factor C: Drug concentration, Y1: Particle size, Y2: PDI and Y3: Entrapment efficiency

II. Particle size and polydispersity

The characteristic stable nano-vesicular shape and consistently sized round vesicles were prepared. Results from the investigation showed that the optimized formulation was credible and plausible, as the predicted values of the formulation particle size, PDI and entrapment efficiency from the Design Expert software were in agreement with the ALT experimental estimates (Table 2). With a constrained size distribution, the results showed that ALT dispersion drastically decreased particle size. The average particle size of the ALT was found to be 165 nm. All agree that PDI values < 0.7 indicate monodispersed systems. A less than 0.7 PDI for ALT indicated a monodisperse system.

Measurement of pH and viscosity: The viscosity of the formulation was determined to be 42976 to 48682 cP. The pH of the formulations is crucial for transdermal delivery. The pH of the transferosomal formulations was 6.2 ± 0.3, which was within the normal pH range of the skin. Hence, it will not cause any skin irritation.

III. FTIR study

FTIR spectroscopy, one of the most important analytical tools for studying drug-excipient interactions, was utilised to study them before designing the formulation. Pure allopurinol spectra showed sharp peaks at the same absorption bands as numerous

independent studies. The FTIR spectra revealed the presence of the drug-polymer combination. When analyzing the FTIR spectra of the pure medication, it was observed that there was a distinct peak at 1442.75 cm^{-1}. This peak belongs to the aromatic C-C ring, which was is located at 2978.09 cm^{-1}. Additionally, the dimer O-H bond was located at 1357.89 cm^{-1}, the alkanes C-H bond was located at 1249 cm^{-1}, the ester C-O stretch was located at 972 cm^{-1} and the =C-H out of plane was located at 972 cm^{-1}. It was noted that the polymer exhibited distinct peaks at 1735.77 cm^{-1} (representing the C=O stretch of alkenes), 2862.36 cm^{-1} (represented the CH stretch of alkanes), 1651.07 cm^{-1} (represented the C=C stretch of alkenes) and 1458.18 cm^{-1} (represented the CH2 and CH3 of alkanes) Figure 1. In the physical mixture of drug and polymer, the distinctive drug peaks were observed, which were comparable to the peaks seen in the spectrum of each particular drug concentration. In addition, there were no alterations found in the FTIR spectra, which was further evident that there were no chemical interactions between the components.

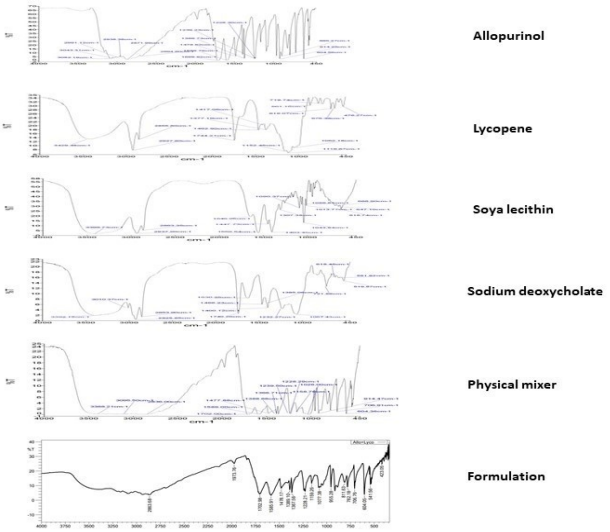

Figure 1. FTIR spectrum of ALT formulation

IV. *In-Vitro* drug release

Results showed drug release patterns for pure drug solutions and ALT at pH 7.4. Allopurinol and lycopene release from formulated ALT was lower than medication solutions. The release graphs showed that 78.01 % of Allopurinol was released within 6 h and 100 % within 12 h. Fortunately, ALT-1 released 85.12 % after 24 h. Lycopene release was 81.12 % for ALT-2 at the end of the release experiment. ALT-1 and ALT-2 formulations released Allopurinol and Lycopene. The *in-vitro* release results of all produced ALT showed no significant difference ($p > 0.05$). The structure and chemistry of ALT surfactants may explain the minor variance in drug release.

V. Acute dermal irritation test of applied formulation

The experimental findings indicate that the formulation demonstrated compatibility with the skin in the context of acute cutaneous irritation. There were no observable clinical indications or alterations in body weight observed in any of the groups that received the formulation, as shown in Table 2. Rabbits did not exhibit any cutaneous responses such as erythema/eschar or edema, as shown in Figure 2.

Table 2 Irritation test of skin by applying formulation at different time interval

Parameter	Edema	Erythema
Dermal irritation study - Acute		
1 h after drug application	0	0
24 h after drug application	0	0
48 h after drug application	0	0
72 h after drug application	0	0
Dermal irritation study - Repeated		
1 h after drug application	0	0
24 h after drug application	0	0
48 h after drug application	0	0
72 h after drug application	0	0

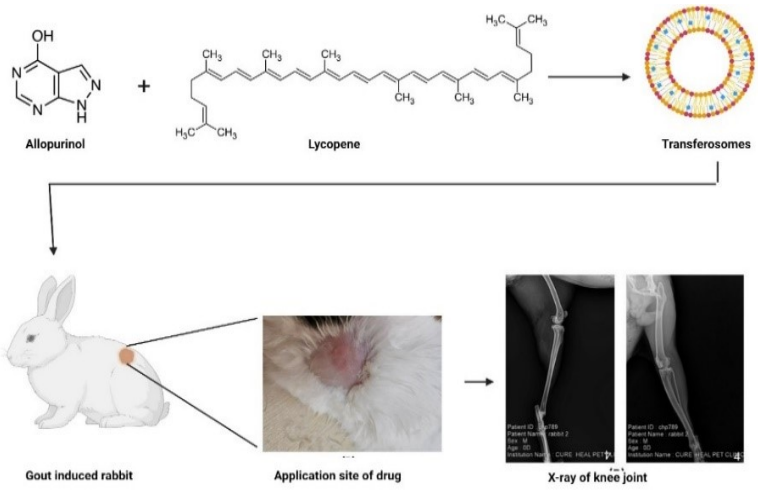

Figure 2. Skin irritation test of rabbits (A) Disease-induced group (B) Blank formulation group (C) Drug-treated group.

VI. Effects of formulations on gout-induced animals

Allopurinol and Lycopene loaded transferosomal formulation significantly reduced inflammation in the joints of rabbits (Table 3). It was 1.18 for the test group on 45 days as compared to the control group (Figure 3). The control group, treated with blank gel, showed no change in joint inflammation scores, maintaining a consistent mean value of 1.22 across all time points. The blank formulation group exhibited a slight reduction from 1.26 on day 0 to 1.21 at day 45, indicating minimal anti-inflammatory activity. In contrast, the drug-treated group-administered a transferosomal gel loaded with allopurinol and lycopene-decreased inflammation, from 1.27 at baseline to 1.18 at day 45. This reduction was statistically significant ($p < 0.05$) compared to the control, suggesting that the combination formulation effectively mitigated MSU-induced joint inflammation.

Table 3 Formulation effects on rabbit knee joint inflammation following MSU crystal injection

Groups	Number of Days		
	0 days	21 days	45 days
Control group	1.22	1.22	1.22
Blank formulation group	1.26	1.25	1.21
Drug Treated group	1.27	1.22	1.18

Data are represented as Mean ±SD (n=3), statistically different at $p < 0.05$ in comparison to the control group. Disease group : MSU crystals; Control group : Blank gel; Test group : Allopurinol and Lycopene loaded transferosomal gel

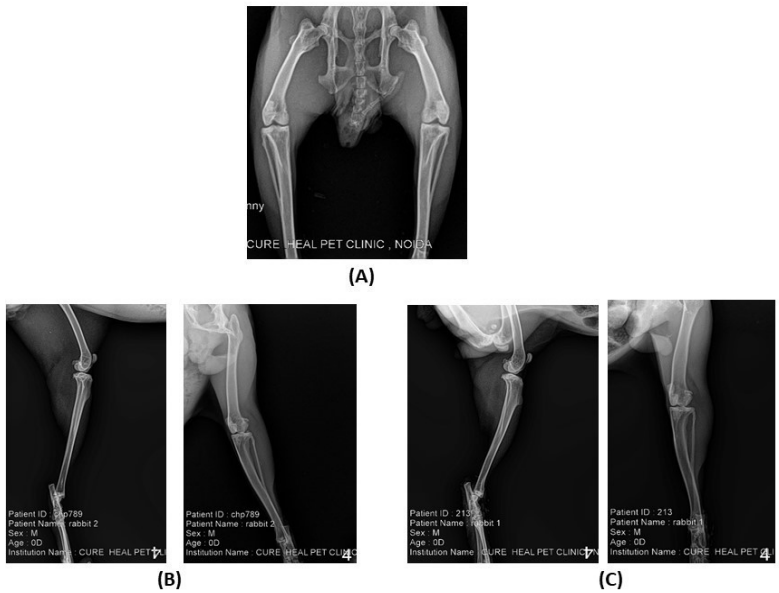

Figure 3. X-ray of knee joint of rabbits (A) Control group, (B) Blank formulation group, (C) Drug-treated group.

VII. Histopathological Analysis

Inflammatory cells infiltrating the joints, bone degradation and synovial hyperplasia are the hallmarks of gouty arthritis. After fixing, sectioning and staining the hind paws with H and E, the histological outcome in the gouty arthritis model was evaluated. Two months after the first induction of gouty arthritis, the model group showed severe arthritic features in their ankle joints, as shown in histological sections (Figure 4).

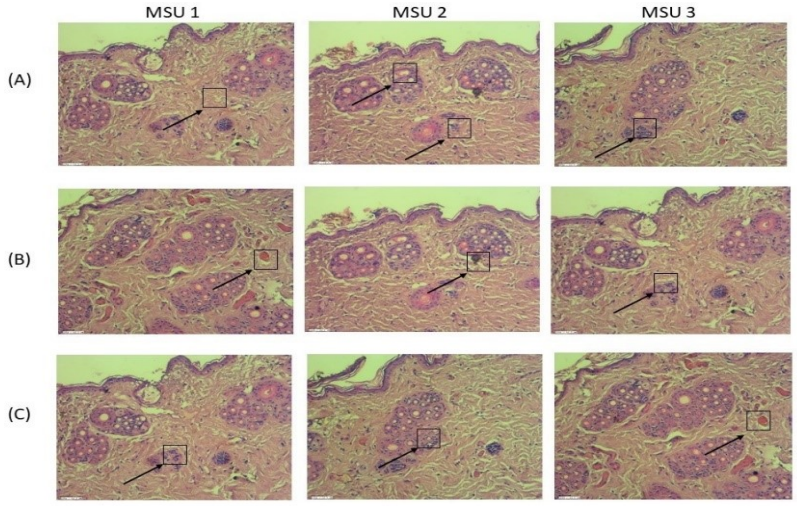

Figure 4. Histopathological analysis of rabbit knee joint injected with monosodium Urate (A) Diseased-induced group, (B) Blank formulation group, (C) Drug-treated group

These features included inflammatory cell infiltration, synovial hyperplasia, bone erosion and cartilage degradation. Focused synovial hyperplasia with modest reactive alterations, vascular congestion, patchy acute inflammatory cellulose infiltrates and rare microcrystalline synovial membranes were found in the histopathological study of the MSU-crystal group on day 1. On the third day, the synovium had thickened and was hyperplastic and there were big clusters of cells with extensive polymorphonuclear infiltration, along with some macrophages and plasma cells. Proteoglycan analysis revealed that all research groups had normal amounts and distributions of sulfated proteoglycans. Both the allopurinol crystal and control groups showed no signs of histological alterations. In contrast to the visible accumulation of MSU-crystals in the gross anatomical specimens, histological sections of the joint cartilage did not reveal this.

CONCLUSIONS

The development and optimisation of transfersomes loaded with allopurinol and lycopene for transdermal applications were successful. The thin-film hydration process was used to prepare the ALT. Concentrations of surfactants and polymers affected the size of transferosomal vesicles. At higher polymer concentrations and lower surfactant concentrations, small ALTs were achieved. The size of vesicles showed statistically

significant changes, indicating that the polymer and surfactant had meaningful effects on the ALT size. Thus, ALT may be an alternate method of administration that improves skin penetration. Therefore, the drug's transdermal distribution was improved by this transferosomal formulation, which was based on highly deformable vesicles. ALT may be used to treat gout after clinical trials.

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AUTHORS' CONTRIBUTIONS

Soumya Mishra Literature collection and writing of the manuscript. Ranjit K. Harwansh: Manuscript conceptualization and Rupa Mazumder: Evaluation and interpretation.

DECLARATIONS

We declare that all authors of this Ms. have made substantial contributions. We did not exclude any author who substantially contributed to this manuscript. We have followed our ethical norms established by our respective institutions.

CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest.

ETHICAL APPROVAL

The authors declare that the study was carried out following scientific ethics and conduct. However, this study involvesthe use of animals; hence, ethical approval has been obtained from the concerned committee.

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