RESEARCH ARTICLE

An Approach to Development and *In-vivo* Evaluation of Beta Sitosterol Phytosomes by Taguchi Orthogonal Array Design

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ABSTRACT

The research mainly focused on developing and optimizing beta-sitosterol phytosomes through a systematic Taguchi orthogonal array design and assessment of their performance in both beta-sitosterol phytosomes. The fabrication of phytosomes containing β-sitosterol was carried out using the rotary evaporation technique. An orthogonal array design was employed in the study to precisely optimize the phytosomes. In this two responses and three independent variables were analyzed. The data was analyzed by using statistical modeling. Characterization of these phytosomes involves Fourier transform infrared spectroscopy, entrapment efficiency, differential scanning calorimetry compatibility study, zeta potential and particle size. Based on the findings, an optimized phytsome formulation was developed to achieve the best results. The results indicated that beta-sitosterol phytosomes responses are significant. It effectively reduced body weight as well as levels of total cholesterol (126.120 ± 0.98 mg/dl), triglycerides (104.23 ± 2.8 mg/dl), HDL (39.31 ± 0.45 mg/dl), VLDL (21.67 ± 0.34 mg/dl), and LDL (76.43 ± 0.34 mg/dl) in rats compared to the other groups. The orthogonal array design study observed that the anti-hyperlipidemic activity of β-sitosterol was nearly as potent as that achieved with standard drug therapy.

Keywords: Beta-sitosterol, phytosome, Taguchi orthogonal array design, total cholesterol, Triglycerides

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INTRODUCTION

Obesity, a metabolic disorder, has wide-ranging effects that can significantly harm physical and mental well-being.[1] Among the various approaches available, herbal products have emerged as a popular treatment option in recent years.[2] One advantage of choosing herbal products over pharmacological preparations for treating obesity is their generally recognized safety and minimal adverse effects.[3] By incorporating these phyto therapeutic supplements into a well-balanced diet and regular exercise routine, individuals can witness physical transformations and promote overall health.[4] β-sitosterol is gaining recognition for its potential as an anti-obesity agent.[5] Adipocytes, which are specialized cells that store fat in the body, were found to be significantly hindered in their ability to form new fat cells when treated with β-sitosterol.[6] As a result, there is a need for novel technologies that enhance the solubility and therapeutic efficacy of these herbal products. In recent years, a novel technology known as phytosomes has gained considerable attention as an innovative approach to delivering phyto-therapeutic products. By encapsulating plant extract within phospholipid complexes, phytosomes protect these bioactive substances from enzymatic degradation and the harsh gastrointestinal tract.[7]

MATERIALS AND METHODS

Beta-sitosterol was obtained from Sigma-Aldrich Private Limited in Bangalore, India, while the L-Alpha-phosphatidylcholine was provided by sigma-Aldrich.co., Healthy Wister albino rats, weighing between 150 to 200 g, were provided by Mahaveer Enterprises in Malakajgiri, Telangana The experimental procedures strictly abide by the rules and regulations established by the institutional animal ethical committee at Nirmala College of Pharmacy.

Preparation of Beta-sitosterol Phytosomes

The beta-sitosterol phytosomes were synthesized utilizing the rotary evaporation technique in three distinct ratios: (1:1) as BSP1, (1:2) as BSP2, (2:1) as BSP3 and (2:2) as BSP4. Beta-sitosterol and L-Alpha-phosphatidylcholine was dissolved in 30 mL of tetrahydrofuran (T.H.F.). This solution was then placed inside a 100 mL round bottom flask connected to a rotary evaporator.[8] The mixture was rotated for 3 hours at a temperature of 40°C. Subsequently, n-hexane was introduced

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Table 1: Taguchi orthogonal array (TAO) layout with experimental values of response variables of beta sitosterol loaded phytosomes

Name	*Std*	*(TAO) Run*	*Factor A. L-Alpha phosphatidylcholine*	*Factor B. Stirring temperature*	*Factor C. Time*	*Response 1(R1) EE*	*Response2 (R2) Particle size*
BSP1	4	1	4	60	60	47.31	467.93
BSP2	2	2	1	60	180	76.41	606..85
BSP3	3	3	4	40	180	75.23	563.94
BSP4	1	4	1	40	60	46.29	528.54

to the resulting thin film, and the mixture was continuously stirred using a magnetic stirrer. The resulting precipitate was collected and stored in an amber-colored glass container.

Taguchi Orthogonal Array Design

Taguchi design was used to prepare and optimize the β-sitosterol phytosome. The principal objective of the Taguchi design is to establish the influence of the excipients employed in the phytosome and their relationship to the chosen responses. In the Taguchi design, the independent variables selected were phospholipids, stirring temperature, and time. As indicated in Table 1, four runs were finalized.[9] The statistical program Design Expert V 11.0 was used to do the process. Entrapment efficiency and particle size were among the responses that were observed. Statistical modeling was used to analyze the collected data, with the process order serving as the design model and the model type as factorial. A half-normal plot, pareto chart, and 3D plot in the bar were used throughout the analysis to identify factors that considerably affected the response.[10]

Characterization of Phytosomes

Evaluation of β-sitosterol phytosomes

The characterization process for β sitosterol phytosomes involved several techniques such as FTIR analysis, DSC, compatibility study, SEM and assessment of various parameters, including entrapment efficiency, particle size distribution, and zeta potential.

FTIR analysis

To analyze the FTIR spectra of the optimized BSP2 phytosome formulation containing β sitosterol, an instrument (Mode Make (IFS 25) by Bruker, Germany) was utilized along with PC-based software for control and data processing. For this analysis, each BSP2 sample was mix with IR-grade potassium bromide at 1:100 ratio and formed into compacted pellets as blanks. In order to identify functional groups present in the sample, Infrared transmittance data was collected within a specific wave number range from 4500 to 500 cm^{-1}.[11]

Differential scanning calorimetry (DCS) analysis

Differential scanning calorimetry (DSC) analyses were conducted on the optimized BSP2 phytosome formulations using a Mettler-Toledo calorimeter. The analysis was performed under controlled conditions, heating from 30 to 360°C/10°C / min while maintaining an ideal environment through nitrogen stream cleaning at a flow rate of 40 mL per minute.[12]

Scanning electron microscope

Additionally, a Hitachi scanning electron microscope (SEM), model S-3400 type II, from Tokyo, Japan, was exploited to observe the surface morphology and shape of the optimized beta sitosterol phytosomes. Samples of beta-sitosterol were dried and mounted on a metal stub in an electron microscope. These samples were coated with gold using a sputter coater.[13] The stub scans at different magnifications capture a digital picture of the formulations.

Detection of surface charge (zeta potential)

The phytosome surface charge was quantified by means of zeta potential detection, employing the ZS-90 Zetasizer from Malvern Instruments (U.K.), which utilizes Dynamic Light Scattering (DLS) technology. Experiments were carried out in a 0.1N aqueous solution of KCL, with triplicate readings taken at a detection angle of 90°.[14] The obtained values were standardized to a reference temperature of 25°C.

Determination of entrapment efficiency (EE)

It was determined using the ultracentrifugation method. An accurately weighed quantity of phytosome complexes (10 mg) was dissolved and diluted in methanol. The phytosomes were subjected to ultracentrifugation at 15000 rpm for 15 minutes at room temperature and subsequently, supernatants were kept aside for 20 minutes. The concentration of beta-sitosterol in the solution was measured by recording absorbance at 202 nm by using a UV-visible spectrophotometer. Thus, EE was determined by calculating the amount of quercetin in the total yield. The percentage entrapment efficiency[R1] was determined by given formula 1:

$$EE(\%) = \frac{Amount\ of\ quercetin\ \ in\ complex}{Total\ amount\ of\ quercetin\ taken} \times 100 \qquad (\ldots 1)$$

Particle size analysis

In order to analyze the particle size of beta-sitosterol phytosome particles, an optical microscope with a trinocular setup was utilized. A total of 100 beta-sitosterol phytosome particles were measured to obtain a representative sample for the analysis. The presented results are the average of triplicate experiments.

In-vivo experimental procedure

Wister albino rats were utilized to conduct all the experiments. The study received authorization from the Institutional Animal Ethics Committee (IAEC) under project proposal number [009/ IAEC/ NCPA/ PhD21-22]. The experimental model involved inducing hyperlipidemia using a cholesterol diet. Rats with 150 to 200 g weight were utilized as subjects.

All animals were acclimatized under specific environmental conditions: a temperature of 24 ± 3°C, relative humidity of 60 ± 10%, a 12/12 hour light-dark cycle and water ad libitum. The treatment lasted for 21 days.[15,16] Based on data from acute toxicity studies, the study dose was determined to be 500 mg per kg body weight (B.W.)

Drug protocol for the study

After a week of acclimatization, the animals were categorized into distinct groups for the experiment, including control, toxic, standard and test extracts of beta-sitosterol pure drug and beta-sitosterol phytosomal group. Each experimental series consists of six animals.

- **Control Group**: Administered normal saline orally.
- **Standard Group**: Received a standard cholesterol diet combined with Atorvastatin (10 mg per kg b. w/day).
- **Toxic Group**: Administered only a cholesterol diet. 4.
- **Pure Drug Group**: Given a cholesterol diet and pure β-sitosterol drug (5 00 mg per kg b. w/day).
- **Phytosomal Group**: Administered a cholesterol diet and β-sitosterol phytosomes (100 mg/ kg b.w /day).

The experiment lasted for 20 days, during which the animals' average weight was regularly monitored· On day 21, blood samples were collected from the arterial lesion to analyze the serum levels of total cholesterol (TC), triglycerides (TG), high-density lipoprotein (HDL), and low-density (LDL) in the serum.

Collection of blood samples and lipid profile analysis

After the experiment concluded, the rats were anesthetized using ether to facilitate the collection of blood samples via intra-cardiac puncture. From the blood samples, separated the serum and collected in to heparinized tubes. The collected serum samples are inverted 2-3 times in order to ensure proper mixing and incubated it 4ºC for 2 to 3 hours. Kits supplied by Beacon Diagnostic Pvt ltd were utilized to measure total cholesterol, triglycerides (TG), and high-density lipoproteins (HDL) in the serum. Friedwald's formula was employed to calculate serum LDL and VLDL levels. This test determines the levels of serum triglycerides in milligrams per deciliter by comparing the test sample absorbance with the standard sample to determine its concentration.

RESULTS AND DISCUSSION

Optimization of Phytosomes by using Taguchi Orthogonal Array Design

The influential factors were screened using a Taguchi design with a randomized subtype, involving a total of 4 runs. The data obtained from the Taguchi-designed formulations model fitting was validated by a factorial type, indicating good fitness for determining main effects. Linear polynomial equations were constructed to evaluate synergistic and antagonistic factors. The model's F-value demonstrated significance, and *p-values* below 0.05 indicated significant model terms. To compare the relative magnitude of effects in the experiment, the half-normal plot was used. In our present research, the half-normal plot (Figure. 1b) represents "entrapment efficiency" where it was significantly influenced by factors "A" (phospholipids) and "C" (Time). Another critical parameter (Figure. 1 d), "particle size", was found to be greatly influenced by factors "B" (stirring temperature) and "C" (Time). Figure 1 shows that for "entrapment efficiency", both "A" and "C" were statistically significant, while for "particle size", factors "B" and "C" were statistically significant. The Pareto chart further supported these findings, displaying t-values where "Time" appeared less significant compared to Phospholipids in the color-coded representation. In the first response (entrapment efficiency), Factor A had a higher impact than Factor C (Time). Additionally, three-dimensional plots were generated to visually depict how changes in variables affected the responses. Figure 1 (e) highlighted higher entrapment efficiency values for formulations with maximum phospholipids, a trend similarly observed in particle size outcomes.

Evaluation of β-sitosterol Phytosomes

FTIR study of optimized β-sitosterol phytosome formulations

From the results of individual FTIR Spectra of beta-sitosterol in [Figure 2], L-Alpha-phosphatidyl choline and their mixture, it is publicized that FTIR of optimized β-sitosterol phytosome formulations BSP2 showed distinct peaks at specific wave numbers. One of the prominent peaks was observed at 3490.36 to 3186.51 $cm^{-1,}$ indicating V(o-H) O-H stretching. It suggests the presence of hydroxyl groups in BSP2. Additionally, peaks at 2864.39 cm^{-1} communicate to V(c-h) C-H stretch or CH3, suggesting the presence of methyl groups. The optimized phytosome BSP2 also exhibited absorption peaks at 1485.54 and 1016.52 cm^{-1} due to C-C and C-O stretching, respectively. These peaks designate the presence of these functional groups in BSP2.

DSC thermogram study of optimized phytosome BSP2 formulation

A DSC thermogram study was conducted on beta-sitosterol, L-Alpha-phosphatidylcholine, the optimized phytosome BSP2

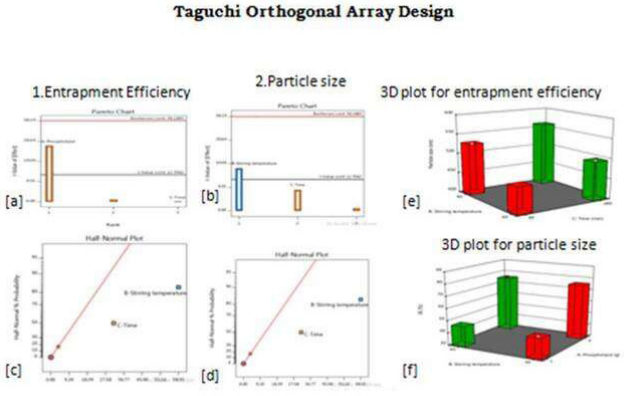

Figure 1: [a] Half-normal plot for entrapment efficiency, [b]Half-normal plot for Particle size, [c] Pareto chart for entrapment efficiency, [d] Pareto chart for Particle size, [e] three-dimensional plot for entrapment efficiency, [f] three-dimensional plot for Particle size

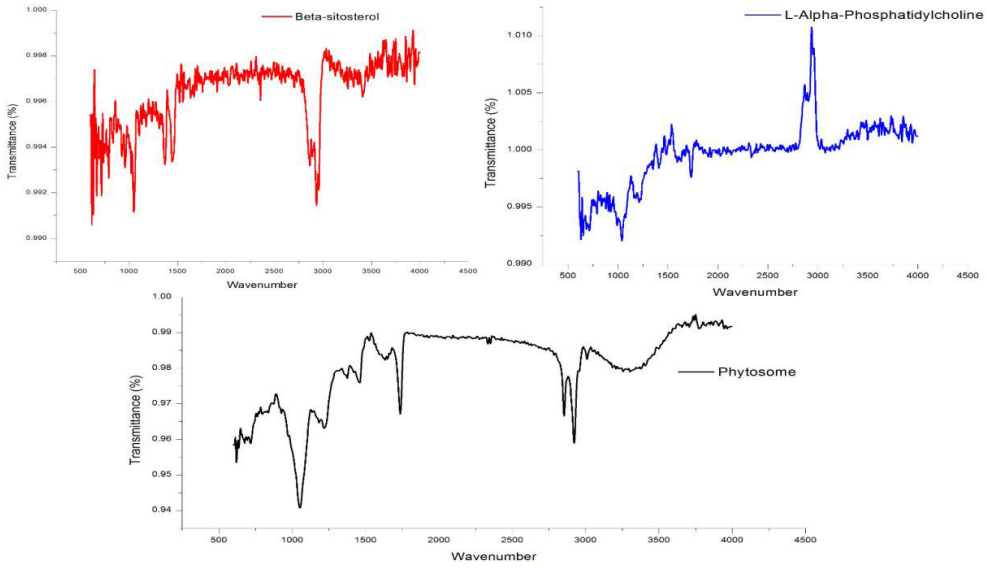

Figure 2: FTIR Spectra of β-sitosterol, L-Alpha-phosphatidyl choline and BSP2 phytosome

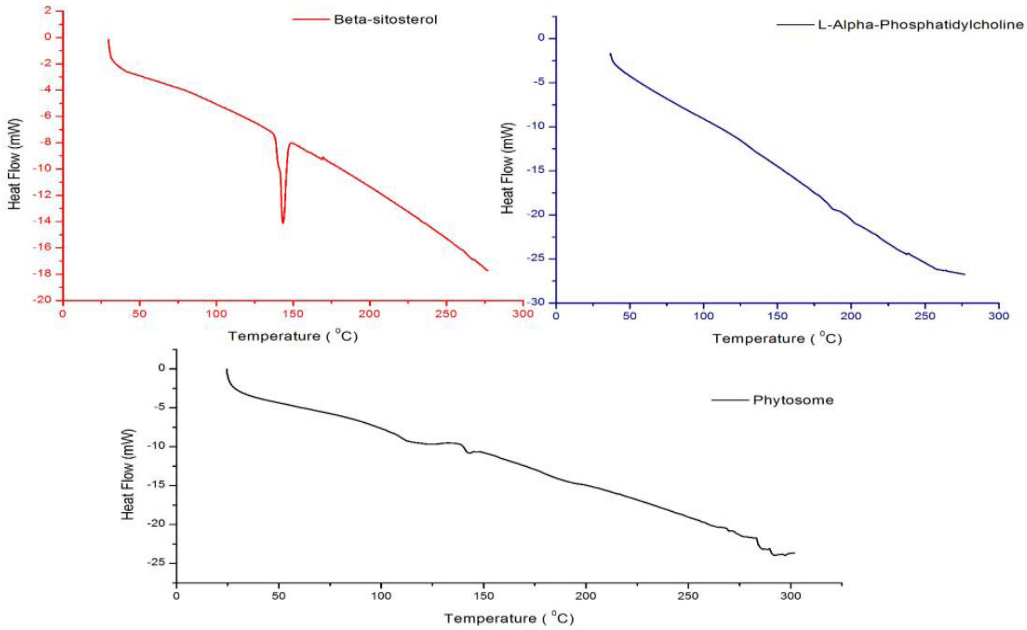

Figure 3: DSC thermogram of β-Sitosterol, L-Alpha-phosphatidyl choline and BSP2 phytosome

formulation, and the results are presented in Figure 3. One notable observation at a temperature of 127.94°C was the presence of a sharp melting endotherm. This endothermic peak indicated no significant interaction occurred between β-sitosterol (a compound found in plants) and L-Alpha-phosphatidylcholine (a phospholipid derived from soybeans).

Scanning Electron Microscope (S.E.M.) of Optimized Phytosomes

Figure 4 illustrates the surface morphology, shape, and structure of the optimized phytosome of β-sitosterol BSP2 at varying magnifications through S.E.M. imaging. This imaging technique allows for a detailed assessment of the phytosome particles. The observation from these images revealed that the β-sitosterol particles are connected to the polar head of soya lecithin. This connection between β-sitosterol and soya lecithin leads to the formation of phytosome BSP2. These phytosomes exhibit regular particle shapes and spherical structures. The SEM images indicate that the surface of these phytosomes is smooth with no apparent irregularities or roughness. Furthermore, at higher magnifications, it can be seen that the particles are tightly packed together, suggesting a high degree of stability within the phytosome structure. The morphological features observed in these S.E.M. images reveal the physical properties and characteristics of the

Figure 4: SEM analysis of optimized β-sitosterol phytosomes BSP2 formulation

optimized phytosome. These findings support previous studies showing enhanced solubility and bioavailability of active compounds when incorporated into a phytosome structure. Moreover, this information can aid in further optimizing its formulation for targeted delivery systems. This SEM analysis provided valuable insights into the surface morphology, shape, and structural details of the optimized β-sitosterol BSP2 phytosome.

Entrapment Efficiency

The entrapment efficiency of different batches of formulations are listed in Table 1.

The maximum value was 76.41% and the minimum was 46.29%

Zeta Potential of Optimized Phytosomes

Depending on the obtained results, it is noted that the carefully chosen ratio of soya lecithin greatly influenced the formation and distribution of phytosomes. Specifically, this ratio played a significant role in promoting the creation of uniformly dispersed nano-sized phytosomes. Furthermore, analysis of the optimized phytosome containing beta-sitosterol BSP1 revealed a zeta potential of -46.8 ± 4.34 mV, BSP2 revealed a zeta potential of -18.9 ± 1.82 mV, and BSP3 revealed a zeta potential of -41.1 ± 3..75 mV as shown in Figure 5. This finding suggests that a well-balanced mixture of soya lecithin helped form phytosomes and maintain their homogeneous distribution at the nano level.

Particle Size of Optimized Phytosomes

The prepared phytosome appeared as spherical vesicles under observation, with an average particle size of 606.8 nanometers (nm) as shown in Figure 6 and results were shown in Table 1. These spherical vesicles are microscopic compartments

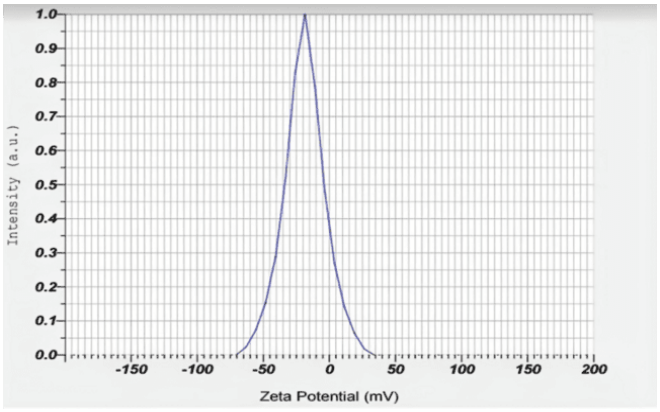

Figure 5: Zeta potential of optimized phytosome

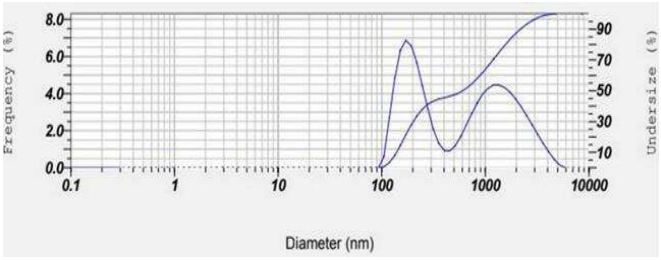

Figure 6: Particle size distribution of optimized β-sitosterol phytosomes BSP2 formulation

enclosed by a lipid bilayer, resembling tiny capsules. This encapsulation method helps enhance the stability and bioavailability of the phytosome, making it suitable for various applications. The mean particle size of 606.8 nm indicates that the phytosome particles are nano-sized, falling into the range of nanoparticles. This size is advantageous as it enables better absorption and cellular uptake compared to larger particles.

In-vivo Experimental Study

Estimation of biochemical parameters

Over the 21-day study period, animals in the cholesterol-induced, standard, and test groups received a cholesterol-rich diet along with their respective treatments. Mean body weight was recorded every 5 days- on day 0, 5, 10, 15, and 20 as detailed in Table 2.

On the 21st day, blood samples were taken from the retro-orbital plexus to measure total cholesterol, triglycerides, HDL, VLDL, and LDL levels for all groups. The toxic group's values were compared to those of the control group, followed by a

Table 2: Results of the body weight of the animals during the treatment process

Groups	*0th day*	*5th day*	*10th day*	*15th day*	*20th day*
Control	142 ± 0.78	142 ± 0.85	143 ± 0.68	144 ± 0.54	144 ± 0.34
Cholesterol	143 ± 0.98	150 ± 0.34	165 ± 0.39	178 ± 0.47	198 ± 0.37
Standard	142 ± 0.75	146 ± 0.29	149 ± 0.26	154 ± 0.29	162 ± 0.65
Beta sitosterol pure drug(500 mg/kg b.w)	142 ± 0.65	146 ± 0.38	149 ± 0.69	154 ± 0.36	162 ± 0.34
Beta sitosterol Phytosomes(100 mg/kg b.w)	141 ± 0.46	143 ± 0.19	145 ± 0.45	152 ± 0.76	162 ± 0.26

Table 3: Results showing all parameters of the animals after the treatment process

Groups	*[TC] Total cholesterol [mg/dl]>200*	*[TG] Triglycerides [mg/dl]>>150*	*[HDL] High-density lipoproteins [mg/dl]>)≤40*	*[VLDL] Very low-density lipoproteins [mg/dl]≥30*	*[LDL] Low-density lipoproteins [mg/dl] ≥100*
Control	65.98 ± 2.12	55.92 ± 0.93	31.21 ± 1.1	12.67 ± 0.4	16.5 ± 2.7
Cholesterol	178.1 ± 2.7**	153.21 ± 2.24**	14.32 ± 0.21**	34.23 ± 0.23**	117.27 ± 0.21**
Standard	143.21 ± 2.83**	94.12 ± 0.21**	37.23 ± 0.12**	20.12 ± 0.32**	50.12 ± 0.71**
Beta sitosterol pure drug (500 mg/ kg b.w)	135.1 ± 0.5*	96.15 ± 0.41*	35.43 ± 0.36*	18.31 ± 0.51*	51.31 ± 0.54*
Beta-sitosterol phytosomes (100 mg/kg b.w)	126.120 ± 0.98*	104.23 ± 2.8*	39.31 ± 0.45*	21.67 ± 0.34*	76.43 ± 0.34*

Represented Values are Mean ± SEM

** $p \leq 0.01$ Values Upon comparison with a control group

* $p \leq 0.01$ upon comparison of values with the standard group

The represented values demonstrate a profound difference when comparing the control n and standard groups.

Statistical analysis was conducted on all the data presented as mean ± SEM. The data was analyzed using ANOVA followed by Dunnett's test and compared to the control group. A significance level of $p < 0.05$ was considered significant, while $p > 0.05$ was considered non-significant (ns).

comparison between the standard and toxic groups. Lastly, the values of the test group were compared to those of the standard group. The results shown in Table 3 reveal that beta-sitosterol phytosome has shown profound action in reducing LDL, VLDL, TG, total cholesterol and increase in HDL levels in compared with the pure drug-treated and toxic groups.

CONCLUSION

The results serve as an exciting conclusion, highlighting the potential of beta-sitosterol phytosome in combating obesity. The discovery that beta-sitosterol treatment yields results comparable to those achieved by standard drugs adds weight to its potential as a natural alternative for managing obesity. Furthermore, this finding offers hope for individuals seeking non-pharmaceutical interventions for hyperlipidemia. Further research is required to draw definitive conclusions about the effectiveness of beta-sitosterol in treating hyperlipidemia in humans. These initial findings provide an optimistic outlook for natural approaches to managing this common health concern, obesity. Herbal drug delivery through phytosomes is gaining more recognition than the traditional approach, leading to improved bioavailability and enhanced patient compliance.

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REFERENCES

1. Batrakoulis A. Psychophysiological adaptations to Pilates training in overweight and obese individuals: a topical review. Diseases. 2022 Sep 29;10(4):71.
2. Ashrafi S, Rahman M, Ahmed P, Alam S, Hossain MA. Prospective Asian plants with corroborated antiviral potentials: Position standing in recent years. Beni-Suef University Journal of Basic and Applied Sciences. 2022 Dec;11(1):1-26.
3. Mendonça-Filho RR. Bioactive compounds: new approaches in the phytosciences. Modern phytomedicine: Turning medicinal plants into drugs. 2006 Sep 20:1-24.
4. McDermott BP, Anderson SA, Armstrong LE, Casa DJ, Cheuvront SN, Cooper L, Kenney WL, O'Connor FG, Roberts WO. National Athletic Trainers' Association position statement: fluid replacement for the physically active. Journal of athletic training. 2017 Sep 1;52(9):877-95.
5. Jakab J, Miškić B, Mikšić Š, Juranić B, Ćosić V, Schwarz D, Včev A. Adipogenesis as a potential anti-obesity target: A review of pharmacological treatment and natural products. Diabetes, Metabolic Syndrome and Obesity. 2021 Jan 8:67-83.
6. Jana P, Shyam M, Singh S, Jayaprakash V, Dev A. Biodegradable polymers in drug delivery and oral vaccination. European Polymer Journal. 2021 Jan 5;142:110155.
7. Waheed S, Li Z, Zhang F, Chiarini A, Armato U, Wu J. Engineering nano-drug bio interface to overcome biological barriers toward precision drug delivery. Journal of Nanobiotechnology. 2022 Aug 31;20(1):395.
8. O'hara F, Baxter RD, O'brien AG, Collins MR, Dixon JA, Fujiwara Y, Ishihara Y, Baran PS. Preparation and purification of zinc sulfinate reagents for drug discovery. Nature protocols. 2013 Jun;8(6):1042-7.
9. Surendra S. Agrawal1 , Govind Soni2 , Anil Pethe1 , Khushwant S. Yadav3 Doxorubicin Hydrochloride-loaded Nanoparticles for Oral Delivery: Optimization using Design of Experiments IJDDT, October - December 2022; 12 (4) :1520-1526
10. Roy H, Maddiboyina B, Rahaman SA, Singh PK, Tripathi AK, Srivastava SK, Chaubey R, Theendra VK, Sankula KR. Implementation and effect estimation by Taguchi orthogonal array design for Metoprolol Sustained Release Tablets. Smart Science. 2023 Oct 2;11(4):649-60.
11. Abd El-Rahman and SN, Suhailah S. Quercetin nanoparticles: Preparation and characterization. Indian J Drugs. 2014;2(3):96-103.
12. Zhang L, Yang X, Li S, Gao W. Preparation, physicochemical characterization and in vitro digestibility on solid complex of maize starches with quercetin. LWT-Food Science and Technology. 2011 Apr 1;44(3):787-92.
13. Abdel Raheem IA, Abdul Razek A, Elgendy AA, Saleh NM, Shaaban MI, Abd El-Hady FK. Design, evaluation and antimicrobial activity of egyptian propolis-loaded nanoparticles:

intrinsic role as a novel and naturally based root canal nanosealer. International journal of nanomedicine. 2019 Oct 21:8379-98.
14. Jiang F, Du C, Zhao N, Jiang W, Yu X, Du SK. Preparation and characterization of quinoa starch nanoparticles as quercetin carriers. Food Chemistry. 2022 Feb 1;369:130895.
15. Mane VB, Killedar SG, More HN, Tare HL. Evaluation of Acute Oral Toxicity of the Emblica officinalis Phytosome Formulation in Wistar Rats. IJDDT, October - December 2022; 12 (4) :1566-1570
16. Singh C, Yashwant, Gupta AK, Garg V. Formulation Development and Evaluation of Divalproex Sodium Extended-release Tablets. International Journal of Drug Delivery Technology. 2022;12(4):1769-1773.DOI: 10.25258/ijddt.12.4.46