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DESIGN, DEVELOPMENT, CHARACTERIZATION AND EVALUATION OF *LAGERSTROEMIA SPECIOSA*-PHOSPHATIDYLCHOLINE COMPLEX: AS A BUDDING ANTIDIABETIC AGENT

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ABSTRACT

Developing the drugs as amphiphilic lipid complexes is a potential approach for improving therapeutic efficacy of the drugs by increasing solubility, reducing drug crystallinity, modifying dissolution behaviour and improving bioavailability. *Lagerstroemia speciosa* (Banaba), plant is a vital source of corosolic acid (2alpha-hydroxyursolic acid), a terpenoid derivative documented to be responsible for anti-diabetic, anti-inflammatory and antihypertensive activities, exhibited by the plant. Major limitation associated with this compound is poor solubility and thus the dissolution restrains its bioavailability. To overcome this limitation, *Lagerstroemia speciosa* phospholipid complex (HAELS) was developed and subjected to pharmaceutical investigation by thermal analysis (differential scanning calorimetry), crystallographic (X-ray diffracto-graphy), surface morphology (scanning electron microscopy), spectroscopic methods (FT-IR), solubility, and the dissolution (*in vitro* drug release) as well as pharmacological evaluation by *in vivo* Streptozotocin-nicotinamide (STN-NCM) induced diabetes model at the dose of 100 mg/kg and 200 mg/kg. The phospholipid complex of *Lagerstroemia speciosa* was found, fluffy and porous with rough surface morphology in the SEM. Further, FT-IR, DSC, and XRD data confirmed the formation of the complex. The improved dissolution was shown by the phospholipid complex. Animal study data revealed dose dependent antidiabetic and antihypolidemic activities. The phytosome may be considered as promising drug delivery system for improving the overall absorption and bioavailability of the *L. speciosa* molecule for the treatment of diabetes.

Keywords: *Lagerstroemia speciosa* extract (HAELS) phospholipid complex, DSC, XRD, SEM, FTIR, STN-NCM.

1. INTRODUCTION

The phytosome technology creates intermolecular bonding between individual polyphenol molecules and one or more molecules of the phospholipids, phos-phatidyl choline (PC) [1]. Phytosomes are produced by a process whereby the standardized plant extract or its constituents are bound to phospholipids, mainly phos-phatidylcholine producing a lipid compatible molecular complex. This phytophospholipid complex (phytosome) resembles a little cell. A Phytosome is generally more bioavailable than a simple herbal extract due to its enhanced capacity to cross the lipid-rich biomembranes and reach circulation and thus exhibits better pharmacokinetic and pharmacodynamics profile than conventional herbal extracts [2-7].

Lagerstroemia speciosa (Lythraceae, banaba, pride of India) is a medicinal plant that grows in the Philippines, China, India and Southeast Asia. Major constituents of *L. speciosa* leaves include corosolic acid, lagertannin, ellagic acid, lagerstroemin, etc. Traditionally, the whole plant and specifically leaves are used to treat diabetes and hyperglycemia (elevated blood sugar). The hypoglycemic (blood sugar lowering) effect of banaba extract is reported to be similar to that of insulin which induces glucose transport from the blood into body cells. This effect is attributed to the various active chemical constituents present like corosolic acid and larger-tannins [8, 9].

Corosolic acid is a naturally occurring pentacyclic triterpene that has displayed a potential anti-diabetic activity, anti-inflammatory, and antihypertensive pro-perties. It has been found out that components of banaba has poor bioavailability and pharmacokinetic profile in dosage form like dry powder extracts, tablets, soft gelatin capsules etc [10]. To resolve this, our aim is to

separately formulate such nanoparticulate formulation with enhanced solubility and efficacy of *L. speciosa*a nd to evaluate *in vivo* antidiabetic activity of optimized formulation by Streptozotocin-Nicotinamide (STN-NCM) induced diabetic model.

2. MATERIAL AND METHODS

Soya Lecithin was procured as gift sample from Indena Pvt. Ltd., Germany. Cholesterol was purchased from Himedia Laboratories Pvt Ltd, Mumbai. Methanol and water were of HPLC grade. All the other reagents and solvents were of the highest purity.

2.1. Procurement of plant *L. speciosa*

Fresh leaves of *Lagerstroemia speciosa L. (L. speciosa)* were collected from Valsad and were confirmed by **Dr. T. G. Gohil**, taxonomist, B.K.M. Science School, Valsad, Gujarat.

2.2. Preparation of Extract for *L.speciosa* (HAELS)

Dried leaves powder of Banaba (100 g) was refluxed in round base jar with half litre of aqueous alocohol (90%) for 2 hr and filtered. The process was repeated thrice till extract obtained was colourless. The extracts were pooled and concentrated under reduced pressure totally to yield reddish brown residue. Percentage yield of fractions was calculated.

2.3. Preparation of Phytosomes by antisolvent precipitation method [10-13]

The specific amounts of plant extract (HAELS) and soya lecithin and cholesterol were refluxed with acetone at a temperature 50-60°C for 2h. The mixture was concentrated to obtain the precipitate which was filtered and collected. The dried precipitate of phytosome complex was placed in amber colored glass bottle and stored in refrigerator.

2.4. Evaluation of trial batch for working method selection

Selection of working method was done on the basis of minimum particle size and maximum entrapment efficiency. A 3^2 factorial design was used to study the effect of Independent variables on the dependent variables (table 1 and table 2).

Design Expert 11.0.4.0 (Trial Version Stat-Ease, Inc, USA) was used for the analysis of effect of each variable on the desired one *i.e.* particle size, span value and entrapment efficiency. ANOVA was used to study the statistical significance.

Table 1: Independent and dependent variables

Independent Variables	Cholesterol concentration, Soya lecithin concentration
Dependent Variables	Particle size, entrapment efficiency and In vitro release
Levels	Low, Medium and High
Constraints	Particle size and maximum Entrapment efficiency

Table 2: Formula used for formulation of Phytosome

Batches	**Extract (g)**	**Soya lecithin (X1) (g)**	**Cholesterol (X2) (g)**	**Temperature (°C)**	**Methanol (ml)**	DCM (ml)	**Hexane (ml)**
F1	5	5	1	40	20	10	20
F2	5	10	4	40	20	10	20
F3	5	7.5	1	40	20	10	20
F4	5	5	2.5	40	20	10	20
F5	5	10	2.5	40	20	10	20
F6	5	7.5	2.5	40	20	10	20
F7	5	5	4	40	20	10	20
F8	5	10	1	40	20	10	20
F9	5	7.5	4	40	20	10	20

2.5. Characterization [14-21]

2.5.1. Entrapment efficiency

Entrapment efficiency of Phytosomes was calculated by centrifugation technique. Phytosome was diluted with methanol and then centrifuged at 10,000 rpm for half an hour at -4°C using high speed cooling centrifuge machine. The supernatant was collected and the amount of free extract was determined by UV visible spectrophotometer at 366 nm for *L. speciosa* extract. The entrapment efficiency was calculated by following formula:

{(Total amt.of drug-Amt. of free drug)/ Total amt.of drug} x 100

2.5.2. Mean Particle Size and Size Distribution

Photon correlation spectroscopy (PCS) using Zeta sizer Nano ZS90 was used to determine particle size and size distribution. 20µl phytosomal suspension was put in a glass cuvette. All estimations were completed in triplicate and performed at 25°C.

Span = [D(90%) -D (10%)]/[D(50%)]

Where D (90), D (10) and D (50) are equivalent volume diameter at 90, 10 and 50% cumulative volume respectively.

2.5.3. Visualization

Visualization of phytosomes was accomplished by utilizing Scanning Electron Microscopy. The samples were sputter-covered with gold/palladium for 120 s at 14 mA under argon air for auxiliary electron emissive SEM (Hitachi-S3400N) and watched for morphology at voltage of 15.0 kV.

2.5.4. Zeta Potential

Zeta potential measurement of the optimized phytosome was done by using the zeta sizer Nano ZS90 (Malvern Instruments Ltd., Malvern, UK). Sample was diluted to 10ml with water, 5ml of this diluted sample was transferred to a cuvette and the Zeta potential was measured.

2.5.5. Differential scanning calorimetry (DSC)

Extract, soya lecithin phospholipids, physical mixture of extract, soya lecithin and cholesterol, phytosome complex of extract were placed in the aluminum crimp cell and heated at 25°C/min from to 300°C in the atmosphere of nitrogen (5ml/min) (TA Instruments, USA). Peak transition onset temperatures were recorded by means of an analyzer.

2.5.6. In Vitro Release Study

In vitro release was measured using dialysis method. Formulation was dispersed in release medium (phosphate buffered saline (PBS), pH 7.4 to form a suspension. The suspension was filled in a cellulose dialysis membrane (Dialysis Membrane-70, Himedia, Mumbai, India), its both ends were tied. It was then suspended in a glass vial containing 10 ml of release medium. The vial was shaken horizontally using water bath shaker at 37°C. In vitro drug release was assessed by intermittently sampling the release medium at predetermined time intervals and was replaced with fresh medium to maintain sink condition. The amount of extract released in each sample was determined using a calibration plot; the reported values are average of three replicates (n= 3). Results of *In vitro* drug release studies obtained are shown graphically as cumulative percentage drug release versus time using power law or Korsmeyer Peppas model.

$$M_t / M_\infty = Kt^n$$

Where, M_t = Amount of drug released in time t,

M_∞=Amount of drug released at infinite time, K = Peppas release rate constant

N = Slope of the line called as release exponent

In Korsmeyer Peppas model, The plot of Log (M_t /M_∞)/Log t gives a straight line with a slope of n and intercept of Log K.

2.5.7. Fourier Transform Infrared (FTIR) Analysis

FTIR (Bruker Alpha FTIR) spectral data were taken to ascertain the structure and chemical stability of phytosome complex, soya lecithin, cholesterol and pure plant extract. Samples were crushed with KBr to get pellets at 600 kg/cm2 pressure. Spectral scanning was done in the range between 3500- 1000 cm^{-1}.

2.5.8. X-Ray Diffraction Analysis

XRD analysis was recorded by PAN analytical X'PERT-PRO Powder X-ray diffractometer. The sample scanning speed at 0.3 s per degree at the anode materials CuK α radiation (1.54060Å) recorded a 2θ range in 10-80° of the spectrum. The X-ray generator was allowed to operate at 35mAtube current and 40kV tube voltages. The scanning angle was adjusted in the range from 3 to 60° in the step scan mode with a step time of 32.8s An individual spectrum was run for plant extract, soya lecithin, cholesterol and also for optimized phytosomal formulation.

2.6. In-*Vivo* Study against Diabetic Activity [33-42]

2.6.1. Experimental animals

Wistar albino rats (150-200 g) were secured and were housed in Shree Naranjibhai Lalbhai Patel College of Pharmacy, Surat, Gujarat, India with 12 h light and 12 h dim cycles. Animals were kept up under at room temperature of 30±2°C and 60-65% relative moisture and fed with standard pellet diet and clean water *ad libitum*. Protocol design was approved by Institutional Animal Ethical Committee. (CPCSEA/VBT/IAEC/35/2014).

2.6.2. Streptozotocin - Nicotinamide (STN - NCM) Induced diabetic model

Thirty six Wistar albino rats were divided in six groups, each group contains 6 animals. All animals has been

treated with nicotinamide (120mg/kg, i.p.) 15 min before streptozotocin (60 mg/kg, i.v) for 21 days. Following were the groups of model.

Group I: STN-NCM induced group

Group II: STN-NCM + Hydroalcoholic extract (200mg /kg, orally)

Group III: STN-NCM+Hydroalcoholic extract (400mg /kg, orally)

Group IV: STN-NCM + Phytosomes of Hydroalcoholic extract (100mg/kg, orally)

Group V: STN-NCM + Phytosomes of Hydroalcoholic extract (200mg/kg, orally)

Group VI: STN-NCM + Glibenclamide (10 mg/kg, orally)

On 0, 7th, 14th and 21st days, all animals were weights and collected the blood by retro-orbital plexus methods for estimation of blood glucose levels.

2.6.3. Determination of biochemical parameters

On 21st days, blood sample were collected by retro-orbital plexus technique from all creatures into EDTA sprinkled tubes and were centrifuged at 3000 rpm for 20 min. Serum was isolated and put away at -20°C until examination was performed. Serum tests were broke down for Cholesterol, High Density Lipoproteins (HDL), Low Density Lipoprotein s (LDL), Very Low Density Lipoproteins (VLDL), cholesterol, total proteins, urea, creatinine and triglycerides utilizing symptomatic unit (ERBA Diagnostics Mannheim, Germany) in Auto analyzer.

2.6.4. Histopathology

Creatures from all gatherings were anaesthetized and dismembered. Pancreas and kidney were extracted out and protected in 10% formalin and sent to histopathology services for Hematoxylin and Eosin staining. Slides were analyzed and pictures were clicked with assistance of binocular magnifying lens fixed with camera.

2.6.5. Statistical analysis

All results were expressed as mean± SEM. Values were considered statistically significant with $P < 0.05$.

3. RESULT AND DISCUSSION [38-43]

The Phytosomes were prepared by antisolvent precipitation technique and particle size and entrapment efficiency was measured as 345.88±0.2 nm and 91.5± 0.63 respectively.

The results tof particle size, span and entrapment efficiency from the experimental trials carried out using 3^2 factorial design are given in table 3.

For all the nine formulations, particle size vary from 143 to 456 nm, span value 0.34 to1.10 and entrapment efficiency from 45.48% to 82.43% respectively. Since the desired goal of the concern phytosomal formulations was to obtained particle size below 300 nm and maximum entrapment efficiency, thereby F5with particle size 296 nm and entrapment efficiency 82.45 % and Zeta potential-19.35 mv was optimized that indicates the formation of stable formulation.

With Design Expert, predicted values of dependent variables (particle size, span and entrapment efficiency) were found to be 278.41nm, 0.585 and 75.95% respectively. A new formulation was prepared by using specified optimized values and results were found to be 295.32±0.33nm, 0.53±0.21 and 82.34±0.05% respectively. The closeness between the observed and predicted values proved the significance of design model (table 4).

Table 3: Phytosomal formulation of *L. speciosa* prepared as per factorial design

Batch	Soya lecithin (g) (X1)	Cholesterol (g) (X2)	Particle size (nm) (Y1)	Span (Y2)	Entrapment efficiency (%) (Y3)
F1	5	1	143±0.43	0.54±0.04	45.48±1.32
F2	10	4	198±1.45	0.66±0.05	52.72±0.85
F3	7.5	1	220±1.51	0.75±0.65	58.69±0.35
F4	5	2.5	255±2.31	1.10±0.23	63.53±1.53
F5	10	2.5	295±0.53	0.53±0.14	82.43±1.65
F6	7.5	2.5	312±1.37	0.80±0.53	77.34±1.43
F7	5	4	343±1.02	0.34±0.58	79.58±1.51
F8	10	1	397±0.56	0.67±0.74	73.34±1.98
F9	7.5	4	456±0.32	0.98±0.32	76.59±1.82

Data are expressed as mean ± SD, n = 3

Table 4: Solution provided by the factorial design for phytosomal formulation of *L. speciosa*

CONSTRAINTS							
Name		**Go al**		**Lower Limit**		**Upper Limit**	
X1: Soya lecithin		Is in range		1		2	
X2: Cholesterol		Is in range		0.2		0.8	
Particle size (nm)		Minimize		143		456	
Span		Less than 1		0.34		1.10	
Entrapment efficiency (%)		Maximize		45.48		82.43	
SOLUTION							
Batch	Soya lecithin (g)	Cholesterol (g)	Particle size	SPAN	Entrapment efficiency	Desirability	Solution
Optimized	2	0.495	278.41	0.585	75.95	0.706	*L.speciosa*

The entrapment efficiency of optimized formulation of *L. speciosa* were found to be 82.43% and 75.1%.The surface morphology, shape and structure of the optimized phytosome complex (Particle size was 295 nm) using SEM is shown (fig. 1).

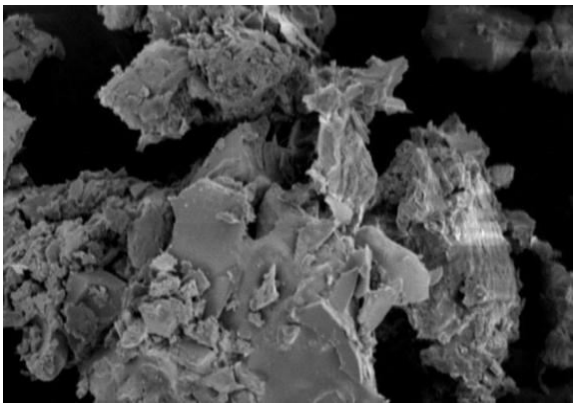


Fig. 1: SEM of optimized formulation of phytosome complex

In DSC study, (fig. 2), the thermogram of soya lecithin gives two distinct peak at 86.71°C and 202.91°C. The first peak indicates melting while second done is attributed to transition temperature. The thermogram of cholesterol gives two peak at 41.87°C and 103.76°C and one sharp peak at 150.90°C. This sharp peak indicates the melting point of cholesterol. The thermogram of *L. speciosa* extract give only one peak *i.e* 97.38°C. Finally, the thermogram of optimized formulation of *L. speciosa* gives two new peaks. DSC study concluded that a stable formulation is formed by some molecular interaction that can be hydrogen bonding between extract and phospholipids that distributed the extract molecularly into phospholipid.

With Korsmeyer-Peppas model, regression value was obtained 0.9935. The release profile was attributed to diffusion process (fig. 3).

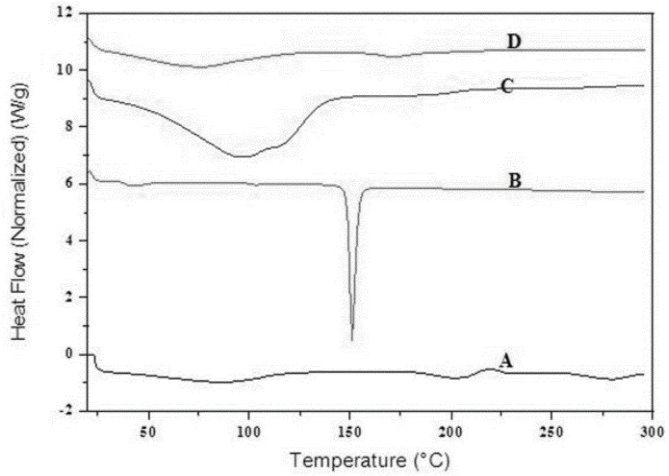


Fig. 2: Comparative DSC thermogram of (A) Soya lecithin (B) Cholesterol (C) *L. speciosa* extract (D) Optimized formulation

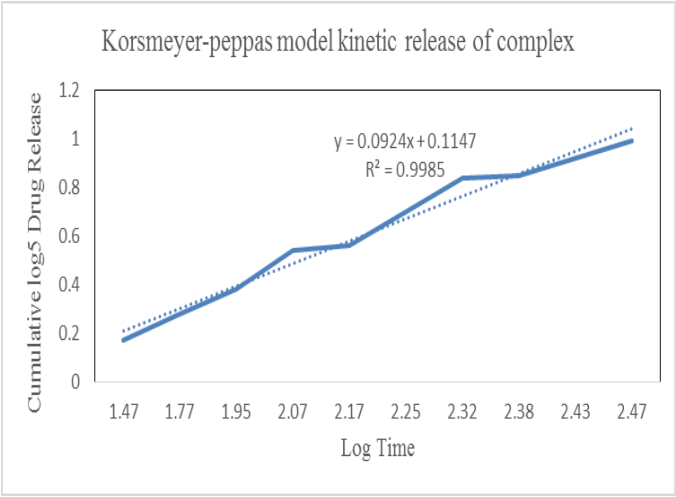


Fig. 3: *In vitro* drug release profile from complex with power model

Comparison of the FTIR spectra shows the changes to specific position in extract due to interaction with

phospholipids. Changes to the stretching frequency in the optimized formulation describes that weak inter-molecular interactions have been occurred during the formation of phytosome (fig. 4).

X-ray diffraction (fig. 5) was carried out to confirm the physical state of L. speciosa, soya lecithin and cholesterol into phytosome in comparison to pure cholesterol, soya lecithin and extract. X-ray diffracto-gram of soya lecithin showed intense sharp diffraction peaks of crystallinity at a diffraction angle (2θ) of 19.6417. The cholesterol showed intense peaks at 2θ value of 16.1415, 17.1538 and also 72.6223 indicating crystalline structure. HECE showed a less intense and broader peak from 2θ values 14.9416 to 44.6383. X-ray diffraction pattern of the phytosome of extract showed peaks from 2θ value 15.1080 to 72.5498 indicating crystalline form.

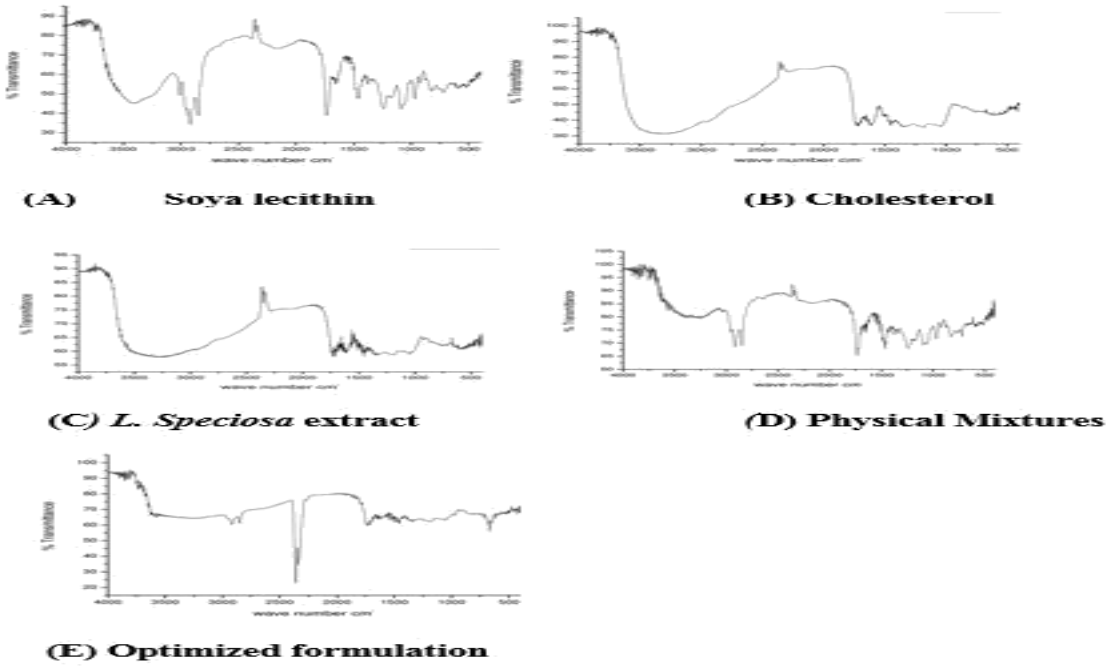


Fig. 4: FTIR spectra

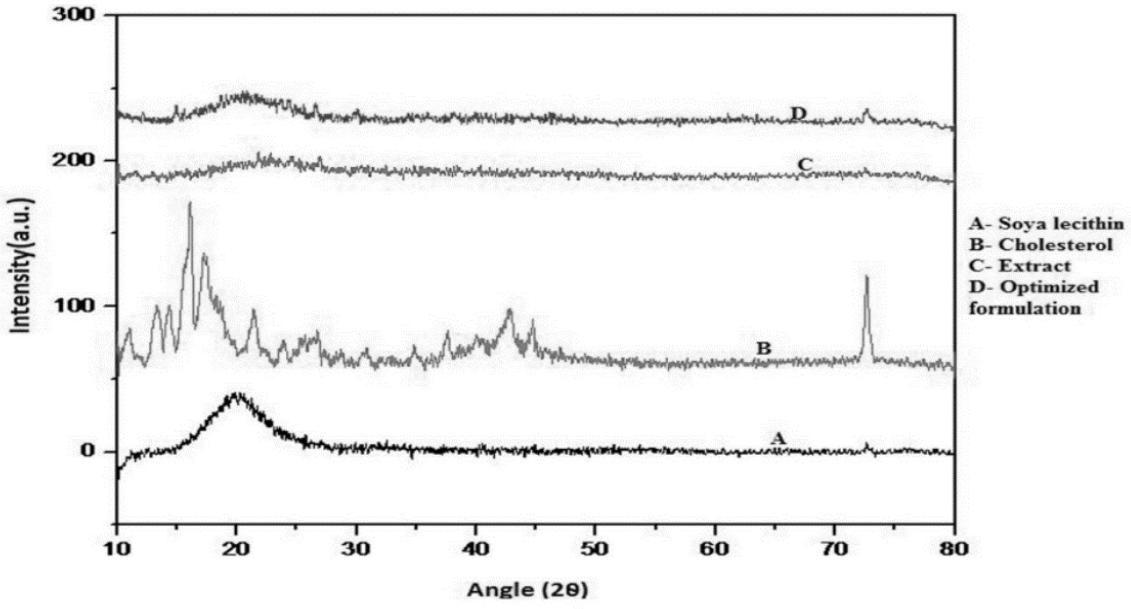


Fig. 5: XRD spectra of (A) SoyaLecithin (B) Cholesterol (C) HAELS (D) Optimized Formulation

3.1. *In vivo* Antidiabetic Activity

Fig. 6 indicated that after 21st day, body weight of extracts and Glibenclamide treated animals has significantly increased body weight as compare to STN-NCM induced animal groups. Decline in body weight of untreated diabetic rats relates to fat catabolism and protein wastage and furthermore might be because of less accessibility of glucose for vitality usage. *L. speciosa* plant extract showed 18% and 20% reduction in serum glucose level at dose of 200 mg/kg and 400 mg/kg respectively in comparison to this optimized phytosomal formulation at dose of 100 mg/kg and 200 mg/kg showed 30 % and 34% reduction in serum glucose level, suggesting improved antidiabetic activity. A sound effect on lipid profile was also observed i.e decrease in serum concentration of cholesterol, triglycerides, LDL, VLDL and increase in HDL level in streptozotocin-nicotinamide induced diabetic rats, when treated, suggesting its hypolipidemic effect. The standard drug, extracts and phytosomes showed a dose related reduction in serum concentration of urea and creatinine but reverse effect on the serum concentration of total protein.

3.2. Histopathology

Microscopy of pancreatic areas of diabetic control group demonstrated breakdown of design of β-cells of pancreas, necrotic changes, β-cell degranulation, pycnotic β-cell cores, and serious vacuolation in islet. Rest all groups indicated checked improvement in anatomical structure of islets. New β-cells arrangement happens and vacuolation was missing. Kidneys of control group indicated variable neurotic changes in glomeruli and renal tangled tubules; there was moderate amplification of glomeruli, dilatation and blockage of glomerular. After treatment, these progressions were improved towards typical condition (fig. 7).

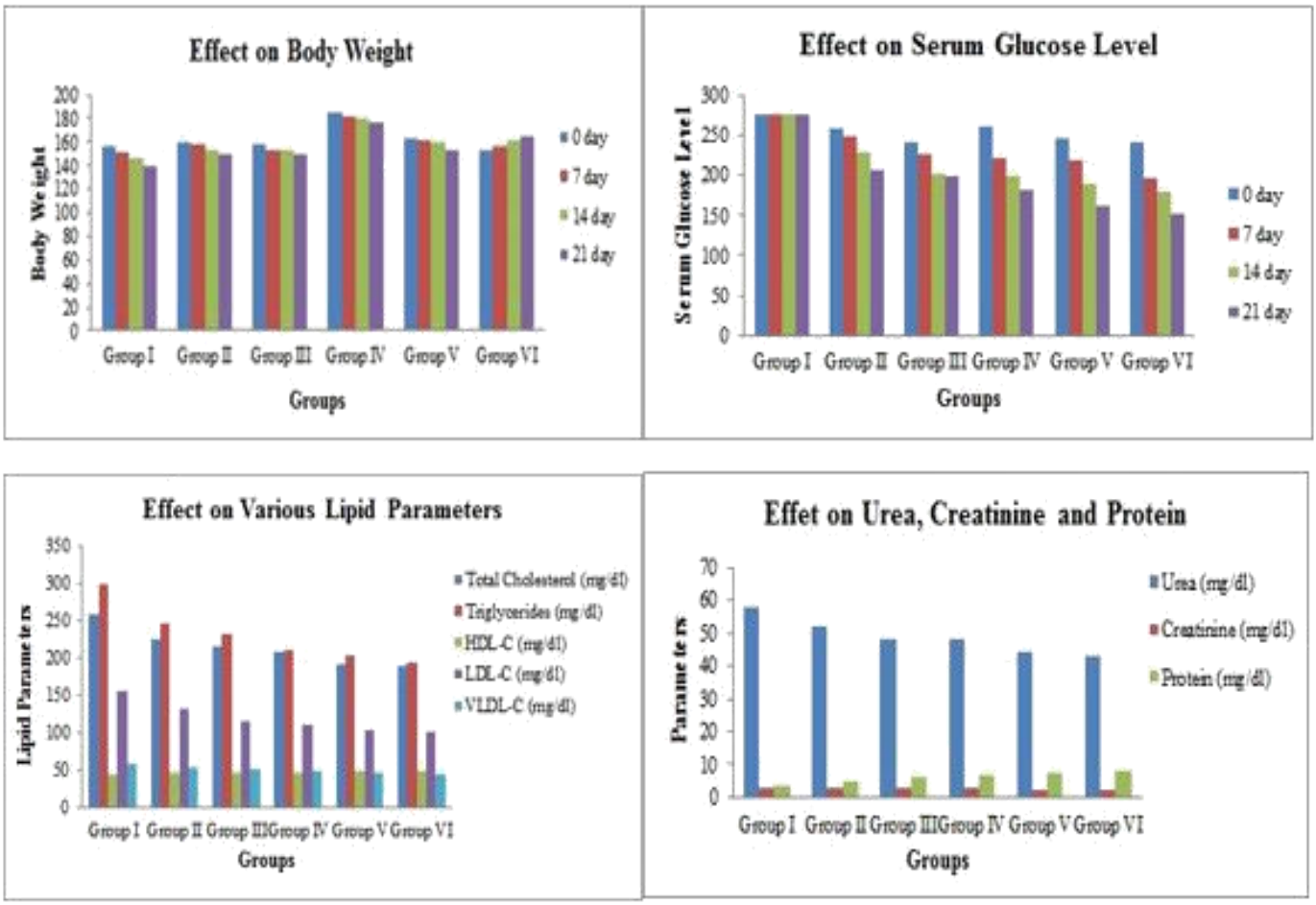
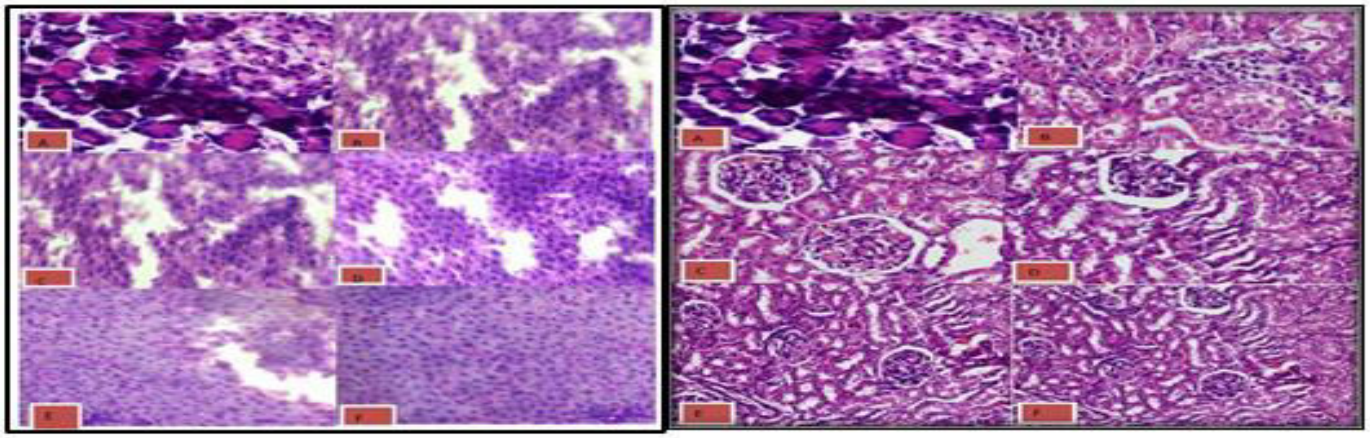


Fig. 6: Comparison of Different Groups on body Weight, Serum Glucose level, lipid parameters, urea, creatinine and protein.



(A) Diabetic control (B) L. speciosa 200mg/kg (C) L. speciosa dose 400 mg/kg (D) Phytosome of L. speciosa dose 100 mg/kg (E) Phytosome of L. speciosa dose 200mg/kg (F) Standard drug

Fig. 7: Photomicrograph of Pancreas and kidney section

4. CONCLUSION

The study revealed the excellent potential of phytosomal (powder form) drug delivery system for improving the *in vivo* antidiabetic activity of hydro-alcoholic extract of leaves of *Lagerstroemia speciosa* (200mg/kg and 400mg/kg) in comparison to optimized phytosomal formulation of plant extract at a lower dose (100 mg/kg and 200 mg/kg). It can be concluded that phytosomal formulation improved the dose efficacy ratio.

Conflict of interest

None declared

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