Formulation and Evaluation of Phytosome Loaded Drug Delivery of Gingerol for the treatment of respiratory infection

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**ABSTRACT**

Respiratory tract infection (RTI) is a well-known issue and influence the working of the lungs and other respiratory organs in winter season, especially in kids and adults. The phyto-constituent antibacterial drug [Gingerol] was used to treat RTI but it exhibits pharmacological issues. To overcome these issues and make home grown treatment more viably for the treatment of RTI, novel drug delivery (nanoparticle) based phytosome loaded complex approach was adopted. The phytosome (GP) was prepared by mixing of gingerol and soya lecithin using anti-solvent precipitation technique. The phytosome loaded complex (LPC) was prepared by loading of phytosome (GLPC) in chitosan aqueous solution and characterized & evaluated by different parameters. The physical compatibility studies by DSC and FT-IR, demonstrated the confirmation of GLPC with soya lecithin and chitosan. The optimized GLPC and GP were irregular particle & spherical structures, with a mean particle size of 254.01±0.05 nm(-13.11 mV) and 431.21±0.90 nm (-17.53 mV), respectively.

The % entrapment efficiency and % drug loading of GLPC (86.02±0.18 %, 08.26±0.72%) and GP (84.36±0.42%, 08.05±0.03%) was found, respectively. The in vitro release rate of GP (86.03±0.06%) was slower than GLPC (88.93±0.33%) in pH 7.4 phosphate buffer up to 24 h by diffusion process (Korsmeyer Peppas model). GLPC has shown the potent antioxidant activity, susceptible antibacterial activity and significant anti-inflammatory activity as compared to GP. GLPC has improved the significant bioavailability and also correlate the hematological values on rabbit blood against the incubation of microorganisms (S. aureus & E. coli). The prepared nanoparticle based complex of phytosome loaded of phyto-constituent drug has the combined effect of chitosan and phytosome which shown better sustained-release profile and also prolonging the oral absorption rate of gingerol with effective antibacterial activity in a better stable way at different storage conditions than phytosome or drug with chitosan.

**KEYWORDS:** Respiratory tract infection (RTI), Complex of phytosome loaded (LPC), Phytosome (P), Gingerol (G)

1. INTRODUCTION

RTI transmits from individual to individual, particularly in youngsters. RTI can make a great deal of difficulties like common cold, tonsillitis, sinusitis, otitis media, pneumonia, bronchitis, bronchiectasis, bronchial asthma and so on. Fortunately, helpful home grown medications can affect respiratory sufferings1. Ginger (rhizome of Zingiber officinale) belongs to the Zingiberaceae family, is widely used as a culinary spice. It is also used in pharmaceuticals, neutraceuticals and in cosmetics. Ginger contains a fusion of an aroma oils both volatile (zingiberene) and non-volatile (oleoresin) oils and phenolic compounds (gingerol and shogaol, zingerone and paradol) 2. Gingerol has less solubility, low bioavailability and rapidly eliminated from the body. To overcome of these problems and extend the retention time of gingerol, it combined with polymer and phospholipid. Phytosome is a patented technology, which improves the absorption and bioavailability of lipid compatible molecular complexes. The objective of the present work was to prepare the complex of phytosome loaded of gingerol (GPLC) using soya lecithin as a phospholipid and chitosan as a polymer and compare it with phytosome of gingerol (GP).

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2. MATERIAL AND METHODS

Preparation of phytosomes of Gingerol (GP):
Phytosomes of gingerol (GP1-GP5) including blank were prepared by anti-solvent precipitation technique using gingerol and soya lecithin with different molar ratios (1:1, 1:2, 2:1 and 2:2). The specific amount of gingerol and soya lecithin were taken in a 100 ml round bottom flask and refluxed with 30 ml of dichloromethane at a temperature not exceeding 60°C for 2 h. The mixture was concentrated to 5 ml and n-hexane (20 ml) was added carefully with continuous stirring to get the precipitate which was filtered, collected and stored in vacuum desiccators for overnight. Powdered phytosomes was placed in amber colour glass bottle and stored at room temperature.

Preparation of Gingerol Phytosome Loaded Chitosan Complex (GPLC):
Gingerol Phytosome loaded chitosan complex (GPLC1-GPLC9) including blank were prepared by anti-solvent precipitation technique using gingerol, soya lecithin and chitosan with different molar ratios (1:1:0.25, 1:1:0.50, 1:2:0.25, 1:2:0.50, 2:1:0.25, 2:1:0.50 and 2:2:0.25, 2:2:0.50). In these formulations, the specific amount of drug and soya lecithin were taken into a 100 ml round bottom flask and refluxed with 30 ml of dichloromethane at a temperature not exceeding 60°C for 2 h. The mixture is concentrated to 5 ml then, this mixture was introduced drop wise (0.2 ml/min) into aqueous glacial acetic acid solution of chitosan using magnetic stirrer with increasing temperature and n-hexane (20 ml) was also added carefully with continuous stirring to get the precipitate. The precipitate was filtered, collected and stored in vacuum desiccators for overnight. Powdered gingerol phytosome loaded complexes was placed in amber colour glass bottle and stored at room temperature.

Optimization and Characterization of GP and GPLC:
GPLC and GP were characterized by FTIR, DSC and SEM and evaluated for % yield, % Entrapment efficiency, % drug loading, particle size analysis, zeta potential, solubility study and in vitro drug release.

Determination of % yield:
Determination of % yield of GP and GPLC was calculated by the following formula: 7-8

\[
\% \text{ Yield} = \frac{\text{Practical yield}}{\text{Theoretical yield}} \times 100
\]

Determination of entrapment efficiency and drug loading:
The entrapment efficiency and drug loading of GP and GPLC were determined by centrifugation method. Both GP and GPLC were centrifuged with 10 ml of methanol at 5000 rpm for 10 min. The free amount of the gingerol in the filtrate was determined by UV-Vis spectrophotometer (Shimadzu-1800, Japan) at 280 nm. The absorption measurements of samples were performed in triplicate. The entrapment efficiency and drug loading was calculated by the following formula: 9-10

\[
\text{Entrapment efficiency (\%)} = \frac{\text{Total amount of drug}}{\text{Total amount of drug - Amount of free drug}} \times 100
\]

\[
\text{Drug loading (\%)} = \frac{\text{Weight of the drug}}{\text{Weight of the phytosome}} \times 100
\]

Determination of particle size and zeta potential:
The average diameter and surface charge property of GP and GPLC were measured by laser diffraction using a Mastersizer 2000 (Malvern Instruments Ltd., UK) at a fixed scattering angle of 90° at 25°C. GP and GPLC were diluted with double-distilled water and particle size and zeta potential was determined.

In vitro drug release:
On the basis of literature survey phosphate buffer pH 7.4 (900 ml) was used as a dissolution medium for 24 hours and maintained at 37 ± 0.5°C. Dissolution study was carried out using dissolution apparatus, USP II paddle method at 50 rpm. The cotton tea bag was used to carry out the in vitro drug release that contains GP and GPLC. Cotton tea bags were dipped into jar containing medium and it was closed to prevent evaporation of the dissolution medium. At predetermined time intervals, aliquots were withdrawn from the release medium and replaced with the same amount of phosphate buffer and analyzed by UV spectrophotometer (Shimadzu-1800, Japan).

In vitro – In vivo Evaluation of optimized GP and GPLC

In vitro Anti-oxidant Activity
To assess the DPPH (1, 1-diphenyl 2-picryl-hydrazyl) radical scavenging effects, 2 mL of each test sample (gingerol, optimized formulations and Ascorbic acid) concentrations of 5, 10, 20, 40, 80 and 100 μg/mL and 2 mL solution of DPPH (0.1 mM in methanol) was included independently. The reaction blend was shaken and incubated in the dark for 30 min, at room temperature and the absorbance was recorded at 517 nm by UV spectrophotometer (Shimadzu-1800, Japan) against methanol as a control with DPPH solution. Vitamin C (Ascorbic acid) was utilized as a standard antioxidant in this method. The assay was performed in triplicate. The % inhibition of the DPPH radical scavenging of the test sample was figured with reference to control absorbance. The percentage of DPPH radical scavenging activity was plotted against the concentration.

% DPPH scavenging = (Abs of control – Abs of Test sample) × 100

(Abs of control)
inoculated by streaking over the entire surface of respected petri plates. At that point, an opening with a distance across of 6 to 8 mm was punched aseptically with a sterile stopper borer or a tip, and a volume (150 μL) of drug, optimized formulations at desired concentrations 100, 150, 200 and 250 μg/mL and Amikacin as a positive control at 10 μg/mL concentration was introduced into the respected inoculated well. DMSO was used as a negative control in each petri plates. Then, agar plates were incubated at 37°C for 24 h. The above procedure was carried out in aseptic condition and the experiment was performed in triplicate. The test sample diffuses in the nutrient agar media and inhibits the growth or diameter of zone of inhibition of the microbial strain was estimated 15–16.

**In vitro anti-inflammatory Activity**

The human red blood cells (HRBC) membrane stabilization method was utilized to assess the anti-inflammatory potential of test samples. Drug, optimized formulations and Aspirin as a reference positive control were used for the ex-vivo anti-inflammatory activity. Adequate amount of blood was withdrawn from typical sound people who were not under NSAIDs treatment for 14 days prior to the study. The same volume of sterilized Alsever solution which was composed of 0.5% anhydrous citric acid, 2% dextrose, 0.42% NaCl, 0.8% and sodium citrate was added to the collected blood. The blood was centrifuged (Remi ElektroTechnik Ltd, Vasai, India) at 3000 rpm and stuffed cells was washed with isosaline (0.9% or 0.85% w/v NaCl, pH 7.2) and a 10% suspension was made with isosaline. The reaction mixture (4 mL) contains different concentrations (10, 20, 40, 60, 80, 100 μg/mL) of the each test samples of drug, optimized formulations was prepared individually using dimethyl sulfoxide (DMSO). Aspirin was prepared in methanol. 1 mL phosphate buffer (pH 7.4, 0.15 M), 2 mL hyposaline (0.36% NaCl), and 0.5 ml HRBC suspension were added to each test sample concentration. After the preparation of reaction mixture, it was incubated at 37°C for 30 min and centrifuged at 3000 rpm for 20 min. The haemoglobin content in the supernatant solution was measured at 560 nm using UV spectrophotometer (Shimadzu-1800, Japan). Similar volume of DMSO for test samples and methanol for reference were served as a negative control. The percentage haemolysis computed by accepting the haemolysis delivered by the control aggregate as 100%. The percentage of HRBC membrane stabilization or protection was calculated using the formula: 17-18

\[
\text{% Inhibition} = \frac{\text{Abs of Control} - \text{Abs of Test Sample}}{\text{Abs of Control}} \times 100
\]

**Abs of Control**

**In vivo Study**

**In vivo Pharmacokinetic Study**

All experimental animals (New Zealand albino rabbits; 1.5 – 3.0 kg) were acclimatized to the laboratory conditions for a period of one week prior to the initiation of experiment. The experiment animals were divided into six groups and each group reserved six rabbits to get statistically meaningful and significant data as well as to avoid the errors. On the day of experiment, rabbits were anesthetized with an i.m. injection of ketamine HCl (50 mg/kg) and the single administration of p.o. of drug (100 mg/kg), optimized phytosome (100 mg/kg), optimized phytosome loaded complex (100 mg/kg), drug with chitosan (100 mg/kg) and chitosan (30 mg/kg) were given to the respective groups. Control group was treated by saline. Then, 0.5 mL of blood was collected in heparinized capillary tubes from marginal ear vein of the rabbit from respective groups at different time intervals (0 - 24 h). The plasma was separated from the collected blood by centrifugation at 3000 rpm for 10 min at 4°C. Each 200 μL sample of plasma was mixed with 5μL of drug concentration (80-180 μg/mL). Extraction of drug was accomplished by adding of 600μL of ethyl acetate to the diluted plasma and vortexed for 2 min. The samples were then centrifuged at 10000 rpm for 10 min and the supernatant was dried under nitrogen gas stream at 40°C. The dried supernatant was dissolved in 600 μL of acetonitrile, vortexed and centrifuged at 10000 rpm for 15 min at 5°C. The supernatant was passed through 0.2 μm membrane filter and 20 μL of the filtrate solution was injected into HPLC 19-21.

**In vivo Pharmacodynamics Study**

To assess the pharmacodynamics or haematological/blood assessment parameters with and without inducing of microbe, 0.5mL of blood was collected at different time intervals (0 -24 h) after the administration of respective samples to respective rabbit groups as mentioned in above procedure. The complete blood counts (CBC) were measured by Automated Haematology Analyser 22-23.

3. RESULTS AND DISCUSSION

The phytosomes of gingerol (GP1-GP5) and gingerol phytosome loaded chitosan complex (GPLC1-GPLC9) were prepared by anti-solvent precipitation technique. After the evaluation, the % yield of GP was found between 37.31±0.04 % to 85.35±0.02 % and GPLC was 28.22±0.10 to 73.96±0.01, respectively. The % EE and % drug loading of GP was between 60.12±0.07 to 84.36±0.42, 40.02±0.06 to 58.05±0.03 and GPLC was 51.09±0.13 to 86.02±0.18, 27.89±0.20 to 59.26±0.72, respectively. The Particle size of GP was between 37.31±0.04 % to 85.35±0.02 µm and GPLC was 193.67±0.04 to 490.19±0.10 nm and GPLC was 161.02±0.05 to 193.67±0.04 to 490.19±0.10 nm and GPLC was 78.29±0.05 to 86.03±0.06 % and GPLC was 79.00±0.34 to 88.93±0.33 % at the end of 24 h, respectively as shown in Fig. 1. The Phytosome of gingerol (GP4) and gingerol phytosome loaded chitosan complex (GPLC4) showed better % yield, % EE, % drug loading, particle size and in vitro cumulative drug release as compared to other GP and GPLC.
To accomplish the main objective of the current work to treat the respiratory infection as well to correlate the *in vitro* and *in vivo* studies together, the *in vivo* pharmacokinetic and pharmacodynamic studies were performed. In the pharmacokinetic study, we observed the use of GLPC4 and GP4 as a more efficient carrier to improve the bioavailability of gingerol followed by the comparing of relevant pharmacokinetic parameters after the p.o. administration of respected treatments. The mean plasma concentration vs time profile of gingerol was shown in Figure 3, showed that the absorption of gingerol increased significantly. After oral administration, gingerol was absorbed rapidly from the gastrointestinal tract, detected the maximum concentration ($C_{\text{max}}$) in the blood at 0.15± 0.05 h and reached maximum $T_{\text{max}}$. These results demonstrated that GLPC4 had a better sustained-release profile and better promoted oral absorption of gingerol than GP4 or gingerol + chitosan. Thus, we conclude that phytosome loaded complex delivery system, that is, a carrier combining chitosan and phytosome, is a more favorable option for oral administration of gingerol.

From the *in vivo* pharmacodynamics study concluded that the values of the white blood cells and the specific differential counts of lymphocytes and neutrophils were in between the normal values. Hence, it was confirmed that the rabbit were in healthy and wealthy stage without presence of any infection. After the incubation of microbes to healthy rabbits, almost all the WBC Differential counts parameters were behind as far as the reference in control group at different time intervals which confirmed the presence of bacterial infection in the blood cells. During the early stage of the infection by *S. aureus* and *E. coli*, GLPC4 was not showing any phenomenal effect to stop the growth of infection and inflammation but after reaching of GLPC4 to the GIT site at its half-life ($T_{1/2}$), it has shown efficiently inhibition activity. Even, from the hematological parameters,
GLPC4 was found to be more accurate against WBC count in the order of GLPC4 < chitosan < gingerol + chitosan < gingerol < GP4 at the end of 24 h. Lymphocytes and neutrophils were in between the reference values as their count concerned and expressed. From the differential WBC count, GLPC4 was found to be more accurate against neutrophils count in the order of GLPC4 < gingerol + chitosan < chitosan < gingerol < GP4 at the end of 24 h. These results confirmed to GLPC4 as an effective sustained antibacterial and anti-inflammatory formulation against the S. aureus and E. coli bacterial organism.

4. CONCLUSION

The above results indicated that the novel approach of GPLC drug delivery system combined the advantages of chitosan with GP, which showed better effects of promoting oral absorption and prolonging retention time of complex of gingerol loaded phytosome than gingerol phytosome or blank complex of phytosome of gingerol.

ACKNOWLEDGEMENT

The author’s are gratefully acknowledging the support extended by Principal and Management, JSS College of Pharmacy, JSS Academy of Higher Education & Research, Mysuru, India

CONFLICTS OF INTEREST

The authors do not have any conflict of interest.

REFERENCE


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