Guggulsterone phytosomes: A novel approach to alleviate hyperlipidemia in high-fat diet-fed rats

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A B S T R A C T

Introduction: Guggulsterone, derived from *Commiphora mukul*, is a potent hypolipidemic medication with low bioavailability and water insolubility. To address these challenges, the study aimed to formulate phytosomes and evaluate the efficacy of guggulsterone phytosomes (GPs) in reducing hyperlipidemia in rats on a high-fat diet.
Methods: GPs were formulated by incorporating soya lecithin with a suitable solvent to enhance their efficacy against hyperlipidemia induced by a high-fat diet in rats. The optimized GPs were characterized, and in vitro drug release pattern was examined. The hypolipidemic effect of GPs (25 mg/kg body weight) was evaluated in Sprague Dawley rats over 28 days.
Results: The GPs demonstrated favorable entrapment effectiveness with a particle size of 145.4 nm and a zeta potential of -17.8 mV. In terms of drug release, the GPs exhibited better stability and bioavailability, with a release of 92.07 ± 1.67% within 24 hours, compared to pure guggulsterone, which only released 28.07 ± 0.81%. GPs elevated the levels of high-density lipoprotein (HDL) and significantly ($P < 0.05$) reduced triglycerides (TG), low-density lipoprotein (LDL) levels, and total cholesterol (TC), compared to their respective control groups. Moreover, GPs showed substantial ($P < 0.05$) antioxidant activity, reduced steatosis, inflammatory cell, and fat cell infiltration in the liver tissue.
Conclusion: GPs exhibited hypolipidemic activity in rats with high-fat diet-induced hyperlipidemia compared to pure guggulsterone. These findings emphasize the potential of GPs as an effective therapeutic intervention for managing hyperlipidemia, surpassing the conventional use of the pure compound.

Introduction

Obesity and hyperlipidemia are significant risk factors for fatal diseases like heart disease and stroke contributing to approximately 74% of all global deaths. Among these deaths, cardiovascular diseases account for a significant share with about 17.9 million deaths occurring annually. It is worth noting that a majority of these deaths are reported in low- and middle-income countries (1). Hyperlipidemia is a medical condition characterized by abnormal elevation of serum lipids, including low-density lipoprotein (LDL), total cholesterol (TC), or triglycerides (TG), or a decrease in high-density lipoprotein (HDL) levels. This condition can lead to the formation of atheroma and other cardiovascular-related problems, such as obesity and myocardial infarction (2). Hyperlipidemia and obesity are recognized as the primary risk factors for cardiovascular diseases and associated deaths worldwide (3). According to data from the World Health Organization (WHO), 39% of adults over 18 years are overweight, and 13% are classified as obese. In India, the prevalence of cardiovascular diseases is increasing annually accounting for 24% of total deaths. Notably, the impact is particularly

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significant among young populations below 40 years of age with rates three times higher than those in developed countries (4).

Statins are the first-line treatment option for hyperlipidemia among many treatment alternatives. They function by blocking the enzyme 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase. However, a challenging adverse effect associated with statins is rhabdomyolysis, which can lead to life-threatening conditions such as hypokalemia and acute kidney failure (5). One of the significant challenges in allopathic treatment for hyperlipidemia is ensuring patient compliance with the available therapies while minimizing potential adverse effects. As a result, patients and physicians are increasingly interested in alternative therapies derived from natural sources, as they can potentially avoid drug interactions and have fewer side effects (6). Medicinal plants and plant-based diets, including vegetables, legumes, and fruits, are rich sources of phytoconstituents that show promise in protecting against cardiovascular disorders, including their potential hypolipidemic effects (7).

Guggulsterone, a significant natural bioactive steroidal alkaloid derived from the oleoresin of *Commiphora mukul*, exists in the form of E-guggulsterone and Z-guggulsterone stereoisomers. It has been recognized for its adjuvant therapeutic role in conditions such as atherosclerosis, obesity, and hyperlipidemia (8,9). Guggulsterone exerts its hypolipidemic effect by blocking the farnesoid X receptor, which plays a critical role in cholesterol and bile acid metabolism (10). In numerous *in vitro* and *in vivo* studies, Guggulsterone and its derivatives have been found to exert their hypolipidemic effects by blocking the farnesoid X receptor, which plays a crucial role in the metabolism of cholesterol and bile acids (10,11).

Achieving optimal bioavailability and maintaining steady-state concentrations of phytocompounds in conventional formulations pose significant challenges in their conversion into therapeutic agents. However, parenteral preparations with 100% bioavailability have limitations such as drug release control and targeting the specific site of action or receptor (12). Despite the potential pharmacological activities of guggulsterone, its low bioavailability and water insolubility hinder its conversion into an effective therapeutic agent.

The application of novel drug delivery systems, such as nanoparticles, liposomes, and phytosomes, to biologically active compounds can enhance the efficiency, control the release pattern, and facilitate targeted delivery, thereby overcoming the challenges associated with conventional formulations (13). Phytosomes, a part of this innovative drug delivery system, involve the entrapment of the drug (phytocompound) within a lipid membrane, thereby enhancing absorption, bioavailability, and ensuring clinical benefits for the treatment of various diseases.

Typically, isolated compounds or standardized plant extracts are used to prepare phytosomes (14).

Based on the hypothesis that entrapping guggulsterone as phytosomes using soya lecithin can enhance its hypolipidemic potential, the present study aimed to develop guggulsterone phytosomes (GPs). These GPs formulations were characterized for entrapment efficiency, zeta potential, particle size, and drug release profiles in *in vitro* studies, demonstrating stability and improved bioavailability. Moreover, the study also aimed to evaluate the efficacy of GPs in reducing hyperlipidemia induced by a high-fat diet in rats, with proper control measures in place to validate the hypothesis.

Materials And Methods
Chemicals
Both guggulsterone and soy lecithin were procured from Sigma Aldrich Co. in St. Louis, Missouri, USA (CAS number: 39025-24-6). Shuddha Guggulu, a standard medication, was obtained from Himalaya Drug Company. The atorvastatin employed in the current investigation was a gift sample provided by Divis Laboratories, India, and all other compounds were of analytical quality.

Preparation of GPs
Phytosomes of guggulsterone were prepared using the method described by Sharma et al (15) with minor modifications. The GPs were prepared by employing the solvent evaporation technique with soya lecithin. In this process, 50 mg of guggulsterone and 150 mg (1:3 ratio) of soya lecithin were taken in a round-bottom flask containing 50 mL of acetone. The mixture was refluxed for 2 hours at 55 °C, followed by the removal of acetone under reduced pressure using a rotary evaporator at 60 °C. The resulting guggulsterone-soya lecithin complexes (phytosomes) were continuously stirred with n-hexane for 2 hours at 40 °C. Subsequently, the hexane was eliminated through filtration, and the formed phytosomes were dried under vacuum to eliminate any residual solvents. Finally, the GPs, appearing as a thin film, were stored in an amber-colored bottle for further studies (15).

In vitro characterization
Scanning electron microscope (SEM) studies
The solid-state and surface morphologies of the optimized GPs complexes were examined using a scanning electron microscope (Carl Zeiss Merlin Compact, Chennai). The GPs sample was applied to an aluminum stub, dried under vacuum conditions, and then sputtered with gold. Images were captured at an acceleration voltage of 3 kV while the coated samples were in a vacuum environment (16).

Entrapment efficiency (EE)
To confirm the formation of the GPs complex and assess its interaction with lecithin lipid, GPs (25 mg) were dissolved

in distilled water and subjected to thorough centrifugation at 3000 rpm for 15 minutes. The supernatant was separated, and the absorbance was measured at 254 nm to determine the concentration of free guggulsterone. The amount of guggulsterone present in the total GPs complex was calculated, and the entrapment efficiency was determined using the following formula (17).

$$\%EE = 100 \times \frac{Total\ amount\ of\ guggulsterone - the\ amount\ of\ free\ guggulsterone}{Total\ amount\ of\ guggulsterone} \quad (1)$$

Where EE = Entrapment efficiency

Particle size and size distribution studies
The particle size distribution and zeta potential of the developed GPs were evaluated using the dynamic light scattering technique with a scattering angle of 90° at a controlled temperature of 25 ± 0.5 °C (Zetasizer, Horiba SZ-100) (18). The zeta potential, representing the electric potential of the GPs, was determined by preparing the test sample at a concentration of 0.5 mg/mL and then diluting it ten times. All measurements were conducted in triplicate at a temperature of 25 °C.

Drug release studies in vitro
The *in vitro* release of guggulsterone from GPs was evaluated using a dynamic dialysis method. The dialysis bags employed had a molecular weight cut-off (MWCO) of 12 000-14 000 g/mol and a pore size of 2.4 nm. To perform the experiment, a 50 mg of GPs was placed inside each dialysis bag and then submerged in 500 mL of buffer solution at pH 7.4 and maintained at a temperature of 37 ± 1 °C. The system was stirred at a rotation speed of 50 rpm using a paddle. At predetermined time intervals, 5 mL samples were withdrawn and replaced with an equal volume of buffer solution. The concentration of guggulsterone in the withdrawn samples was determined using an ultra-violet (UV) spectrophotometer at a wavelength of 254 nm. Based on these measurements, the percentage of drug release was calculated (19).

MTT (3-[4,5-dimethylthiazol-2-yl]-2,5 diphenyl tetrazolium bromide) assay
Viability and compatibility of optimized GPs with cells were evaluated through *in vitro* cell line studies. For this study, mouse fibroblasts (3T3-L1) from the National Center for Cell Sciences (Pune, India) were chosen as the cell lines. The 3T3-L1 cells were cultured in Dulbecco's modified eagle's medium (DMEM) supplemented with 10% (v/v) bovine calf serum and 1% (v/v) penicillin-streptomycin. Throughout the experiments, the cells were maintained at a temperature of 37 °C and 5% CO_2. The cells were grown and seeded in 96-well plates at a density of 1×10^4 cells per well. Subsequently, the cells were treated with various concentrations of GPs (1, 5, 10, 20, 40, 60, 80, and 100 µg/mL) and incubated with MTT solution at 37 °C for 3 hours. Following the incubation, formazan formation was stabilized using 150 µL of dimethyl sulfoxide (DMSO) for 30 minutes, and the absorbance was measured at 540 nm using a UV spectrophotometer. This allowed the assessment of cell viability (1).

In vivo studies
Animals
In this study 36 male Sprague-Dawley rats weighing between 170 and 200 g were randomly assigned to 6 groups. All of the animals were maintained in regular settings during the experiment and were given free access to water and formulated food. The rats were kept in polypropylene cages that were maintained at a humidity of 60 ± 5%, a temperature of 25 ± 2 °C, and 12-hour light/dark cycle.

The experimental methods and procedures conducted in this study were reviewed and approved by the Institutional Animal Ethical Committee of the Sri Padmavathi School of Pharmacy, following the guidelines set by the Committee for the Purpose of Control and Supervision of Experiments on Animals (CCSEA). The approval reference number for this study is SPSP/1016/PO/Re/S/06/CPCSEA/IAEC/2022/03.

Acute toxicity
Acute toxicity study was conducted according to the organization for economic co-operation and development (OECD) 423 guidelines in female rats. Single dose of GPs was administered at 2000 mg/ kg body weight to 3 female animals. Animals were observed individually after dosing at 30 minutes, 1 hour, 2 hours, 4 hours and 24 hours for behavioral, motor, and autonomic changes. Thereafter, they were observed for mortality or moribundity for 14 days (20).

Preparation of high fat diet (HFD)
HFD was prepared following the method described by Iftikhar et al. (15) with minor modifications. The composition of HFD was casein (20%), corn starch (15%), sucrose (20%), beef tallow (35%), Corn oil (5%), and a vitamin and mineral mixture (5%). These ingredients were mixed thoroughly and homogenised with warm water; pellets were prepared, dried in a hot air oven, and stored for further use (21).

Experimental schedule
The treatment schedule (Table 1) for investigating the anti-hyperlipidemic effects of GPs was designed for a duration of 28 days. The drug treatment was administered at the same time each day. To deliver the drugs, a 0.5% carboxymethyl cellulose solution was used as the vehicle.

Blood sample collection and analysis
After completing the 28-day treatment with the test drug,

Table 1. Experimental design

Group	Treatment	Purpose
Group 1	0.5% Carboxymethyl cellulose with normal rat pellet diet	Served as normal control
Group 2	HFD	Served as disease control
Group 3	Atorvastatin (10 mg/kg) + HFD	Served as standard treatment control
Group 4	Shuddha Guggulu (25 mg/kg) + HFD	Served as marked formulation control
Group 5	Guggulsterone phytosomes (25 mg/kg) + HFD	Served as test (formulation)
Group 6	Pure guggulsterone (25 mg/kg) + HFD	Served as test control

HFD, high fat diet.

blood samples were collected from all animals through cardiac puncture. The blood collection was performed under anesthesia induced by thiopental sodium (20 mg/kg body weight, intraperitoneal). The collected blood samples were then centrifuged at 3000 rpm to separate the serum, which was subsequently stored at -20 °C for future use.

Body weight changes & serum biochemical parameters
The study assessed the impact of GPs on changes in body weight, measured at the beginning and end of the study (Figure 1). Serum lipid levels, including TC, TG, and HDL, were analyzed using diagnostic test kits from AGAPPE diagnostics, Kerala, India, and measured using a spectrophotometer. LDL and very low-density lipoproteins (VLDL) levels were calculated based on the obtained data. Liver biomarkers, namely alkaline phosphatase, serum glutamic-oxaloacetic transaminase (SGOT), serum glutamic pyruvic transaminase (SGPT), and total protein were estimated using diagnostic test kits (22).

$$Atherogenic\ Index\,(AI) = \frac{Total\ cholesterol - high\ density\ lipoproteins}{high\ density\ lipoproteins} \quad (2)$$

Determination of serum antioxidants
At the end of the experimentation, serum was used for the estimation of *in vivo* antioxidant enzymes like superoxide dismutase (SOD), reduced glutathione (GSH), catalase (CAT), and lipid peroxidation (LPO) (21-24).

Histopathological studies of aorta and liver
After isolating the liver tissue, it was rinsed with ice-cold saline to eliminate all blood and preserved in a 10% buffered neutral formalin solution. Tissues were embedded in paraffin after fixation and their serial sections were cut to 0.5 μm. Hematoxylin and eosin were used to stain each segment. Under a light microscope, the slices were evaluated for histological alterations.

Statistical analysis
Data were represented as mean ± standard deviation (SD). Data were subjected to one-way analysis of variation (ANOVA) followed by Bonferroni's multiple comparison as post hoc test using graph pad prism version 5.0. A $P < 0.05$ was considered statistically significant.

Results
Morphology, particle size, and electro kinetic potential
It was found that the optimised GPs had a spherical, uniform distribution, somewhat rough, and porous texture. Zeta potential and particle size of the optimised GPs were determined to be 145.4 nm and -17.8 mV, respectively (Figure 1).

Drug release in in vitro studies
The percentage of cumulative drug release was assessed for 24 hours in 0.01M phosphate-buffered saline (PBS) at pH 7.4. The percent cumulative release of guggulsterone from optimized GPs was found to be 92.07 ± 1.67 and the pure guggulsterone was 28.07 ± 0.81 (Figure 2).

Cell viability of GPs in in vitro studies
To assess the impact of the marketed formulation, GPs, and pure guggulsterone on 3T3-L1 cell viability, an MTT assay was conducted. The cells were treated with concentrations ranging from 1 to 100 μg/mL of the respective formulations. Following the effective treatment, it was observed that the cell viability decreased as the drug concentration increased across all three formulations (Figure 3).

Effect of GPs on body weight
When compared to a normal control group, the body weights of rats fed an HFD alone, showed a substantial ($P < 0.05$) increase from day 0 to day 28. The body weights of rats treated with GPs decreased significantly ($P < 0.05$) compared to the pure guggulsterone group (Table 2).

Effect of GPs on serum lipid profile
After 28 days of treatment with the test medication, changes in the serum lipid profile were assessed. Rats on HFD alone showed a significant increase ($P < 0.05$) in blood TC, TG, LDL, and VLDL and a decrease in HDL level when compared to the normal control group. When compared to the HFD group, treatment with GPs for 28 days caused a substantial decrease ($P < 0.05$) in blood TC, TG, LDL and VLDL levels and an increase in HDL level (Table 3).
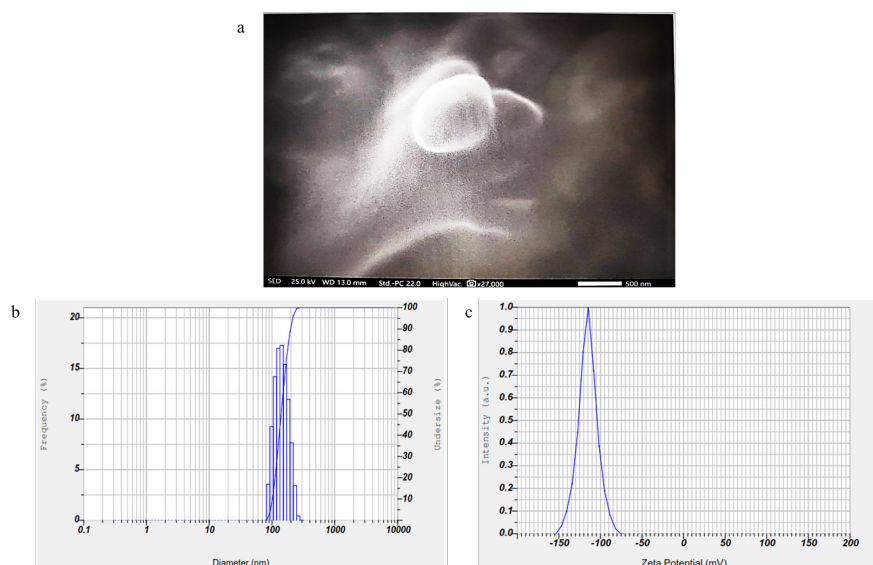
Figure 1. Physical characterization of guggulsterone phytosomes (GPs) by scanning electronic microscope (SEM). The analysis revealed spherical shaped GPs (a); the particle size found to be 145.4 nm (b); the zeta potential of GPs found to be -17. 8 mV.
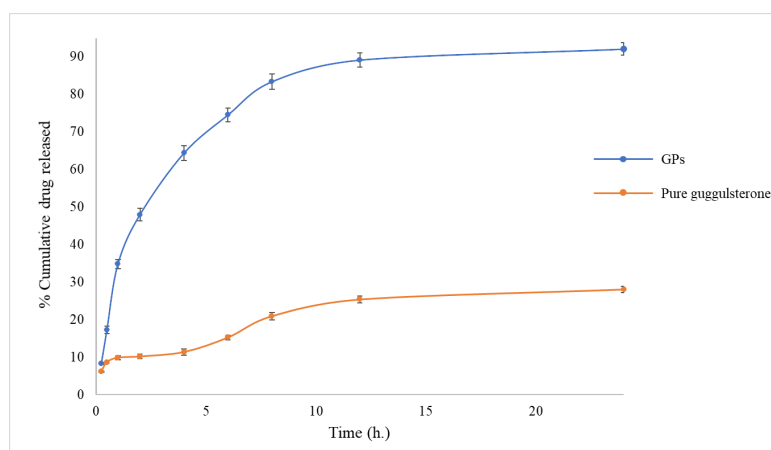

Figure 2. Release pattern of guggulsterone phytosomes (GPs) and pure guggulsterone.

Effect of GPs on atherogenic index

Animals in the HFD control group had an atherogenic index that was significantly higher ($P < 0.05$) than those in the normal group. When compared to the HFD control group, the same was considerably decreased in all pharmacological treatment groups, including the GPs group (Figure 4).

Effect of GPs on serum antioxidant enzymes

Changes in serum antioxidant enzyme levels were evaluated to estimate their role in hyperlipidemia. The results showed a significant ($P < 0.05$) decline in GSH, CAT, and SOD levels and a substantial increase in LPO in the HFD-fed group compared to the normal control group. Animals treated with GPs and pure guggulsterone significantly showed elevated serum antioxidant levels and reduced LPO levels (Figure 5). Animals treated with

GPs showed a predominant antioxidant effect compared to other formulations.

Effect of GPs on histopathology of liver

Upon the microscopic examination of the H&E-stained liver tissue, a radial arrangement of hepatocytes and normal-appearing sinusoids around the central vein were observed in the normal control group. Contrarily, the HFD group displayed the presence of micro and macro vesicular lipid droplets in the cytoplasm of hepatocytes surrounding the central vein. Additionally, moderate central vein dilation, congestion, fat globules, and hepatocyte necrosis were observed. However, treatment with GPs reversed these histopathological changes. The hepatocytes and sinusoids exhibited a normal arrangement around the central vein, a smaller number of lipid droplets in the hepatocytes, and a decrease in the
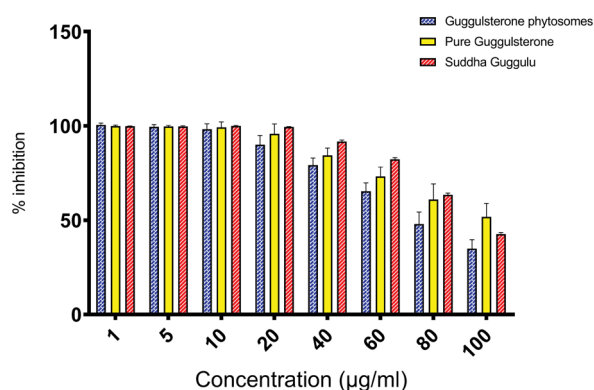
Figure 3. Effect of marketed formulation, GPs (guggulsterone phytosomes), and pure guggulsterone on 3T3-L1 cell viability at 1 to 100 µg/mL concentrations.
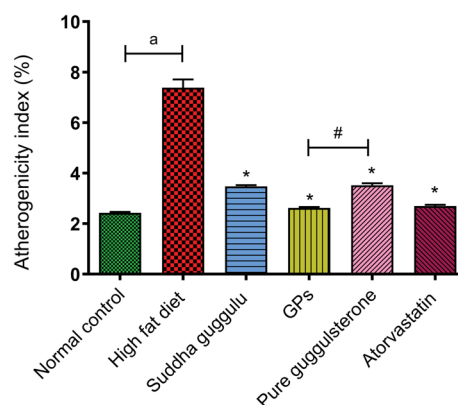

Figure 4. Effect of guggulsterone phytosomes (GPs) on atherogenic index. [a] $P < 0.05$ when high-fat diet (HFD) group was compared with the normal control group. * $P < 0.05$ when test groups were compared with the HFD group. # $P < 0.05$ when GPs group was compared with the pure guggulsterone group.

Table 2. Effect of guggulsterone phytosomes (GPs) on body weight

Groups	0 day	28th day
Normal control	183.5 ± 9.979	203.3 ± 8.819
High fat diet	180.0 ± 7.303	323.3 ± 13.33[a]
Shuddha guggulu	181.7 ± 5.270	268.2 ± 4.282*
GPs	186.7 ± 6.667	252.5 ± 7.630*
Pure guggulsterone	185.8 ± 5.231	277.7 ± 6.667[#]*
Atorvastatin	185.5 ± 3.416	245.1 ± 4.282*

[a] $P < 0.05$ when HFD group was compared with normal control group. * $P < 0.05$ when test groups were compared with HFD group. # $P < 0.05$ when GPs group compared with pure guggulsterone group.

infiltration of inflammatory cells indicating a restoration of liver health (Figure 6).

Discussion

Guggulsterone, an essential bioactive compound found in Commiphora species, has been recognized for its anti-hyperlipidemic properties (9). In the present study, an attempt was made to enhance the hypolipidemic potential of guggulsterone using a phytosomes formulation, and the results were promising. These advancements aim to overcome the challenges associated with conventional formulations and improve the therapeutic outcomes (23).

Soybean, being a rich natural source of phosphatidylcholine, contributes to the stability of GPs due to the resistance of the choline group to oxidation (24). The solvent evaporation method, utilizing acetone, successfully enhances the complexation between guggulsterone and soya lecithin, ensuring uniform drug entrapment. Scanning electron microscope analysis confirms that the optimized GPs exhibit a porous texture, spherical shape, and uniform distribution. The spherical shape of the phytosomes maintains the amphiphilic properties of soya lecithin, facilitating contact with the cell membrane and enhancing absorption (25). The particle size and zeta potential of the phytosomes play crucial

Table 3. Effect of guggulsterone phytosomes (GPs) on serum lipid profile against high-fat diet (HFD) fed induced hyperlipidemia in rats

Group	TC		TG		HDL		LDL		VLDL	
	Day 0	Day 28	Day 0	Day 28	Day 0	Day 28	Day 0	Day 28	Day 0	Day 28
Normal control	99.24 ± 2.19	105.58 ± 2.64	79.06 ± 2.96	81.54 ± 2.39	46.08 ± 1.17	43.38 ± 0.88	71.88 ± 1.54	73.56 ± 0.89	31.92 ± 1.3	32.89 ± 1.3
HFD	99.13 ± 3.21	182.63 ± 4.5[a]	78.5 ± 2.83	172.39 ± 3.31[a]	45.36 ± 1.66	24.93 ± 2.66[a]	72.21 ± 1.39	148.01 ± 4.18[a]	32.47 ± 1.07	75.38 ± 3.25[a]
Shuddha guggulu	100.8 ± 1.66	130.5 ± 2.73*	77.78 ± 2.68	92.59 ± 2.19*	46.08 ± 1.7	37.56 ± 1.7*	73.18 ± 1.62	84.18 ± 1.05*	31.9 ± 1.52	45.28 ± 2.37*
GPs	102.06 ± 2.71	115.23 ± 1.51[*#]	80.51 ± 1.43	85.59 ± 1.48[*#]	45.8 ± 1.24	43.88 ± 1.61[*#]	75.08 ± 1.43	73.73 ± 2.07[*#]	31.66 ± 0.66	31.1 ± 1.85[*#]
Pure guggulsterone	98.92 ± 2.71	132.89 ± 2.39*	78.48 ± 3	97.6 ± 3.66*	46.47 ± 1.6	37.81 ± 2.36*	72.11 ± 2.01	94.99 ± 2.41*	32.64 ± 1.17	51.86 ± 3.43*
Atorvastatin	98.57 ± 2.11	117.67 ± 1.99*	79.75 ± 2.42	87.29 ± 1.16*	45.12 ± 1.44	43.55 ± 1.36*	74.45 ± 2.21	74.52 ± 1.32*	32.35 ± 0.82	32.27 ± 1.14*

[a] $P < 0.05$ when HFD group was compared with the normal control group. * $P < 0.05$ when test groups were compared with the HFD group. # $P < 0.05$ when GPs group was compared with the pure guggulsterone group.
TC: Total cholesterol; TG: Triglycerides (TG); HDL: High-density lipoprotein; LDL: Low-density lipoprotein; VLDL: Very low-density lipoprotein.
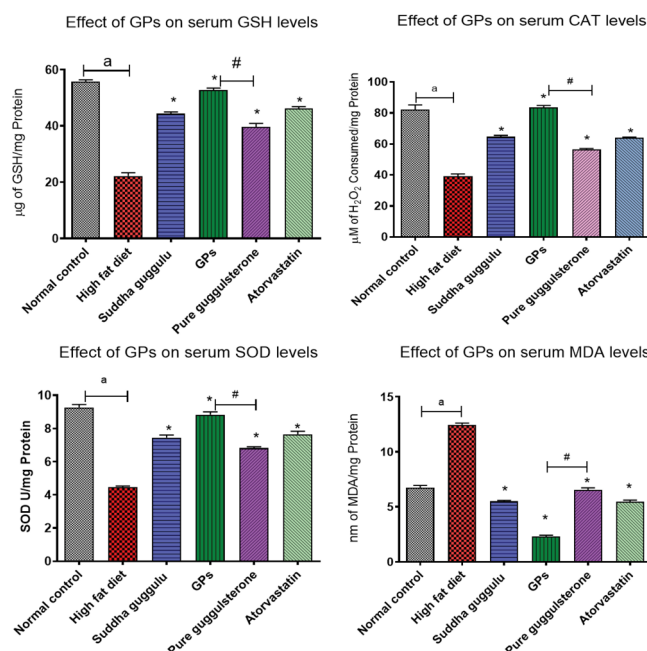
Figure 5. Effect of guggulsterone phytosomes (GPs) on serum antioxidant enzymes. [a] $P < 0.05$ when high-fat diet (HFD) group was compared with the normal control group. * $P < 0.05$ when the test groups were compared with the HFD group. # $P < 0.05$ when GPs group was compared with the pure guggulsterone group. MDA: malondialdehyde; SOD: superoxide dismutase; GSH: glutathione; CAT: catalase.

roles in their stability and reproducibility (26). Repulsion between charged particles aids in maintaining the stability of the formulation, while phytosomes with a zeta potential greater than 30 mV are considered stable and effective (27). The GPs prepared in this study have a particle size of 145.4 nm and a zeta potential of -17.8 mV. These characteristics significantly improve the bioavailability and absorption of guggulsterone, thereby enhancing its therapeutic effects compared to pure guggulsterone, which exhibits lower bioavailability.

Rapid drug release makes maintaining the study state concentration challenging to achieve the desired therapeutic effect (28). The percentage of cumulative drug release for GPs and pure guggulsterone was studied, and GPs found to be significantly (92.07 ± 1.67) higher than pure guggulsterone (28.07 ± 0.81). The increase in *in vitro* release of the GPs resulted from improved solubility due to complex formation with soya lecithin. Apparently, the release of GPs showed a very rapid initial burst, followed by a slow drug release, while pure guggulsterone shows a
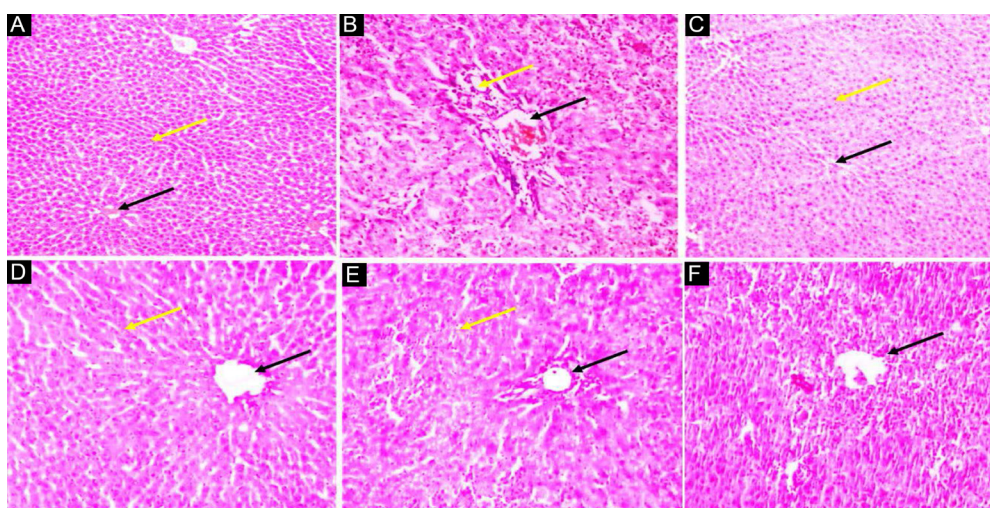

Figure 6. Photomicrographs of the rats' livers stained with H&E stain at 40X. A): Normal rat's liver showing the standard texture of hepatocytes with central vein. B) High-fat-diet (HFD) alone fed rat's liver showing inflammatory cell infiltration and steatosis. C) Shows Atorvastatin protection. D) guggulsterone phytosomes (GPs) treated rats showing restored inflammatory cell infiltration and steatosis. E-F) Moderate protection with marketed formulation and pure guggulsterone. Black arrows indicate central vein; yellow arrows indicate sinusoids and hepatocytes arrangement.

prolonged release from the membrane due to a low water solubility. The initial fast release of guggulsterone suggests that a section of drug was confined on the surface of the phytosomes. Hence, the initial rapid releasing pattern followed by the sustained release of active drug from GPs not only maintains bioavailability but also enhances therapeutic efficacy (29,30).

The hypolipidemic activities of guggul have been well studied in experimental animal models and cell line studies. The body weight and lipid profile of high-fat diet-fed rats and rabbits, along with guggul and purified guggulsterone, significantly produced hypolipidemic effects (31). Phytosomes have incredible benefits like good drug encapsulation, stability, release, and bioavailability to enhance the therapeutic effects of polar drugs (14). A vital interaction occurs between the active drug and phospholipid of formulation, which is responsible for the beneficial effects of phytosomes (32). The present study's results were consistent with the previous reports of crude and purified guggulsterone regarding body weight and lipid profile (33,34). However, in the present study, the hypolipidemic effect was more effective with GPs than pure guggulsterone. The steady-state concentration and targeted delivery of the active drug help the noteworthy results of GPs to regulate the elevated lipid levels in high-fat-fed rats. HDL is the protective type of cholesterol that helps reduce TG and LDL production. GPs significantly improved the DHL cholesterol levels in serum. The atherogenic index was also significantly decreased in rats treated with GPs, which further verify its hypolipidemic effect.

The contributory factors of oxidative stress in obesity, including chronic inflammation, hypercholesterolemia, and endothelial dysfunction lead to atherosclerosis and cardiovascular diseases. Oxidative stress is associated with an imbalance of pro-oxidant radicals and the antioxidant enzyme system of the cell (35). Tissue-based antioxidant enzyme system consisting of SOD, CAT, and GSH significantly protects against the perilous effects of oxidative free radicals. However, during the active disease condition, the malondialdehyde (MDA) levels were high and involved in the dysfunction of cellular activities to cause cell death (36). The present study showed that in a high-fat diet alone fed rats, elevated MDA and decreased SOD, CAT, and GSH levels in serum contribute to developing obesity and hyperlipidemia-associated adverse events. GPs treatment alters these serum enzyme levels near normalcy, and the effect was steadier than other conventional formulations. This specifies that GPs hold better therapeutic action and are beneficial in managing hyperlipidemia and obesity.

Liver is the crucial organ where lipids are synthesized and assembled, and any histological changes notably impact the disease state and or protection of the test drug (37). The anti-hyperlipidemic effect further corroborates the accumulation of fatty cells and inflammatory cells, and steatosis was significantly reduced in GPs treated rats than in high-fat diet-fed rats. The present study further confirms the previous studies that guggulsterone and raw resin of Commiphora species possess a hypolipidemic effect (38).

Conclusion

In conclusion, the optimization of GPs led to the development of a formulation with favorable characteristics such as efficient drug entrapment, appropriate particle size, optimal zeta potential, and controlled drug release. These properties contribute to the enhanced stability and improved bioavailability of guggulsterone. Additionally, the study demonstrated a significant increase in hypolipidemic activity with GPs treatment in high-fat-diet fed rats.

Further research should focus on evaluating the bioavailability and other relevant pharmacokinetic parameters involved in achieving steady-state concentrations. Additionally, efforts should be made to explore potential approaches for the clinical application of GPs. These investigations will provide valuable insights for future clinical studies and the development of GPs as a therapeutic intervention.

Authors' contribution

Conceptualization: Jamal Basha Dudekula, Damodharan Narayanasamy.
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Resources: Jamal Basha Dudekula.
Software: Jamal Basha Dudekula.
Supervision: Damodharan Narayanasamy
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Writing–review & editing: Jamal Basha Dudekula, Jebastin Koilpillai, Damodharan Narayanasamy.

Conflict of interests

The authors declare no conflict of interest.

Ethical considerations

The experimental methods and procedures conducted in this study were reviewed and approved by the Institutional Animal Ethical Committee of the Sri Padmavathi School of Pharmacy with approval reference number of SPSP/1016/PO/Re/S/06/CPCSEA/IAEC/2022/03.

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None.

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