Frontiers in Health Informatics ISSN-Online:2676-7104 www.healthinformaticsjournal.com
2024; Vol 13: Issue 3 Open Access

In-Vivo Antihyperlipidemic Potentials Of Guggul Lipid Loaded Chitosan Nanoparticles Designed And Optimized By Box-Behnken Design

Dr B.R.srinivasmurthy[*] ,Dr R.Gayathri[1] ,Dr Barish[2] ,Dr A.Dinesh raja[3] M.Jegathis kumar[4] , S. kiruthika [5],S.Ragul[6], Dr.S. Muthukumar[7]

[1]Professor,Dept of Pharmaceutics,KMCH College of Pharmacy,Coimbatore,Tamilnadu
[2]Professor,Dept of Pharmaceutics,RVS College of Pharmaceutical Sciences,The Tamilnadu Dr.M.G.R Medical Univeristy,Chennai
[3]Assistant Professor, Dept of Pharmaceutics,KMCH College of Pharmacy,Coimbatore,Tamilnadu
[4]Lecturer,Dept of Pharmacy Practice,KMCH College of Pharmacy, Coimbatore, Tamilndu
[5]Assistant Professor, Faculty of Pharmacy, Karpagam Academy of Higher education, Coimbatore, Tamilnadu
[6]Exceutive, Maiva Pharma Pvt Ltd, Hosur, Tamilnadu
[7]Manager, Maiva Pharma Pvt Ltd, Hosur,Tamilandu

*Corresponding Author:
Dr.B.R.Srinivas Murthy,
Assistant Manager-Formulation Research and Development,
Maiva Pharma Pvt Ltd,
Hosur, Tamilnadu
Email: seenu46@gmail.com
ORCID: 0000-0001-8124-8566

Cite this paper as: B.R.srinivasmurthy, R.Gayathri ,Barish , A.Dinesh raja, M.Jegathis kumar,S.kruthika,S.Ragul, S. Muthukumar (2024) In-Vivo Antihyperlipidemic Potentials Of Guggul Lipid Loaded Chitosan Nanoparticles Designed And Optimized By Box-Behnken Design. *Frontiers in Health Informatics, 13* (3), 9441-9452

ABSTRACT

Oral application of guggul lipid for the treatment of hyperlipidaemia/obesity is limited due to extensive hepatic metabolism and lack of suitable drug delivery carriers/systems.
Guggul lipid loaded chitosan nanoparticles (GNPs) were designed, optimized and evaluated to address the above mentioned limitations. GNPs were designed, optimized by three factor-three level Box-Behnken experimental design employing ionic gelation technique. Further GNPs were evaluated for in-vitro characteristics and in-vivo parameters like assessment of body weight, lipid profile like triglycerides, total cholesterol, high density lipoproteins, low density lipoproteins, very low density lipoproteins along with atherogenic index and total protein in obese rats using high fat diet induced model. GNPs produced better in-vitro characteristics with high entrapment efficiency (92.98 ± 0.47%) and desired biphasic drug release (95.12 ± 0.36%) with additional in-vitro lipase inhibition and cell viability respectively. Oral administration of GNPs displayed promising in-vivo potentials with significant ($p<0.05$) reduction of body weight and lipid levels along with increased levels of high density lipoproteins and total proteins in obese rats at the end of 28th day. Histopathological examinations of liver and adipose tissues of GNPs treated rat's revealed normal architecture as that of normal group rats. Conclusively GNPs were found to be promising nanocarriers for effective treatment of hyperlipidaemia/obesity and related disorders like atherosclerosis.

Key words: *Atherosclerosis, Box–Behnken design, Guggul lipid, Hyperlipidaemia, Nanoparticles.*

INTRODUCTION

Hyperlipidaemia is characterized by presence of abnormal levels of lipids in blood marked by elevated cholesterol, low-density lipoprotein (LDL) and low or unaltered high-density lipoprotein (HDL) levels [1]. Hyperlipidaemia leads to coronary heart diseases, especially atherosclerosis and atherosclerosis-related vascular diseases, like ischemic cerebrovascular disease, and peripheral vascular disease. It also leads to fatty liver, stroke, cerebral infarction, hemiplegia, myocardial infarction, ischemic stroke etc., and alarmingly accounts for the majority of morbidity and mortality among different age group of people [2,3].

Commonly used synthetic hypolipidemic agents like statins, fibrates, bile acid sequesterants on chronic use, witnessed many potential adverse effects like myopathy, rhabadomyolysis, myoglobinuria, renal failure, hepatic toxicity (elevated hepatic transaminase enzymes) and even death [4,5]. On the other hand, herbal hypolipidemic agents like garlic, onion and coriander have been found to be useful in treating hyperlipidaemia effectively [3,6,7]. Researchers have explored antihyperlipidemic activities of various bioactive compounds viz., gamma oryzanol, ursolic acid, *Leucas aspera, Centella asciatica, Taraxacum officinale, Protorhus longifolia* [8,9]. Guggul resin obtained from *Commiphora mukul* has been reported to possess anti hyperlipidemic activity with E- and Z-guggulsterones as bioactive compounds. Recent research indicates that guggulsterones are antagonists of the farnesoid X receptor (FXR) and the bile acid receptor, nuclear hormone receptors involved in bile acid regulation and cholesterol metabolism[10–12] but its therapeutic action is limited by lack of effective delivery carriers/ systems. Therefore natural antihyperlipidemic agents designed in suitable dosage form/ carriers comprising biocompatible integrants would be a better alternative to allopathic treatments with improved therapeutic efficacy and reduced side effects.

In order to facilitate effective delivery of guggul lipid, we have developed a novel guggul lipid nanoparticles in our previous study, in which guggul loaded chitosan nanoparticles (GNPs) was designed, optimised using 3-factor 3-level Box-Behnken experimental design. Optimised GNPs produced promising results with successful encapsulation of guggul along with desired in-vitro drug release with additional lipase inhibition and improved viability against selected cell lines (Mouse fibroblast 3T3-L1 preadipocytes, NSC-34-Mice motor neuron like, Kidney epithelial cells-Vero cells) [13]. However the in-vivo efficiency of GNPS is to be evaluated, hence the present research is aimed to explore in-vivo antiobesity potentials of optimized GNPs in obese rats using high fat diet induced model.

2. MATERIALS AND METHODS

Guggul lipid was procured from M/s. Chemilloids (Vijayawada, India), Chitosan (MW 150 kDa, degree of deacetylation >85%) was purchased from Aura Biotech Pvt ltd (Chennai, India). Sodium tri polyphosphate (TPP) was purchased from Sigma-Aldrich (Bangalore, India). Milli Q water was used throughout the study. Biochemical assay kits for estimation of lipid profile were purchased Span Diagnostics Ltd., (India). Anaesthetic ether and ethanol was purchased from SD Fine Chemicals (Mumbai).All other chemicals and materials used in the study were of analytical grade and generally recognized as safe with pharmaceutical grade.

2.1 Preparation and optimization of GNPs-Box- Behnken Design

In our previous study, guggul lipid loaded chitosan nanoparticles (GNPs) were processed by ionic gelation technique. GNPs were designed, characterized and optimized by a three factor-three level Box-Behnken design (with five centre points). A total of 17 formulations (with varying proportions of independent variables) were prepared and evaluated for various in-vitro parameters like particle size, zeta-potential, surface morphology, percent entrapment efficiency (EE), in-vitro drug release (DR) by dialysis tubing, x-ray diffractometer (XRD), differential scanning calorimetry (DSC), lipase inhibition (in-vitro), stability studies, in- vitro cell line study using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT assay). The effect of independent variables i.e., amounts of chitosan (X_1), sodium tripolyphosphate (X_2) and guggul lipid (X_3) on dependent variables like %Entrapment efficiency (Y_1) and %Drug release (Y_2) were studied [13]. Numerical optimization technique was used for optimizing the formulation variables to achieve desired responses. Further experiments were repeated in triplicate to determine the dependability of optimized conditions. Mean values of experimental data were compared against predicted values and percent error was determined.

2.2 In-vivo antihyperlipidemic study

The in-vivo/pharmacodynamic study was carried out to explore antihyperlipidemic potentials of optimized GNPs and to compare its pharmacodynamic (PD) profile with standard marketed product (Yograj guggulu). Male albino rats were procured from the animal house of Biogene laboratory Pvt. Ltd. Bangalore. Albino Wistar rats (150–200 g) were kept in polyacrylic cages maintained under standard conditions of 22±2 °C and 12 h light/dark cycle. Animals had free access to standard chow diet and water, ad libitum [14].

The study was approved by the Institutional Animal Ethics Committee (SPSP: 1016/PO/Re/S/06/CPCSEA/2019/014) Tirupati and animals were treated according to the guidelines of Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA).

Study design

A total of 30 male albino rats of wistar strain weighing 150-200g were used throughout the study. The animals were randomly divided into five groups (six animals in each group, n=6). All rats were fed with commercially available normal pellet diet (NPD) and had access to water ad libitum prior to dietary manipulation. Except for the animals of the normal control group (group 1) which were continued to be fed with NPD, all other animals (groups 2–5) were fed with high fat diet (HFD) till end of the study. The HFD comprised 58% fat, 25% protein and 17% carbohydrate as a percentage of total kcal [15, 16]**.** After seven weeks feeding with HFD, body weights and lipid profiles such as total cholesterol (TC), triglycerides (TG), low density lipoproteins (LDL), very low density lipoproteins (VLDL), high density lipoproteins (HDL), of all the rats were assessed for their elevation to confirm the induction of hyperlipidaemia. After confirmation of induction of obesity, each group received the assigned treatments and the study lasted for a period of 28 days. The treatment groups are as follows

Group 1: NPD (Normal group); Group 2: HFD (Disease control group); Group 3: HFD & Standard Guggul Marketed product (GMP); Group 4: HFD & optimized (GNPs); Group 5: HFD & Guggul pure drug (GPD);

2.3 Drug administration and dosing.

The Guggul Marketed product (Yograj guggulu) tablets were crushed and dispersed in water by sonication immediately before administration. A volume of suspensions equivalent to 30 mg/kg of guggul was given to the animals through the oral route using an oral gavage needle. Optimized GNPs (equivalent to 30 mg/kg of guggul) were re-dispersed in water by sonication immediately before administration then a volume (2ml) of GPD suspensions (equivalent to 30 mg/kg guggul) was given orally. Dosing was given daily for GMP, GNPs and GPD [17].

2.4 Blood sampling

Blood sampling was done initially following the seven-week HFD right before starting treatments (for confirmation of hyperlipidaemia i.e., elevated lipid profile) and later every week during treatment period. The blood (200-300 µl) was collected from retro orbital sinus puncture under mild ether anaesthesia into heparinised tubes.

Clotted blood samples were immediately centrifuged (Remi) and were centrifuged (2400 ×g for 10 min) at 4 ºC. Serum samples obtained were separated and were analysed for lipid profiling using biochemical kits and autoanalyzer

2.5 Assessment of biochemical parameters

2.5.1 Determination of body weights

Body weights of all groups of rats were weighed and noted prior to the commencement of treatment (with elevated body weights of group 2-5 animals fed on HFD, confirming the induction of obesity). Since then body weights of all groups of rats were measured and noted every week after the commencement of treatment period [18].

2.5.2 Lipid profile

Biochemical parameters related to hyperlipidaemia were analyzed in the collected serum from all groups using commercial kits (Span Diagnostics Ltd., India). The serum samples were analyzed for estimation of triglycerides (TG), total cholesterol (TC), high density lipoproteins (HDL), low-density lipoprotein (LDL) and very low-density lipoprotein (VLDL). The lipid parameters were estimated as per the standard procedures [9]. Atherogenic Index (AI) and Total protein (TP) were also estimated in the serum samples. Atherogenic Index (AI) was calculated by using the following formula.

$$\text{Atherogenic Index (AI)} = \frac{\text{TC}}{\text{HDL}} \quad (1)$$

Statistical analysis

Data was analyzed using one-way analysis of variance (ANOVA) followed Dunnett's multiple comparison test using Graph Pad Prism version 5.0. The results are presented as mean± standard error of the mean (SEM). Values of $p < 0.05$ were considered to be statistically significant.

Histopathological studies

The livers and visceral adipose tissues were excised at the end of the in vivo study and preserved in 10% buffered formalin solution for histopathological studies. Tissue was processed as per routine histological procedure such as washing, sectioning, staining with haematoxylin and eosin (H and E) and evaluated under light microscope to observe any morphological changes [19].

3. RESULTS

3.1 Characterization and optimization of GNPs

Numerical optimization technique based on the desirability approach was employed for optimization of GNPs to achieve maximum entrapment efficiency and desired drug release. Optimized GNPs composed of concentrations of chitosan (X_1-1733.94 mg), TPP (X_2-1833.97 mg) and dose of guggul (X_3-342.69 mg) with resultant EE and DR of 92.98 ± 0.47% and 95.12 ± 0.36% DR and overall desirability of 1. The % yield of optimized GNPs was found to be 85.84%. Optimized GNPs possessed particle size of 96.5 nm, electrokinetic potential of -15.4 mV and were with spherical, uniformly distributed slightly porous textured morphology. Solid state characterization studies (X ray diffraction & Differential scanning calorimetry) of GNPs revealed existence of characteristic peaks of guggul within the range but with slight amorphization. A biphasic mode of release of guggul from GNPs was witnessed with initial burst release and subsequent sustained release. GNPs were found to be stable over stability studies with enhanced lipase inhibition and better viability against selected cell lines as reported in our previous study [13].

3.2 Pharmacodynamic profile

3.2.1 Effect of GNPs on body weight

A significant increase ($p<0.05$) in the body weight was observed in rats maintained on high fat diet (HFD) when compared to normal control (NPD). Treatment with GMP, GNPs and GPD (30 mg/kg) had shown a marked ($p<0.01$) decrease in the body weights. GNPs were found to be equipotent to GMP in reducing body weight as shown in Table 1. However rats treated with GPD did not show prominent reduction in the body weights as depicted in Figure 1.

3.2.2 Impact of GNPs on serum HDL levels

Rats of disease control group had shown a statistically significant ($p<0.05$) decrease in the HDL-C levels compared to normal group. Whereas, rats treated with GNPs had shown marked increase ($p<0.01$) in HDL levels when compared with that of rats treated with GMP and GPD (Table 1 and Figure 1).

3.2.3 Effect of GNPs on serum TG

A statistically significant ($p<0.05$) increase in the serum TG levels was seen in rats treated with HFD. Treatment with GMP, GNPs and GPD (30 mg/kg) had shown a marked ($p<0.01$) decrease in the serum TG levels. GNPs were found to be equipotent to GMP in reducing triglycerides levels as shown in Table 1 and Figure 1.

3.2.4 Effect of GNPs on serum TC

Total cholesterol, a prominent marker of hyperlipidaemia was significantly increased ($p<0.05$) in disease control rats compared to normal group rats. GNPs promisingly reduced elevated TC levels in rats compared to GMP and GPD treated groups. The TC values of treated rats are depicted in Table 1 and Figure 1.

3.2.5 Impression of GNPs on serum LDL levels

GNPs significantly ($p<0.01$) reduced LDL levels compared to GMP. LDL levels were significantly ($p<0.05$) increased in rats fed with HFD compared to rats fed with normal pellet diet (NPD) as shown in Table 2. However prominent decrease in LDL levels was not observed in rats treated with GPD (Figure 2).

3.2.6 Impact of GNPs on serum VLDL levels

VLDL levels were significantly ($p<0.05$) raised in rats of disease control compared to rats of normal control (group-1). Treating rats with GMP, GNPs and GPD had shown a marked ($p<0.01$) decrease in the serum TG levels. However compared to GNPs and GPD, GMP was superior in reducing VLDL levels in rats as shown in Table 2 and Figure 2.

3.2.7 Role of GNPs in reducing serum total proteins

As a measure of liver function, total protein levels were also estimated in the serum samples of rats of all groups along with lipid parameters. Total proteins levels were significantly ($p<0.05$) reduced in disease control rats compared to rats of normal group. On the other hand, Total protein levels were increased and maintained (as that of normal group rats) in the rats treated with GNPs and GMP, where GNPs were effective compared to GMP (Table 2). However, GPD was unable to raise/restore total proteins levels and was closer to disease control rats as shown in Figure 2.

3.2.8 Impact of GNPs on Atherogenic index (AI)

Atherogenic index, a consequence of hyperlipidaemia/obesity, was drastically and significantly ($p<0.05$) elevated in rats fed with HFD. Whereas GNPs and GMP were found to be effective in reducing AI compared to GPD as shown in Table2. However GNPs were equipotent as that of GMP in reducing AI (Figure 2).

3.3 Histopathological studies

Histology of liver tissues of all groups of rats is depicted in figure 3 where hepatocytes of rats treated with GNPs resembled with that of hepatocytes of untreated groups (normal rats). Similarly histomicrographs of adipose tissues of all rats are elucidated in figure 4, where the adipocytes of GNPs treated rats retained their normal architecture.

4. DISCUSSION

GNPs were processed by ionic gelation technique where chitosan was proved as an appropriate choice of polymer that resulted with desired entrapment efficiency and drug release. BBD was proved as an efficient logistic tool in predicting and elucidating the influence of independent variables [20] (chitosan, TPP, guggul) on desired responses (EE and DR). Guggul being an FXR blocker also possessed lipase inhibition action that could be a synergistic effect in the treatment of hyperlipidaemia. GNPs also possessed better cell viability against selected cell lines as assayed by MTT assay [13].

Optimized GNPs also demonstrated their efficiency in in-vivo studies as indicated by obtained results. As hyperlipidaemia is prominently characterised by increased bodyweight, it becomes imperious to determine bodyweights throughout treatment period to evaluate the efficiency of the designed formulation. However GNPs displayed their promise in reducing and controlling the elevated body weights of obese rats throughout the study. GNPs also showed their promise in significantly reducing the elevated lipid profile and this could be attributed due to successful inhibition of cholesterol synthesis and lipase action. Rats fed with HFD (group-2) witnessed marked elevation in their lipid profile compared to rats fed with normal pellet diet (group-1). On the other side, GNPs were found to be more potent compared to standard product (GMP) in reducing the elevated lipid levels of obese rats. GNPs also significantly reduced atherogenic index of obese rats and finds their application in reducing cardiovascular risk factors associated with obesity. However pure form of guggul (group-5) was not capable to reduce the elevated lipid profile and body weights in obese rats and this could be due to inadequate concentrations at the target site.

Histopathological features of liver and adipose tissues are illustrated in figure 3 and figure 4. In rats of normal control group (group-1), the liver was seen with normal architecture without any inflammation, fibrosis, fatty changes and necrosis. On contrary, the liver tissue of rats fed with HFD (group-2) evidenced altered architecture where the hepatocytes with fatty vacuoles, mononuclear cell infiltration, facilitated granular degeneration was observed. However, no changes/abnormalities were observed in liver histology of rats treated with GNPs.

Regeneration of cells (indicated by binucleated cells) was also evidenced with GNPs treated rats. Rats treated with GMP exhibited normal architecture without any alterations. However GPD treated rats exhibited mild proliferation of fibroblasts indicating slight regeneration of cells as shown in figure 3. The histopathological examination of adipose tissue revealed that the adipocytes were spherical to polyhedral shaped cells with eccentric nucleus in rats maintained on NPD. Whereas, swollen adipocytes with thick membrane and slight eosinophillic granules with cytosteato necrosis was observed in rats fed with HFD. Adipocytes with spherical and eccentric nucleus were seen in rats treated with GNPs and resembled with that of normal adipocytes. Similarly, adipocytes with normal architecture were also noticed in rats treated with OMP, however slightly swollen adipocytes were seen in OPD treated rats, the histopathological observations of adipose tissues are depicted in Figure 4.The most critical challenge for targeted drug delivery is to preserve the designed formulation during its transport through the stomach and upper part of the small intestine. Such strategies would assure not just direct treatment at the target site, but also the possibility of a reduction in administered dose and any associated systemic adverse effects. The key factor and rate determining step as indicted in our study is to improve the absorption of guggul and this was successfully achieved by GNPs as indicated by the results obtained.

In conclusion, GNPs designed and optimized by BBD had revealed promising in-vivo antihyperlipidemic potentials. A significant reduction in the serum TG, TC, LDL, VLDL, AI, body weights and a significant increase in the levels of HDL and total protein was accomplished, thus signifying the potential use of GNPs in the treatment of hyperlipidaemia and in other associated disorders like atherosclerosis. GNPs processed by ionic gelation method employing chitosan as polymeric material was a promising novel approach for targeted drug delivery with the results achieved. GNPs were found to be superior and equipotent with that of GMP at lower dose. Further pharmacokinetic and preclinical studies using suitable models are necessary to demonstrate the antihyperlipidemic potentials of GNPs.

Acknowledgements

The authors are grateful to Sri Padmavathi School of Pharmacy, for rendering generous support to carry out the research work.

Ethics approval

The study was approved by the Institutional Animal Ethics Committee, (1016/PO/Re/S/06/CPCSEA/2019/014).

Conflict of Interest

The authors declare that they have no competing interest.

Author Contribution

All authors contributed to the study conception and design. Design of study, data collection, analysis and manuscript preparation was performed by Srinivas Murthy BR. Drafting of manuscript was done by Lakshmayya, Rekha Devi A and Vishakha K. Manuscript was revised and approved by Prasanna Raju Y and Devanna N. All authors read and approved the final manuscript.

REFERENCES

1. Nelson RH. Hyperlipidemia as a risk factor for cardiovascular disease. Prim Care Clin off Pract 2013;40(1):195-211.
2. Thomas A, Horn L Van, , Tracy P, Lloyd-jones DM. Lifetime Risks of Cardiovascular Disease. N Eng J Med 2012; 366(4):321–9.
3. Kumari S, Deori M, Elancheran R, Kotoky J, Devi R. In vitro and In vivo Antioxidant , Anti-hyperlipidemic Properties and Chemical Characterization of Centella asiatica (L .) Extract. Front Pharmacol 2016;7:1–12.
4. Harchaoui KEL, Visser ME, Kastelein JJP, Stroes ES. Triglycerides and Cardiovascular Risk. Curr Cardio Rev 2009; 5: 216–22.
5. Shattat GF. A review article on hyperlipidemia: Types, treatments and new drug targets. Biomed Pharmacol J 2014; 7(2):399–409.
6. García-Carrasco B, Fernandez-Dacosta R, Dávalos A, Ordovás J, Rodriguez-Casado A. In vitro Hypolipidemic and Antioxidant Effects of Leaf and Root Extracts of Taraxacum Officinale. Med Sci 2015; 3(2):38–54.
7. Kazmi I, Afzal M, Rahman S, Iqbal M. Antiobesity potential of ursolic acid stearoyl glucoside by inhibiting pancreatic lipase. Eur J Pharmacol 2013; .709(1–3):28–36.

8. Machaba KE, Cobongela SZZ, Mosa RA, Oladipupo LA, Djarova TG, Opoku AR. In vivo anti-hyperlipidemic activity of the triterpene from the stem bark of Protorhus longifolia (Benrh). Lip Health and Disease 2014; 13 (132): 1–7.
9. Rawal T, Mishra N, Jha A, Bhatt A, Tyagi RK, Panchal S. Chitosan Nanoparticles of Gamma-Oryzanol: Formulation, Optimization, and In vivo Evaluation of Anti-hyperlipidemic Activity. AAPS PharmSciTech 2018; 19(4):1894–907.
10. Das S, Datta A, Bagchi C, Chakraborty S, Mitra A, Tripathi SK. A comparative study of lipid-lowering effects of guggul and atorvastatin monotherapy in comparison to their combination in high cholesterol diet-induced hyperlipidemia in rabbits. J Diet Suppl 2016; 13(5):495–504.
11. Deng R. Therapeutic Effects of Guggul and Its Constituent Guggulsterone: Cardiovascular Benefits. Cardio Drug Reviews 2007; 25(4):18-24.
12. Mohiuddin R, Dar MA, Masoodi MH. Guggulipid as an adjuvant therapy for Hyperlipidemia : A review. Inter J of Med Res 2019; 3(1): 17-22.
13. Murthy BRS, Yelavarthi PR, Devanna N, Basha DJ. Design of Guggul Lipid Loaded Chitosan Nanoparticles Using Box-Behnken Design – An Evaluation Study. Asian J Pharm Clin Res 2021; 33(18):53–67.
14. Semalty M., Kumar R. and Semalty A. Formulation and characterization of herbal formulation for antihyperlipidemic activity in diet induced obese mice. Indian Drugs 2016; 53 (07):30-34.
15. Ahmed IS, El-Hosary R, Shalaby S, Abd-Rabo MM, Elkhateeb DG, Nour S. PD-PK evaluation of freeze-dried atorvastatin calcium-loaded poly-ε-caprolactone nanoparticles. Int J Pharm 2016; ;504(1–2):70–9.
16. Srinivasan K, Viswanad B, Asrat L, Kaul CL, Ramarao P. Combination of high-fat diet-fed and low-dose streptozotocin-treated rat: A model for type 2 diabetes and pharmacological screening. Pharmacol Res 2005. 52(4):313–20.
17. Shaik J, Khan Z. Antihyperlipidemic activity of Commiphora mukul against atherogenic diet- induced hyperlipidemia in experimental rats diet-induced hyperlipidemia in experimental rats. Asian J Pharm Clin Res 2018; (6): 25-31.
18. Kazmi, I, Afzal, M., Rahman, S, & Iqbal, M. Antiobesity potential of ursolic acid stearoyl glucoside by inhibiting pancreatic lipase. Eur J Pharmacol 2013:709: 28–36.
19. Kanoujia J, Singh M, Singh P, Saraf SA. Novel genipin crosslinked atorvastatin loaded sericin nanoparticles for their enhanced antihyperlipidemic activity. Mater Sci Eng C 2016;69:967–76.
20. Laxmi PS, Vidyavathi M, Kumar SVR. DoE approach for development of localized controlled release microspheres of vancomycin for treatment of septic arthritis. Fut J Pharm Sci2021; 7(235): 1-15.

TABLES

Table.1 EFFECT OF GNPS ON BODY WEIGHT, HDL, TG AND TC AT THE END OF 28TH DAY

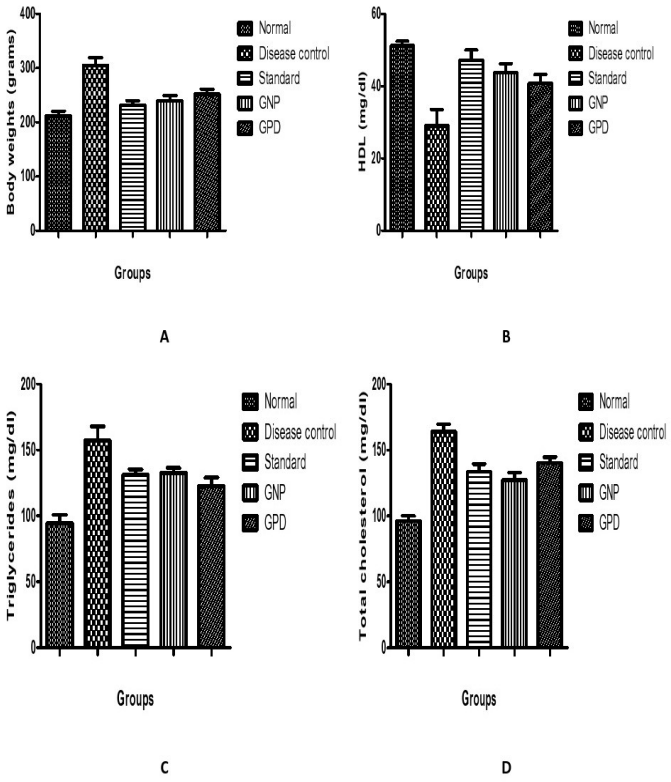
S. No	Group	Body Weight	HDL	TG	TC
1	Normal control	212.15±2.09	50.59±1.62	94.61±0.74	90.06±1.47
2	Disease control	305.19±5.49*	29.09±1.31*	157.47±1.05*	164.36±2.01*
3	GMP (30mk/kg)	230.57±2.84**	45.65±0.71**	131.74±1.10**	133.5±1.02**
4	GNPs (30mk/kg)	239.85±0.97**	47.62±1.09**	132.3±1.224**	127±1.30**
5	GPD (30mk/kg)	292.95±2.64**	33.59±0.66**	138.71±1.03**	145.4±0.50**

*P<0.05 (when compared with control), **P<0.01 (when compared with disease control)

Table.2 EFFECT OF GNPS ON LDL, VLDL, TP AND AI AT THE END OF 28TH DAY

S. No	Group	LDL	VLDL	TP	AI
1	Normal control	63.4±0.77	24.33±0.62	8.24±0.21	1.68±0.20
2	Disease control	123.9±1.15*	58.23±1.15*	4.46±0.82*	6.17±0.81*
3	GMP (30mk/kg)	86.63±0.91**	45.4±0.98**	6.42±0.36**	2.46±1.01**
4	GNP (30mk/kg)	84.43±1.06**	43.62±0.95**	7.2±0.16**	2.67±0.47**
5	GPD (30mk/kg)	97.23±0.71**	47.46±1.07**	4.49±0.17**	4.32±1.03**

*P<0.05 (when compared with control), **P<0.01 (when compared with disease control)



FIGURES

Fig.1 Body weights, HDL, TG and TC profiles of Normal control, Disease control, GMP, GNPs and GPD treated groups.

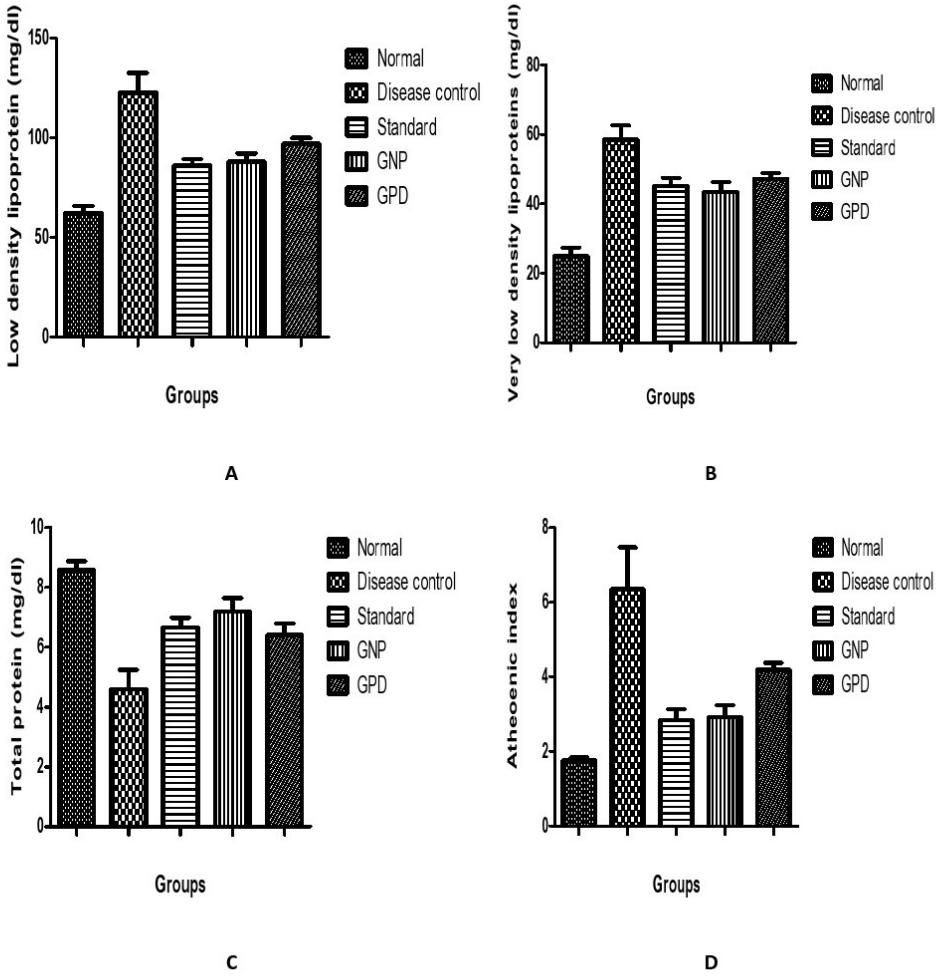


Fig. 2 LDL, VLDL, TP and AI profiles of Normal control, Disease control, GMP, GNPs and GPD treated groups.

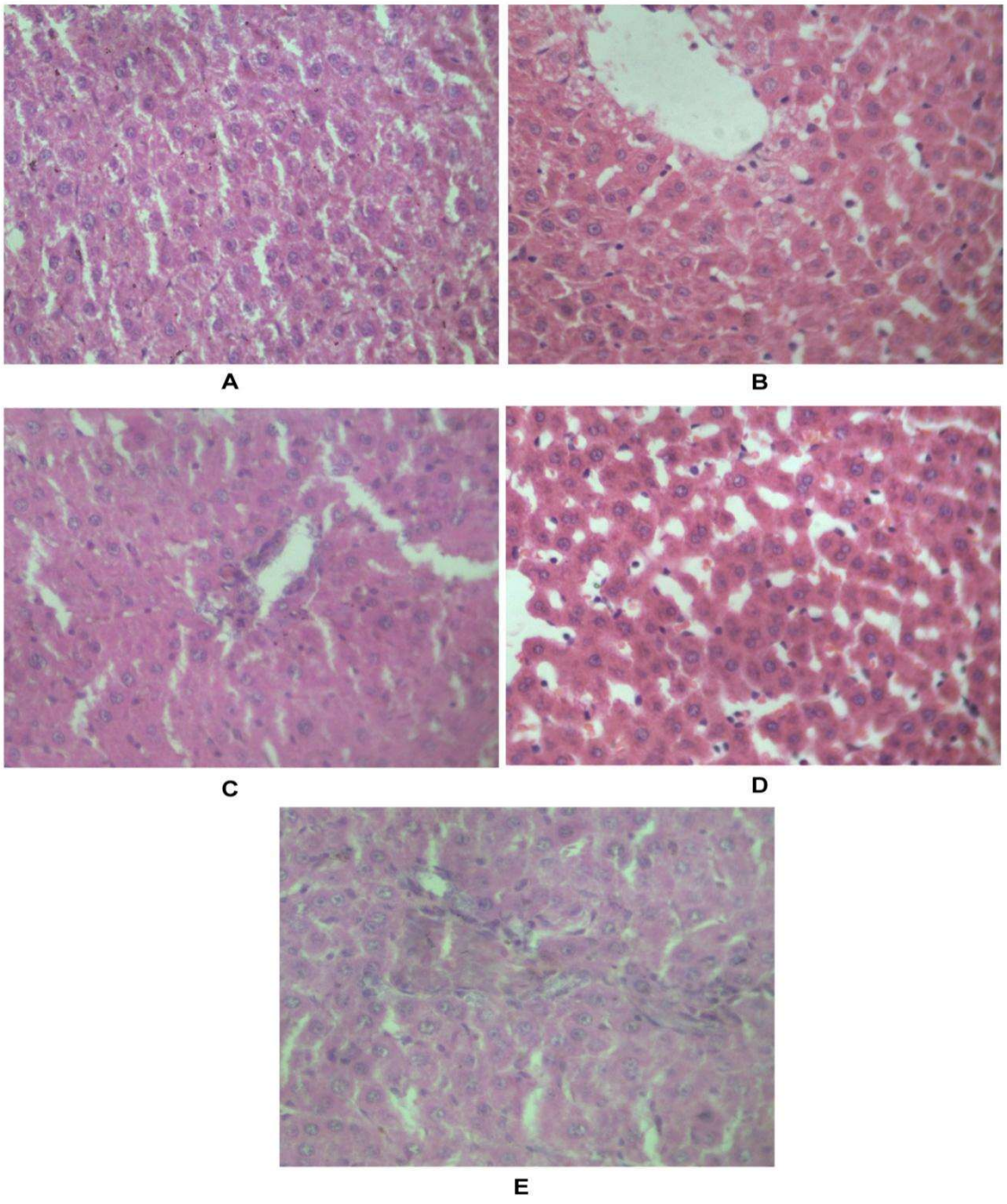


Fig. 3 Histomicrographs showing histopathological changes in Livers of Normal control (A), Disease control-HFD (B), GMP (C), GNPs (D) and GPD (E) treated rats.

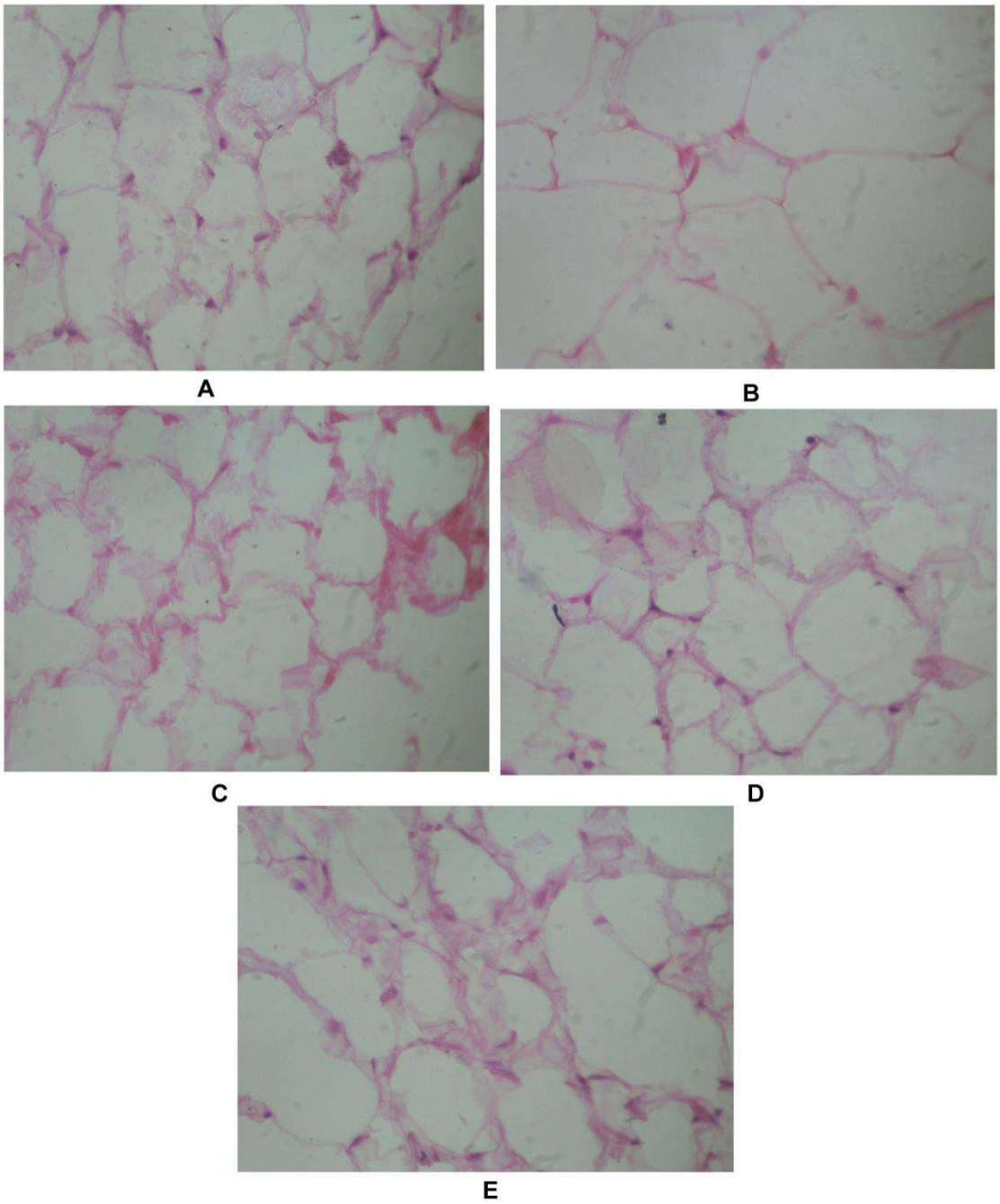


Fig. 4 Histopathological investigations of adipose tissues of Normal control (A), Disease control-HFD (B), GMP (C), GNPs (D) and GPD (E) treated rats.

TABLES AND FIGURES TITLES AND LEGENDS

Table.1 EFFECT OF GNPS ON BODY WEIGHT, HDL, TG AND TC AT THE END OF 28TH DAY

*P<0.05 (when compared with control), **P<0.01 (when compared with disease control)

Table.2 EFFECT OF GNPS ON LDL, VLDL, TP AND AI AT THE END OF 28TH DAY

*P<0.05 (when compared with control), **P<0.01 (when compared with disease control)

Fig.1Body weights, HDL, TG and TC profiles of Normal control, Disease control, GMP, GNPs and GPD treated groups.

Fig. 2 LDL, VLDL, TP and AI profiles of Normal control, Disease control, GMP, GNPs and GPD treated groups.

Fig. 3 Histomicrographs showing histopathological changes in Livers of Normal control (A), Disease control-HFD (B), GMP (C), GNPs (D) and GPD (E) treated rats.

Fig. 4 Histopathological investigations of adipose tissues of Normal control (A), Disease control-HFD (B), GMP (C), GNPs (D) and GPD (E) treated rats.