RESEARCH ARTICLE

Formulation and Characterization of Pomegranate Peel Extract-Loaded Phytosomes for Anti-diabetic Activity

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ABSTRACT

Pomegranate, or *Punica granatum* L. (Lythraceae), is a plant with a variety of pharmacological characteristics. Also, pomegranate peel's medicinal potential appears to be multifaceted due to its rich content of phenolic and flavonoid compounds. The study's objective was to create phytosomes of pomegranate peel extract to get over the drawback of low bioavailability. The phytosomes of pomegranate peel extract were developed by an antisolvent precipitation technique using soya lecithin as a lipid and n-hexane as an antisolvent. The formed phytosomes were examined for particle size, zeta potential, incorporation efficiency, and In-vitro dissolution study. The chemical and thermal stability of phytosomes were studied using FTIR analysis and Differential Scanning calorimetry. Pomegranate peel extract and phytosome formulation were also examined for oral acute toxicity study and antidiabetic study in low dose and high dose concentration. On the 28th day of treatment blood glucose level, cholesterol, HDL cholesterol, and triglycerides were examined. The encapsulation efficiency of the optimized formulation was 86.37±0.21% with a particle size of 953.0±34.1nm. The production of phytosomes is verified by SEM pictures of the formulation. FTIR and DSC studies showed no interaction. The in vitro dissolution study showed 87.66±2.15% CDR which is greater than the peel extract 42.73±0.29% CDR. During the acute toxicity study, the animals' mortality was assessed, and no reported deaths were noted. There is a substantial decline in the amounts of glucose, cholesterol, and triglycerides observed when treated with bioactive extract and phytosomes. Evaluation results of phytosomes suggest that bioavailability can be improved by phytosome formulation. Also, Formulation can give effective results against diabetic conditions.

Keywords: Pomegranate peel extract, phytosomes, acute oral toxicity study, In vivo antidiabetic study

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INTRODUCTION

Since ancient times, humans have used pomegranate (*Punica granatum* L.), a member of the Lythraceae family that grows in tropical and subtropical climates, as a crop.[1] Because the temperature there is conducive to its growth, it is especially grown in West Asia and the Mediterranean region. The shrub typically reaches a height of 5 m, although, except for dwarf varieties that can reach 1-2 m, it can occasionally attain the shape of a tree up to 10 m.[2] One well-known source of important nutrients is pomegranates. It contains phenolic and organic acids, flavanols, anthocyanins, hydrolyzable tannins, and condensed tannins compounds that have been researched and linked to a host of health advantages against illnesses. Half of the fruit is edible, with the remaining portion being the exocarp or skin, which is inedible. Complex polysaccharides, minerals, and phenolics can all be found in peels.[3] High concentrations of hydrolyzed ellagitannins, specifically referred to as "pomegranate ellagitannins" and consisting of punicalins, punicalagins, and pedunculagins, are found in pomegranates, particularly in their rind or peel. Together with ellagitannins, pomegranate rind consists of flavonoids (such as kaempferol, luteolin, and quercetin), anthocyanidins (like cyanidin, pelargonidin, and delphinidin), and hydroxybenzoic acids (including gallic acid, ellagic acid.[4] These phytochemicals are responsible for improving its nutritional value. Pomegranates' punicalagin and ellagic, gallic, and oleanolic acids have been shown to have anti-diabetic effects. Pomegranate extracts have been shown to reduce irritation and low-density lipoprotein (LDL) cholesterol in rats on a high-fat diet. In many mouse intervention experiments, the extract also lowers serum glucose levels and hepatic lipid peroxidation in healthy rats, enhances glycaemic control, and raises the relative number of beta cells in alloxan-induced diabetic rats.[5,6] These phytochemicals have low solubility; hence the study focussed on developing and evaluating phytosomes containing bioactive from pomegranate peel to increase pomegranate peel extract's bioavailability and solubility for efficient diabetic treatment. "Phyto" signifies the plant, whereas "some" indicates cell-like. The vesicular drug delivery method known as phytosomes (or herbosomes) augments the bioavailability and absorption of medications that are not particularly soluble.[7] Phytosomes are compounds of lipids and organically potent phytochemicals bound in their

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Table 1: Coded levels of independent variables.

Independent Variables	Levels		
	-1	0	+1
Soy lecithin and extract ratio (X_1)	1:1	2:1	3:1
Reaction Temperature (X_2)	40	50	60
Dependant Variable			
Entrapment efficiency (Y)			

Table 2: Factorial design experimental trials

Experimental Batches	X_1	X_2	Entrapment Efficiency (%)
1	-1	-1	64.38 ± 1.23
2	-1	0	67.17 ± 0.19
3	-1	+1	72.98 ± 1.23
4	0	-1	84.98 ± 1.39
5	0	0	92.39 ± 1.24
6	0	+1	93.16 ± 0.58
7	+1	-1	82.05 ± 1.23
8	+1	0	85.58 ± 0.20
9	+1	+1	88.90 ± 1.33

Table 3: Dose concentration and Dosage rate.

Step	Animal ID	Test Dose (mg/kg)	Test Concentration (mg/ml)	Dosage Rate (ml/kg)
I	1	300	30	10
	2	300	30	10
	3	300	30	10
II	1	300	30	10
	2	300	30	10
	3	300	30	10
III	1	2000	200	10
	2	2000	200	10
	3	2000	200	10
IV	1	2000	200	10
	2	2000	200	10
	3	2000	200	10

structures that are created when phosphatidylcholine (or any hydro group) reacts with botanical extracts in an aprotic solvent.[8,9] Due to their improved ability to pass through lipid biomembranes and ultimately enter the bloodstream, they become more permeable than straightforward herbal extracts. These formulations have superior pharmacological and pharmacokinetic properties in comparison to commonly used preparations.[10] As the choline and phosphatidyl moieties are hydrophilic and lipophilic, respectively, phosphatidylcholine is a bifunctional molecule. In particular, following the choline head's binding to these molecules, the phosphatidylcholine molecule's body and tail, which are made up of the fat-soluble phosphatidyl component, wrap the choline-bound material. Thus, a lipid-compatible molecular complex called the phyto-phospholipid complex is formed by the bioactive and phospholipids. Certain spectroscopic methods can show that molecules are chemically bonded to the polar choline head of the phospholipids.[10,11] There are many advantages of phytosomes over old dosage forms:

Table 4: Group of animals for antidiabetic study

Sr. No.	Group	No. of Animals	Treatment
1.	I: Normal Control	06	No STZ, No Treatment
2.	II: Disease Control	06	STZ + No treatment
3.	III. Standard	06	STZ + Glibenclamide (0.5 mg/kg)
4.	IV: Pomegranate Peel Extract (Low Dose)	06	STZ + 200 mg/kg of Pomegranate Peel Extract
5.	V: Pomegranate Peel Extract (High Dose)	06	STZ + 400 mg/kg of Pomegranate Peel Extract
6.	VI: Phytosomes (Low Dose)	06	STZ + 200 mg/kg of Phytosomes Formulation
7.	VII: Phytosomes (High Dose)	06	STZ + 400 mg/kg of Phytosomes Formulation

Enhanced absorption from the gastrointestinal tract is made possible by their penetration of the lipophobic botanical extract.[12] The Phytosome constituents have all been approved for usage in medicinal and cosmetic applications, and the dosage form is safe.[13] Because they may be readily rendered accessible by phytosomes, they have been employed to administer flavonoid compounds that protect the liver. Furthermore, phosphatidylcholine has a synergistic effect on safeguarding the liver because it is also hepatoprotective.[14] This method provides delivery that is economical.[15] Drug entrapment during formulation production is not an issue. As the drug itself produces vesicles after conjugating with lipids, the entrapment efficiency is also high and more predefined. Because the phosphatidylcholine molecules and phytoconstituents create chemical bonds, they provide a superior stability profile.[16]

MATERIALS AND METHODS

Materials

Pomegranate peel extract was obtained using the Soxhlet extraction technique and used for the formulation of phytosomes. Ethanol, Methanol, Soya lecithin, Dichloromethane (DCM), and n-hexane, were purchased from Loba Chemie (Mumbai, India). All chemicals and solutions used were of analytical quality.

Methods

Preparation of Phytosomes

The phytosomes were formulated using the antisolvent precipitation technique. Extract and soya lecithin using different molar ratios (1:1, 1:2, 1:3) were dissolved in a 40 ml mixture of ethanol and dichloromethane. For three hours, the temperature of the reflux reaction mixture was maintained at 40°C, 50°C, and 60°C. The solution was then concentrated to 5-10mL. The addition of n-hexane was done with stirring. Precipitated phytosomes were dried in a desiccator for further use.[17,18]

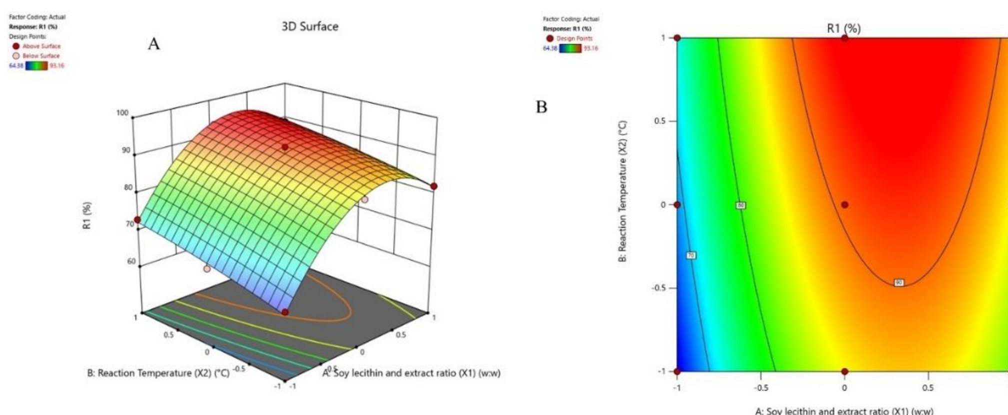

Figure 1: A] 3D response surface plot and B] contour plot of entrapment efficiency (Y, %).

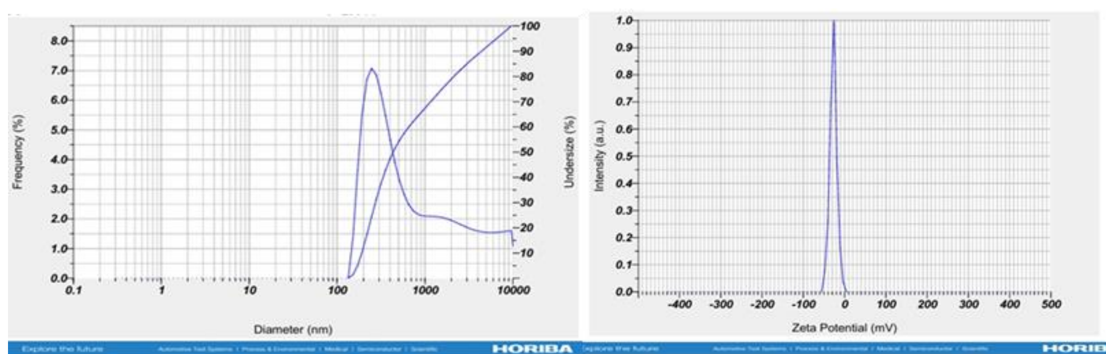

Figure 2: Particle size and Zeta potential of phytosomal complex.

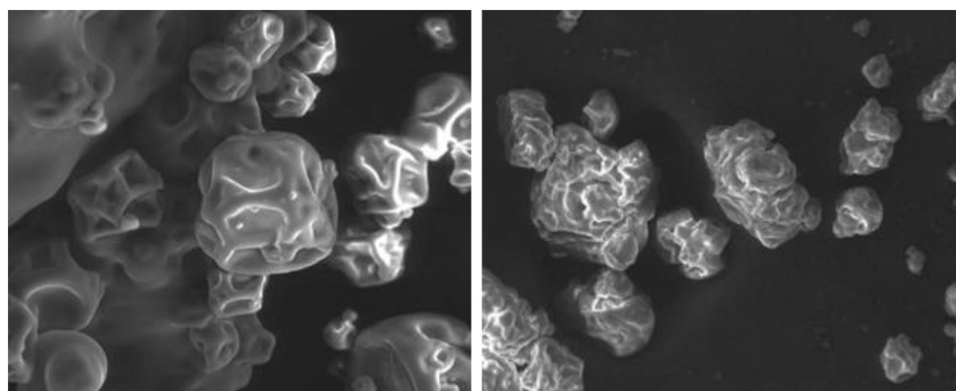

Figure 3: SEM Images of Phytosomal complex.

Determination of Entrapment Efficiency

A UV spectrophotometer was used to assess the phytosome encapsulation effectiveness. Ten milliliters of methanol were used to dissolve ten milligrams of phytosomes. To guarantee that the phytosomes were completely dissolved and that the encapsulated medication was released, the liquid was sonicated for fifteen minutes. The entrapped active in phytosomes was then measured at 258 nm after the mixture had been further diluted. The provided formula was used to compute the encapsulation efficiency.[19]

$$\text{Entrapment efficiency} = \frac{\text{Amount of entrapped drug}}{\text{Amount of initial drug}} \times 100$$

Optimization of processed variables

The overall effects of two independent variables-the ratio of soy lecithin to extract (X1, W: W) and the reaction solution temperature (X2, °C)-on entrapment efficiency (% yield) were investigated using a full factorial approach. Both independent variables (X1 and X2) were coded as low, middle, and high, and three stages of selection were used to construct 3^2 factorial designs with nine potential combinations. The dependent variable was determined to be the entrapment efficiency (%yield). All nine feasible combinations of the chosen variables were used in the experimental trials. Table 1 displays the coded levels of the independent variables, whereas Table 2 displays the batch composition with entrapment efficiency (%) data.

Evaluation of Physicochemical Properties

Particle size and Zeta potential

The zeta potential, polydispersity index, and particle size of the phytosomes were measured using the Horiba Scientific SZ-100-Z2 particle size analyzer. It uses dynamic light scattering (DLS) to quantify particle size and distribution width. To determine the particle size, five milligrams of the

material were dissolved in ten milliliters of deionized water. Every time more dilution was needed, this was done.

Scanning Electron Microscopy

Scanning electron microscopy is a very versatile technique for acquiring detailed information about the surfaces of samples and high-resolution images. Using a concentrated electron beam to scan a specimen's surface, this type of electron microscopy produces images with a far greater resolution than optical microscopy. The surface morphology of phytosomes can be studied by SEM ((Model: ECNAI 12, Netherlands, Software: Tecnai imaging & Analysis; Source – Tungsten Filament).

Fourier Transform Infra-red spectrophotometry (FTIR)

A Bruker FT-IR Alpha II Fourier Transform Infrared Spectrophotometer was used to record the FT-IR spectra of pure extract, soy lecithin, drug-excipient mixture, and phytosomes. A tiny sample (5–10 mg) was put directly onto an infrared diamond crystal, and pressure was applied after the pressure arm was adjusted over the sample. The program was used to record the spectra. For the purpose of a drug-excipient compatibility research, the spectra were examined for any interactions between the extract and the excipients in the physical mixture. The formulation did not exhibit any physicochemical interactions.

Differential Scanning Calorimetry (DSC)

Perkin Elmer's DSC 4000 model was used to measure the heat stability of the phytosomes that had developed. To cover the temperature range of interest, it employs several rapid heating and isothermal hold phases. An aluminum pan containing 9 mg of the sample was sealed, and a DSC thermogram was taken between 30°C and 400°C at a heating rate of ten degrees Celsius per minute.

In vitro dissolution studies

Phytosomes were dissolved in vitro at 37°C and 50 rpm using the USP Type I dissolving test device (LABINDIA DS 8000). The 100 mg of phytosomes that were measured were put into a size 0 capsule, which was then put in a basket with 900 cc of phosphate buffer (pH 6.8). To maintain stable sink conditions, samples (5 ml each) of

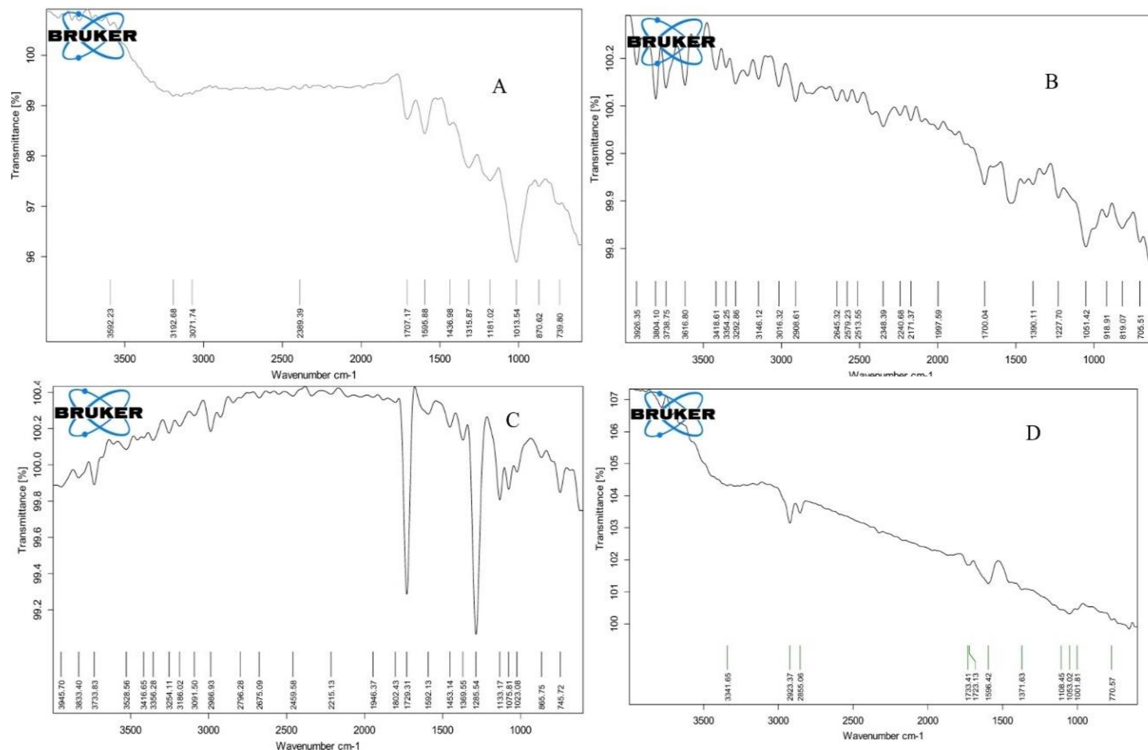

Figure 4: FTIR Spectra of A) Extract, B) Soya lecithin C) Phytosomes, D) Extract- Lecithin Mixture.

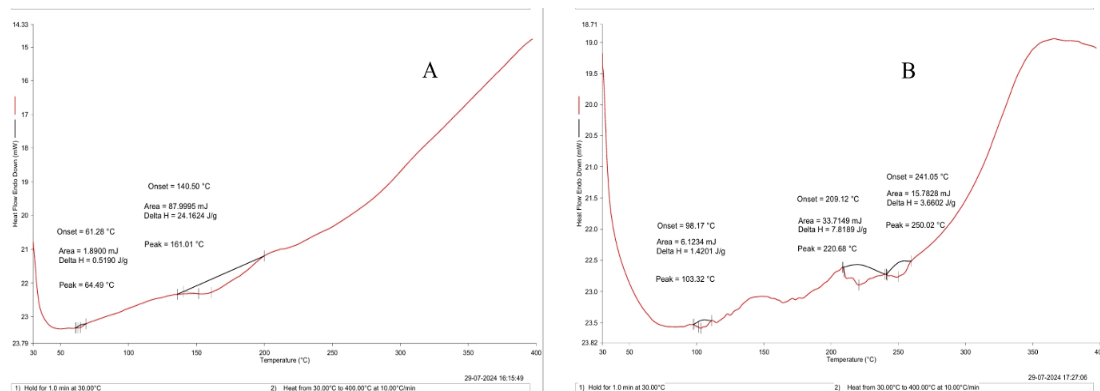

Figure 5: DSC Spectrum of Extract and Phytosomes.

Table 5: Summary of mortality result.

Step	Dosage (mg/kg)	No. of Treated Rats	Terminally Sacrificed	Found Dead(X)
1	300	3	3	0
2	300	3	3	0
3	2000	3	3	0
4	2000	3	3	0
Total	-	12	12	0

Table 6: Effect of Phytosomes on body weight of female Wistar rat.

Step & Dosage	Animal code	Day 0	Day 7	Day 14
Step I 300 mg/kg	1	190.50	196.00	201.50
	2	191.00	195.50	201.50
	3	192.50	197.00	202.50
Step II 300 mg/kg	1	189.50	194.00	199.50
	2	190.00	193.50	199.40
	3	194.50	199.00	205.00
Step III 2000 mg/kg	1	192.00	196.50	201.50
	2	190.00	193.00	197.00
	3	189.50	195.50	200.50
Step IV 2000 mg/kg	1	192.00	197.00	202.00
	2	189.50	192.50	196.00
	3	192.50	199.00	205.00

dissolving fluid were taken out at 1-hour intervals for 12 hours and replaced with an equivalent volume of fresh medium. The drug release from the phytosomes was assessed using UV spectroscopy at 258 nm after the extracted samples were filtered.[20,21]

Acute Oral Toxicity of Phytosomes

The study was conducted following the OECD 423 guideline, Acute Oral toxic class method. Female Wistar rats weighing 180-200 gm were used for the experiment.

The animals were kept in an experimental room at 22± 3°C with 50-65% humidity and a 12-hour. light/dark cycle. Enough data on the test substance's acute toxicity is gathered using a step-by-step process that uses just a few rats per step to allow for classification. A set of experimental animals are given the drug orally at one of the specified dosages. Three animals of the same sex, typically females are used in each phase of the methodical process used to evaluate the chemical. The next stage will be determined by whether compound-related death of creatures dosed at one step is present or absent; if no more testing is required, three more animals will be dosed with the same dose at the subsequent elevated or next reduced dose level.

Experimental Procedure

The research was carried out in phases. Three female rats were given 300 mg/kg body weight of phytosomes suspended from distilled water as part of the first experimental stage. Three to four hours before and two hours following medication, the rats were not fed. Unlimited water was available. All of the animals were observed for 14 days following the dosage. In the second trial stage, three female rats were given a dose of 300 mg/kg body weight of phytosomes suspended in distilled water. Three to four hours before and two hours following medication, the rats were not fed. Unlimited water was available. After the dose, all of the animals were supervised for 14 days. Formulation suspended in distilled water was given to three female rats at a dose of 2000 mg/kg body weight in the third trial phase. Three to four hours before and two hours following medication, the rats were not fed. Unlimited water was available. After the dosing, all of the animals were examined for 14 days. Formulation suspended in distilled water was given to three more female rats in step four of the trial at a dosage of 2000 mg/kg body weight. Three to four hours before and two hours following medication, the rats were not fed. Unlimited water was available. After the dose, all of the animals were monitored for 14 days.[22] Each rat received a single oral gavage of phytosomes from the test product at the concentration listed in Table 3. An intubation needle attached to a properly graded syringe was used to administer the doses to the animals. The dosage volume given to each rat was modified based on its most recent body weight, as shown in the table below.

Body Weight

Animals' weights were measured just before the test substance's administration (pre-administration fasted weight) and at least once per week thereafter, that is, on days seven and fourteen after treatment or at the time of death.[23]

In vivo Antidiabetic Effect

Animals: For the experiment, female albino rats weighing between 200 and 250 grams were employed. Throughout the experiment, the animals had unrestricted access to food and water. The mice were housed in an experimental room with a 12-hour light/dark cycle, 22± 3°C, and 30–70% humidity. Diabetes Induction: Streptozotocin at a dose of 60 mg/kg was used to induce diabetes. Streptozotocin was administered intraperitoneally at a dosage of 10 ml/kg after being dissolved in Citrate Buffer (pH 7.4). Following a 72-

Table 7: Effect of treatment on Body Weight.

Weekly Body Weight					
Group No.	Day 0	Day 7	Day 14	Day 21	Day 28
1	222.63 ± 3.08	227.90±2.55	236.17 ±3.74	245.89±6.19	272.93±41.81
2	219.80±3.92	215.06±5.50	219.76±17.02	220.78±23.93	211.83±27.44
3	214.30±5.91	215.36±8.87	222.25±10.04	228.48±10.38	235.89±8.54
4	231.61±5.83	239.34±7.68	243.74±5.07	248.58±9.80	253.81±12.33
5	241.63±6.37	249.20±7.38	253.93±9.22	258.55±9.54	264.47±15.16
6	224.69±4.97	225.40±4.74	226.16±7.06	231.88±6.74	236.09±7.32
7	222.99±3.86	222.69±6.40	228.14±5.57	231.87±5.51	237.30±5.45

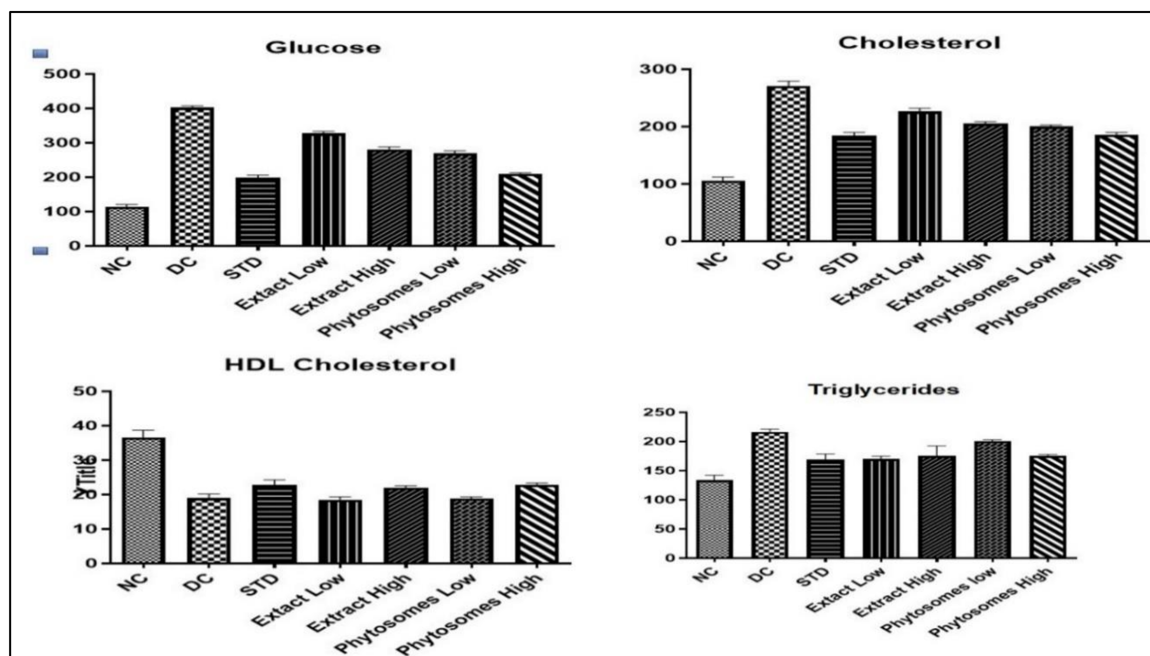

Figure 6: Effect of 28 days treatment on glucose, cholesterol, HDL cholesterol and Triglycerides.

hour STZ injection, the animals' blood glucose levels were measured. The animals chosen for the investigation were those whose blood sugar levels were found to be higher than 250 mg/dl. These animals were randomly placed in the following groups as shown in Table 4. According to weight, oral dosing for control, test extracts, and formulation was carried out once daily to the respective group for 28 days. Weekly blood glucose and body weight were monitored. When the study was finished duration, the animals were sacrificed and blood was collected. The collected blood was allowed to form a clot, and centrifuged at 3000 rpm for 15 mins. The supernatant serum was collected. Clinical biochemistry was conducted on collected serum, the following parameters were evaluated glucose, Cholesterol, Triglycerides, and HDL Cholesterol.

RESULTS AND DISCUSSION

Preparation and Optimization of Phytosomes

The phytosomes were formulated using the antisolvent precipitation technique. Polyphenolic extract incorporated with chemical bonding in soya lecithin formed a phytosomal complex. In the study, encapsulation efficiency was in the range of 64.38 ± 1.23% to 93.16 ± 0.58%. Table 2 lists entrapment efficiency for each formulation batch. A quadratic model was built employing these data to relate the variables that were investigated to the degree of extract incorporation (Y, yield, %). The following mathematical equation is the result of the developed formulation.

$$Y = 90.66 + 8.66X_1 + 3.93X_2 - 0.43X_1X_2 - 13.33X_1^2 - 0.63X_2^2$$

As the p-value (0.0033) is less than 0.0500, indicates model terms are significant. The observed value of the correlation coefficient (R2 =0.9911), caused us to determine that this was the study's most representative model. The 3D response graph and contour graph demonstrate how strongly independent variables affect dependent variables using STAT EASE 360 software. Figures 1 display the % EE graphs. The results showed that the entrapment efficiency was significantly influenced by both X_1 and X_2 variables. Based on these observations and calculations from the built quadratic model, the optimal values for the variables under investigation-the drug: phospholipid ratio (X1, w: w) and the reaction temperature (X2, °C)-were found to be 1:2 and 60 °C, respectively.

Particle size, polydispersity index, and zeta potential determination

The physical stability of nanocarrier dosage forms in liquid media is significantly influenced by particle size and zeta potential. The formed phytosomal complex had a polydispersity index of 0.444 and a mean particle size of 953.4 ± 12.37nm shown in Figure 2. For phytosomal complex dosage forms with a low polydispersity index, which denotes a limited distribution of particles, the particle size should be in the range of 50nm to 10mm. With a zeta potential of -27.2 mV, the phytosomes were found to be extremely stable.

Scanning Electron Microscopy (SEM)

SEM images of the phytosomal formulations are shown in Figure 3 Rounded shape of phytosomes was observed.

Fourier Transform Infra-red spectrophotometry (FTIR)

FTIR spectra of Extract, Soya lecithin, and phytosomal formulation are shown in Figure 4. FTIR spectra of Extract, soya lecithin, extract-lecithin mixture, and phytosomal complex are shown in Figure 4. The FT-IR absorption peaks of extract, 1013 (C-H bending), 1315 (O-H stretch), 1595-1707 (C=C stretching, C=O stretching), 1334.89 (Phenol O-H bend), 1046.02 (C-O-C stretch), 2389 (O-H stretch). The FTIR spectrum of Soya lecithin shows the identified peaks at 1051 (C-H bending), 1700 (C=O stretch), and 3146-3418 (O-H stretch). The phenolic O-H stretch of the extract shifts from 3326.54 to 3342.64 and 3485.66 in formulation when the IR spectra of the crude extract and phytosomes are compared, indicating the establishment of weak molecular

interactions. The phytosomal complex (1600.79) shifts the aromatic C=C stretch of soy lecithin (1578.73) and shows 740-865 (cis C-H and Trans -CH stretch). This suggests weak intermolecular interactions as a result of the extract and lipids' hydrogen bond reaction. The formulation had all of the drug and excipient matching peaks, indicating that there was no significant chemical interaction between the two.

Differential Scanning Calorimetry (DSC)

The experimental method known as differential scanning calorimetry (DSC) has made a substantial contribution to our knowledge of the stability of macromolecular assemblies and biomacromolecules. This method involves subjecting a substance and a reference to a temperature program that is controlled while measuring the difference in the heat flow rate into each as a function of temperature. It also provides information on how the medicine interacts with other formulation ingredients and how the newest molecules have evolved. Figure 5A shows two endothermic peaks at 64.49°C and 161.01°C while Figure 5B shows three peaks at 103.32°C, 220.68°C and 250.02°C. Figure 5B spectrum shows that the melting and transition temperatures in the extract are absent, at 64.49°C and 161.01°C, respectively. This suggests that the extract and excipients have formed a chemical connection and soy lecithin has been fully incorporated into the phytosomes matrix. A glass transition in the formulation is suggested by a little exothermic peak. The DSC thermogram of prepared phytosomes is seen in Figure 4. New peaks appeared in the phytosomal complex indicating that the extract was distributed uniformly throughout the phospholipid matrix by some molecular cooperation between the formulation ingredients, which may have taken the form of hydrogen bonds or van der Waals' force of attraction.[24]

In vitro dissolution studies

In vitro drug release was determined for optimized formulation. The extract and phytosomes had distinct drug-release mechanisms. Studies on the in vitro breakdown of phytosomes revealed that a set quantity of medication was released at predetermined times. The phytosomes displayed 87.66±2.15% CDR, whereas the extract displayed 42.73±0.29% CDR. This indicates a rise in the extract's solubility in the phytosomal complex formulation. High bioavailability will result from the formulation's high solubility.

Acute oral toxicity study

This study required a total of 12 female rats to be tested a dose levels. Phytosomes caused NO Mortality at the dose of 2000 mg/kg. At Step I and II: 300.00 mg/kg as well as Step III and IV: 2000.00 mg/kg, the test material caused NO death. Until the study's conclusion, every animal seemed healthy and showed no outward symptoms of intoxication. Table 5 shows a summary of mortality results.

Body weight

In an acute toxicity study, Table 6 displays how the phytosomal complex formulation affected the body weight of female Wistar rats. On Days 7 and 14 after formulation administration, as well as during the fasting period, or Day 0, the body weight of the animals used for research in the treatment groups was recorded. The body weight of the rats showed no discernible changes in weight increase. Over the 14 days, the body weight of rats were raised gradually and normally.

***In-vivo* Antidiabetic Effect**

After receiving approval from the Institutional Animal Ethical Committee (proposal number. IAEC02/2023/RP-04), the in vivo antidiabetic potential investigation was carried out utilizing the methodology described in the literature. The effect of treatment on body weight are showed in Table7. Body weight of STZ induced female albino rats was decreased when no treatment was given while other treated groups of extract and phytosomes showed a significant ($p<0.05$) increase in body weight as compared to first day of treatment.

Impact on the level of Glucose, Cholesterol, HDL Cholesterol and Triglycerides

There is significant decrease in the levels of glucose, cholesterol and triglycerides was observed when treated with pomegranate peel extract and phytosomes. HDL Cholesterol levels observed to be increased untreated rats. The treated groups with extract and phytosomes showed significant ($p<0.05$) decrease in glucose, cholesterol and triglyceride level. The impact of all groups on these parameters are shown in Figure 6.

CONCLUSION

Because they have a higher therapeutic 2 value and fewer side effects than modern drugs, herbal remedies have been used for millennia and are valued by both patients and medical experts. To improve the solubility and bioavailability of natural components, phytosomes or phytosomal complexes are nanocarrier dosage forms. To enhance bioavailability, the bioactive of pomegranate peel was successfully extracted in this study and formed into phytosomes. The phytosomes were evaluated for various parameters such as Entrapment efficiency, Particle size, Zeta potential, SEM, FTIR, DSC, In vitro dissolution study, acute oral toxicity, and In vivo antidiabetic study. It can be inferred from the evaluation parameters' findings that the formulation of phytosomes from bioactive can be effective for its bioavailability, as it gives 87.66±2.15% CDR which is greater than the peel extract 42.73±0.29%. The acute toxicity of phytosome formulation was evaluated using OECD 423 recommendations. When the toxicity was acute, the animals' mortality was assessed, and no reported deaths were noted. During observation, the experimental animals showed no discernible changes in body weight or clinical symptoms. The body weight of the diabetic control group significantly decreased, which could be attributed to fat breakdown or the inability to obtain glucose for energy use. Due to increased glucose metabolism, the treated groups showed a significant increase in body weight. In the treated groups, these parameters' levels returned to almost normal, indicating that phytosomes may have antidiabetic effects. Its hypolipidemic action was also suggested by a noticeable impact on lipid levels. Therefore, the current investigation indicates that this phytosomal preparation is safe to employ in vivo and has important clinical applications for delivering bioactive chemicals.

STATEMENT OF ETHICS

This experiment was conducted under the Institutional Animal Ethics Committee (proposal number IAEC02/2023/RP-04)

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