Preparation and therapeutic evaluation of rutin-phospholipid complex for antidiabetic activity

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ARTICLE INFO

ABSTRACT

The objective of the present work to prepare Rutin-phospholipid complex (RPC), a phytoformulation and characterization, evaluation for its antidiabetic activity in streptozotocin induced diabetic model. Prepared RPC was characterized and screened for antidiabetic activity by OGTT in normoglycemic and diabetic rats for RT and RPC at different time intervals. Effect of RT and RPC (50 and 100 mg/kg b.w. p.o. respectively) in STZ induced diabetic rats for one day and fifteen days was studied. This is followed by estimation of Estimation of SG, and lipid parameters. Histopathology studies of pancreatic tissue and bioavailability studies of RT & RPC were also carried out. SEM data showed that RPC has irregular size vesicles consisting of phosphatidylcholine. RPC showed a single endothermal peak at 147.68°C in DSC studies. OH group has shifted to lower frequency in phytosome compared to rutin phospholipid in FTIR spectra. Treatment with RPC (50 and 100 mg/kg b.w. p.o.) significantly reduced the blood glucose levels and restored the altered lipid parameters as compared to RT. Histopathological studies revealed that RPC also restored back the size of pancreatic islets and maintained the normal β-cells. A higher serum concentration of RT (13.20 μg/mL) in RPC treated group was observed in bioavailability studies as compared to RT. RPC maintained effective concentration of rutin for a longer period in rat serum.

INTRODUCTION

Diabetes mellitus (DM) is a metabolic disease in which there are high blood sugar levels over a prolonged period. It leads to decrease in both insulin secretion and insulin action. It is associated with the development of vascular diseases which include neuropathy, nephropathy, cardiovascular and cerebrovascular diseases (Rao *et al.*, 2010). The world wide prevalence of diabetes for all age groups is projected to rise from 382 million in 2013 to 592 million in 2035 (Guariguata *et al.*, 2014).

Presently, the growing interest in herbal remedies is associated with the oral hypoglycemic agents for treatment of DM. So, the traditional medicines obtained from the plants play an important role in the management of diabetes mellitus. The active constituents of plants are either polar or water soluble. The water soluble phytoconstituents as flavanoids, tannins, polyphenols etc. are poorly absorbed due to their poor lipid solubility and large molecular size. So, the ability to transport across lipid membranes results in poor bioavailability (Kidd, 2009). Bio-flavanoids consists of a group of polyphenolic secondary plant metabolites that are wide spread in nature containing a unique C6-C3-C6 structure. Flavanoids that have well categorized structure function relationships are: flavans, flavanones, flavones, flavanols, flavanonols, catechins, anthocyanidins and isoflavones. Bio-flavanoids are having well-known multidirectional biological activities including antidiabetic efficacy (Goutam, 2011).

Phytosome is a patented technology which is used to increase absorption and bioavailability. A phytoformulation obtained by incorporation of standardized plant extracts or water soluble phytoconstituents into phospholipids to produce lipid compatible molecular complexes is termed as a phytosome (Yang *et al.*, 2005). Rutin, a very common quercetin glycoside was recognized to decrease the permeability of capillaries; it has also been reported to scavenge free radical, to lower hepatic cholesterol levels, showed antiplatelet activity and antidiabetic activity (Yang *et al.*, 2005).

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As the efficacy of any herbal formulation depends on the concentration of the active compound and cell membranes are made up of phospholipids, an effort has been made in the present study to prepare, characterize and to evaluate phospholipid complex of rutin (RT) for antidiabetic effect to substantiate the claim that complexation between standard rutin and phospholipid can enhance the therapeutic efficacy of the parent molecule along with bioavailability studies in rats.

MATERIALS AND METHODS

Materials

Standard Rutin was obtained as a gift sample from Shreya Life Sciences Pvt Ltd. Waluj, Aurangabad, Maharashtra, India. Qualitative analysis of sample was confirmed by HPLC and FTIR analysis. Hydrogenated soya phosphatidyl choline (HSPC) and Streptozotocin (STZ) purchased from Hi-media, Mumbai. Standard drug Glibenclamide (GLB) was obtained from Aventis pharma Ltd.

Preparation of rutin phospholipid complex (RPC)

RT and HSPC were refluxed with 30 mL of dichloromethane at a temperature not exceeding $60^{o}C$ for 3h in a 100 mL round bottom flask. The mixture was then concentrated to 5-10 mL. To the concentrated mixture 30 mL of n-hexane was added carefully with continuous stirring to get the precipitate. The precipitate was filtered and stored in vacuum desiccator for overnight. The dried precipitate was crushed in mortar and sieved in #100 mesh. Powdered complex was kept in amber colored bottle flushed with nitrogen and stored at room temperature (Prasanna *et al*., 2013).

Physicochemical properties of RPC
Microscopic view

Trinocular microscope (LX 300, LABOMED Inc, USA) was used for characterization of the complex. The complex was suspended in distilled water and a drop was placed on a slide and covered with a cover slip. Microscopic view of complex was observed at a magnification of 100x, 200x and 400x (Prasanna *et al*., 2013).

Scanning electron microscopy (SEM)

Scanning electron microscopy (JOEL JSM-6360 scanning microscope Japan) has been used to determine the particle size distribution and surface morphology. Samples were placed on an electron microscope brass stub and coated with gold in an ion sputter. Photographs of RT, RPC and physical mixture of RT and RPC were taken by random scanning of the stub at 100x, 500x and 1000x magnifications (Prasanna *et al*., 2013).

Differential scanning colourimetry (DSC)

RT, RPC, and physical mixture of RT and RPC were placed in the aluminum crimp cell and heated at $10^{o}C$/min from 0 to $400^{o}C$ in an atmosphere of nitrogen (TA instruments USA, model DSC Q10 V24 4 build 116). Peak transition onset temperatures were recorded by means of an analyzer (Prasanna *et al*., 2013).

FTIR

FTIR (α-series, Bruker, Germany) spectral data were taken to ascertain the structure and chemical stability of RPC, phosphatidylcholine and RT. Samples were crushed with KBr to get pellets at 600 kg/cm^2 pressure. Spectral scanning was done in the range between 4000 and 500 cm^{-1} (Prasanna *et al*., 2013).

Apparent solubility

Solubility determination of rutin material and phospholipid complex was carried out by adding excess of rutin material and phospholipids complex to 6 mL of water or *n*-octanol in sealed glass containers at 25 °C. Each experiment was performed in triplicate.

The liquids were agitated for 24 h, then centrifuged to remove excessive rutin (15-20 min, 4000 rpm).The supernatant was filtered through a 0.45 μm membrane. The 1 mL filtrate was mixed with 9 mL of solvent A and a 20 μL aliquot of the resulting solution was injected into a HPLC and detected at a wavelength of 340 nm, the concentration of rutin was measured (Yanyu *et al*., 2006).

Determination of content of RT in RPC by HPLC

The content of RT in RPC was determined using HPLC. About 50 mg of RT/RPC was placed into 50 mL volumetric flask. 35mL of methanol was added and mixed on cyclomixer for 5 min, and sonicated to dissolve for 10 min. Final volume was made with methanol and a 20 μL aliquot of the resulting solution was injected into Shimadzu LC-2010 HPLC system.

The stationary phase, hypersil ODS, C-18 250 mm x 4.6 mm, 5 μm was kept at $25^{o}C$. The mobile phase was a mixture of 5% phosphoric acid: acetonitrile: methanol (60:30:10). The flow rate was 1.5 mL/min. Effluent was monitored at 340 nm (Maiti *et al*., 2007).

Dissolution studies

The dissolution studies were carried out according to a dissolution test apparatus (paddle method). The dissolution flasks were immersed in a water bath at $37^{o}C$. The dissolution medium (pH 1.2 HCl or pH 6.8 phosphate buffer saline, 900 mL) was continuously stirred at 100 rpm. RPC which is equivalent to 185 mg of rutin was added on the surface of the stirred dissolution medium at the beginning of the study.

At different time intervals, 10 mL samples were withdrawn and filtered using cellulose nitrate membrane, 10 mL fresh mediums were added into the flask. The 1 mL filtrate was mixed with 9 mL of solvent A and a 20μL aliquot of the resulting solution was injected onto a HPLC and detected at a wavelength of 340 nm, the concentration of rutin was measured (Maiti *et al*., 2007).

Screening of RPC for antidiabetic activity
Animals

Male Wistar rats weighing 190-210 g were used for the present study. The animals were purchased from Sri Venkateshwara Enterprises, Bangalore, India. They were maintained in the animal house of SET's College of Pharmacy, Dharwad, India for experimental purpose. The animals were maintained under controlled conditions of temperature ($22 \pm 2°C$), humidity (50 ± 5 %) and 12h light-dark cycles. They were fed commercial stock diet and water, *ad libitum*. The animals were housed individually in sanitized polypropylene cages containing sterile paddy husk as bedding.

Animals were habituated to laboratory conditions for 48h prior to experimental protocol to minimize if any of non-specific stress. All the studies conducted were approved by the Institutional Animal Ethical Committee (IAEC) of SET's College of Pharmacy, Dharwad, India.

Effect of RPC in normoglycemic rats
Oral glucose tolerance (OGTT) in normal rats

The OGTT was performed in overnight-fasted (18h) normal rats. The experimental rats were divided into six groups of six each and treated as follows:

  Group 1: Normal control (NC) received water (10 mL/kg, p.o.)
  Group 2: NC rats treated with glibenclamide (0.5 mg/kg, p.o.)
  Group 3: NC rats treated with RT (50 mg/kg, p.o.)
  Group 4: NC rats treated with RT (100 mg/kg, p.o.)
  Group 5: NC rats treated with RPC (50mg/kg, p.o.)
  Group 6: NC rats treated with RPC (100 mg/kg, p.o.)

All the rats were fasted for 18 h and given different doses of RT, RPC, glibenclamide (GLB) and vehicle orally. After 30 min glucose (2 gm/kg in distilled water) was administered orally. Blood samples were collected before glucose load (0 min) and 30, 60 and 120 min afterwards. Serum glucose (SG) levels were estimated using a glucose oxidase-peroxidase reactive strips and a glucometer (SD Check Gold Blood Glucose Meter, Standard Diagnostics, Korea). The results were expressed as integrated area under curve for glucose ($AUC_{glucose}$) by trapezoid rule (Shirwaikar *et al.*, 2006).

Effect of RT and RPC in STZ-induced diabetic rats
Induction of diabetes mellitus (DM)

Diabetes was induced by single intravenous injection of streptozotocin (STZ) (45 mg/kg). STZ was dissolved in ice-cold citrate buffer (pH-4.5) and injected immediately within few minutes to avoid degradation. Rats were provided with 10% glucose solution after 6h of STZ administration for the next 24 h to prevent hypoglycemia as STZ is capable of inducing fatal hypoglycemia as a result of massive pancreatic insulin release. Blood samples were collected 48 h later, and SG levels were determined to confirm the development of diabetes. Only those animals which showed blood glucose level more than 250 mg/dL were used for the experiment (Muruganandan *et al.,* 2005).

Diabetic rats were randomized into different groups based on their SG levels.

Experimental design for Single-dose one-day study

The experimental rats were divided into seven groups of six each and treated as follows

  Group 1: Normal control (NC) received water (10 mL/kg, p.o.)
  Group 2: Diabetic control (DC) received water (10 mL/kg, p.o.)
  Group 3: DC rats treated with glibenclamide (0.5 mg/kg, p.o.)
  Group 4: DC rats treated with RT (50 mg/kg, p.o.)
  Group 5: DC rats treated with RT (100 mg/kg, p.o.)
  Group 6: DC rats treated with RPC (50 mg/kg, p.o.)
  Group 7: DC rats treated with RPC (100 mg/kg, p.o.)

Blood samples were collected at 0, 2 and 4 h after RT, RPC and GLB administration. SG was estimated by using a glucose oxidase-peroxidase reactive strips and a glucometer. Percent reduction in glycemia was calculated with respect to the initial (0 h) level according to: Percentage reduction in glycemia=[(Gi-Gt)/Gi] x 100; where Gi is initial glycemia and Gt is glycemia at 2 and 4 h.

Experimental design for Multiple-dose fifteen-day study

The animals were treated with respective doses of RT, RPC and GLB for 15 consecutive days (Kumar *et al.,* 2006) and SG were measured as explained above using glucometer.

  Group 1: Normal control (NC) received water (10 mL/kg, p.o.)
  Group 2: Diabetic control (DC) received water (10 mL/kg, p.o.)
  Group 3: DC rats treated with glibenclamide (0.5 mg/kg, p.o.)
  Group 4: DC rats treated with RT (50 mg/kg, p.o.)
  Group 5: DC rats treated with RT (100 mg/kg, p.o.)
  Group 6: DC rats treated with RPC (50 mg/kg, p.o.)
  Group 7: DC rats treated with RPC (100 mg/kg, p.o.)

OGTT in diabetic rats

At the end of 15 days of treatment, glucose (2 g/kg body weight) was administered to 12 h fasted rats and blood samples were collected at 0 (immediately after glucose load), 30, 60 and 120 min after glucose administration. Glucose was estimated and results were expressed as integrated area under curve for glucose ($AUC_{glucose}$) calculated by trapezoid rule (Liu *et al.*, 2005).

Estimation of biochemical parameters

At the end of the treatment, blood samples were collected from retro-orbital plexus. Serum was separated and analysed spectrophotometrically for triglycerides (TG), total cholesterol (TC), HDL-cholesterol (HDL-c) using diagnostic reagent kit ERBA diagnostics Mannheim GMBH, Germany. VLDL-c, LDL-c in serum were calculated as per Friedewald's equation (Friedewald *et al.*, 1972).

$$(\text{VLDL-c}) = \frac{\text{Triglyceride}}{5}$$

$$\text{LDL-c} = \text{Total cholesterol} - \frac{\text{Triglyceride}}{5} - \text{HDL-c}$$

The markers of dyslipidemia such as TC/HDL-c & LDL-c/HDL-c ratios were also calculated.

Histopathological examination

Pancreas of individual animal were excised quickly, fixed in 10% buffered neutral formalin, and fixed in bovine solution. They were further processed for paraffin embedding following standard microtechnique. Sections were stained with alum-haematoxylin and eosin, were observed photomicroscopically for histopathological changes (Galigher, 1971).

Bioavailability experiments in rats

Serum sample preparation

Twelve wistar albino male rats were divided into two groups and fasted for 12 h, but allowed to take up water freely. RT and RPC equivalents to 40 mg/kg were suspended into distilled water and Tween 20 % and administered orally to rats. The rats were anesthetized with ether and 500 µL blood was withdrawn from retro-orbital plexus in heparin tubes at an interval of 0, 1, 2, 3, 4, 5 h.

The blood was centrifuged at 3000 rpm for 10 min and plasma was separated. 10 µL of aliquot was taken into 10 mL volumetric flask; methanol was added to transferred to 15 mL volumetric centrifuged at 5000 rpm for 10 min. the supernatant liquid was used to estimate the content of rutin by HPLC.

Estimation of rutin content in serum

The serum concentration of rutin was determined by HPLC (Shimadzu LC-2010, Japan) system. The stationary phase, Hypersil ODS, C-18 250 mm x 4.6 mm, 5 µm was kept at 25°C. The mobile phase was a mixture of 5% phosphoric acid: acetonitrile: methanol (60:30:10). The flow rate was 1.5 mL/min. Effluent was monitored at 340nm and peaks were noted down (Prasanna *et al.*, 2013).

Statistical evaluation

The data were expressed as Mean ± S.E.M. Statistical comparisons were performed by one-way ANOVA followed by Tukey's post-test. $P<0.05$ were considered was considered significant.

RESULTS

Preparation of rutin phospholipid complex

Rutin phospholipid complex was prepared according to different quantity ratio of phospholipids and drugs.

The results showed that when the ratio was less than 1, the stability of phospholipid complexes was worse, when the ratio was more than 1 the appearance of resultant material was viscous and we prepared rutin phospholipid complex in terms of the quantity ratio 1 the content of RT in the phospholipids complex was 53.17 % w/w.

Physicochemical properties of rutin phospholipid complex
Microscopic view

The surface morphology of the RPC at various magnifications showed that the drug particles are associated with the phospholipid forming the complexes with irregular size. The microscopic view indicated the presence of spherical structures of the complex. The spherical structures consisted of HSPC and RT which was seen to be interpolated between the lipid layers.

Scanning electron microscopy (SEM)

The surface morphology of RPC as examined by SEM is shown in Figure 1. RPC was appeared as irregular shape. We could see that phospholipids did not exit on the appearance of drugs but RT was uniformly dispersed in phospholipids and formed the structure of irregular shape.
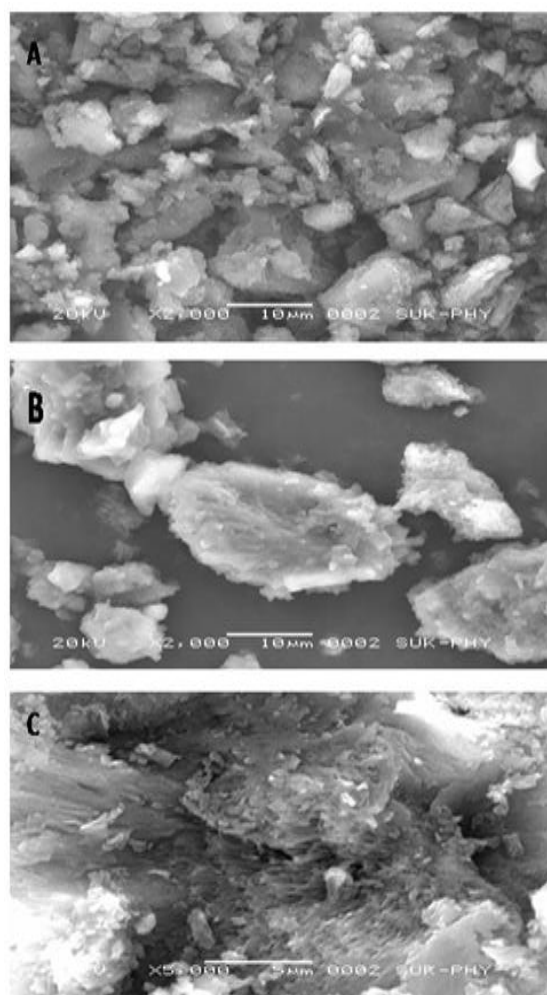

Fig. 1: Scanning electron microscopy (SEM) surface morphology and particle size distribution of RPC (SEM) at A) 200X, B) 2000X and C) 5000X magnifications. RT was found to be intercalated within the lipid layers.

Differential scanning colourimetry (DSC)

DSC is a reliable method to screen drug excipient compatibility and provides maximum information about the possible interactions. In DSC, we can conclude the interaction by

elimination of endothermic peak (s), appearance of new peak (s) area, change in the peak shape and its onset, peak temperature/ melting point and relative peak area. Figure 2 shows the DSC thermograms of phospholipid (A), pure rutin (B), rutin phospholipid complex (C) and physical mixture of rutin and phospholipid (D).

The DSC thermogram of Phospholipid showed a two peak, the first endothermal peak was milder and was considered that the formation of this peak was due to hot movements of phospholipid molecules to polarity parts.

The second endothermal peak at 159.4°C appears pointed. RT shows a endothermal peak at 181.03°C, the physical mixture of RT and PC shows two endothermal peaks at 150.76°C and 173.42°C. Further RPC showed a broad endothermal peak at 147.68°C which was more or less than physical mixture (150.76°C). Therefore it can be concluded that RT molecules were dissolved in the phospholipids and formed the complex when the temperature was increased. This could be explained through the theory of preparation by melt-out method.

FTIR

The FTIR spectroscopy revealed shifting of hydroxyl (OH) group to a lower frequency in the RPC spectra as compared to that of RT, indicating the formation of strong hydrogen bonding between hydroxyl groups of the phospholipids and rutin constituents in the phytosome form.

Solubility studies

The solubility of RT, physical mixture, and RPC in water or n-octanol was studied. The data shows that solubility of RPC in n-octanol was more than in water Table 1.

Determination of content of RT in RPC by HPLC

Figure 3 (A) and (B) showed the HPLC chromatograms of pure RT and RPC respectively. Content of rutin in the complex was estimated by HPLC and was found to be 53.17%

Dissolution studies

Figure 4 shows the dissolution profile of rutin from rutin phospholipid complex and rutin in HCl (pH 1.2) and phosphate buffer saline (pH 6.8), respectively. The dissolution of rutin from phospholipid complex in pH 6.8 phosphate buffer saline was not complete until 60 min; The amount about 167.8 mg; however at about 30 min, the dissolution in 0.1 N HCl was complete and the amount only 7.8 mg. The dissolution procedures are greatly influenced by the pH of media, and the dissolution amount of rutin was increased with increase in pH of media.
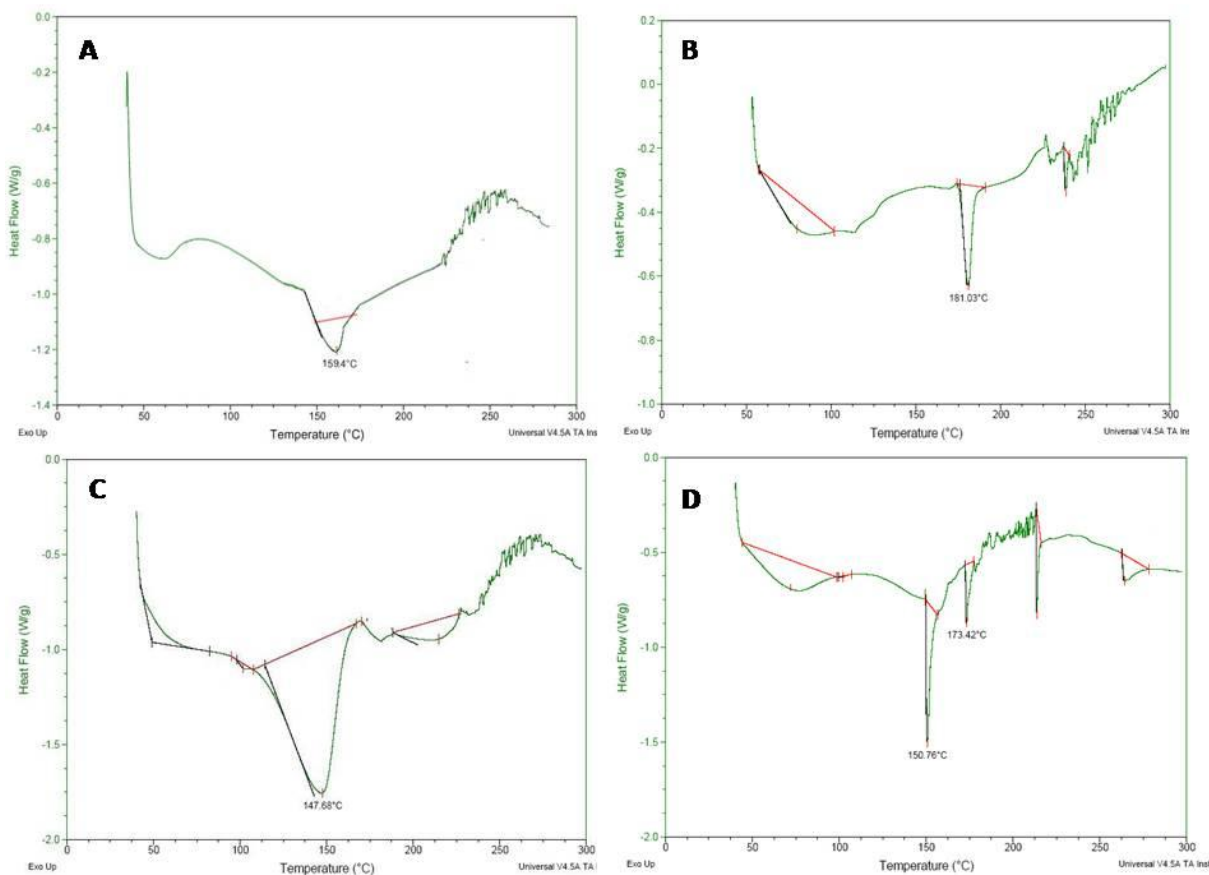

Fig. 2: Differential scanning colourimetry (DSC) of A) HSPC, B) Rutin, C) RPC and D) Physical mixture of RT & RPC
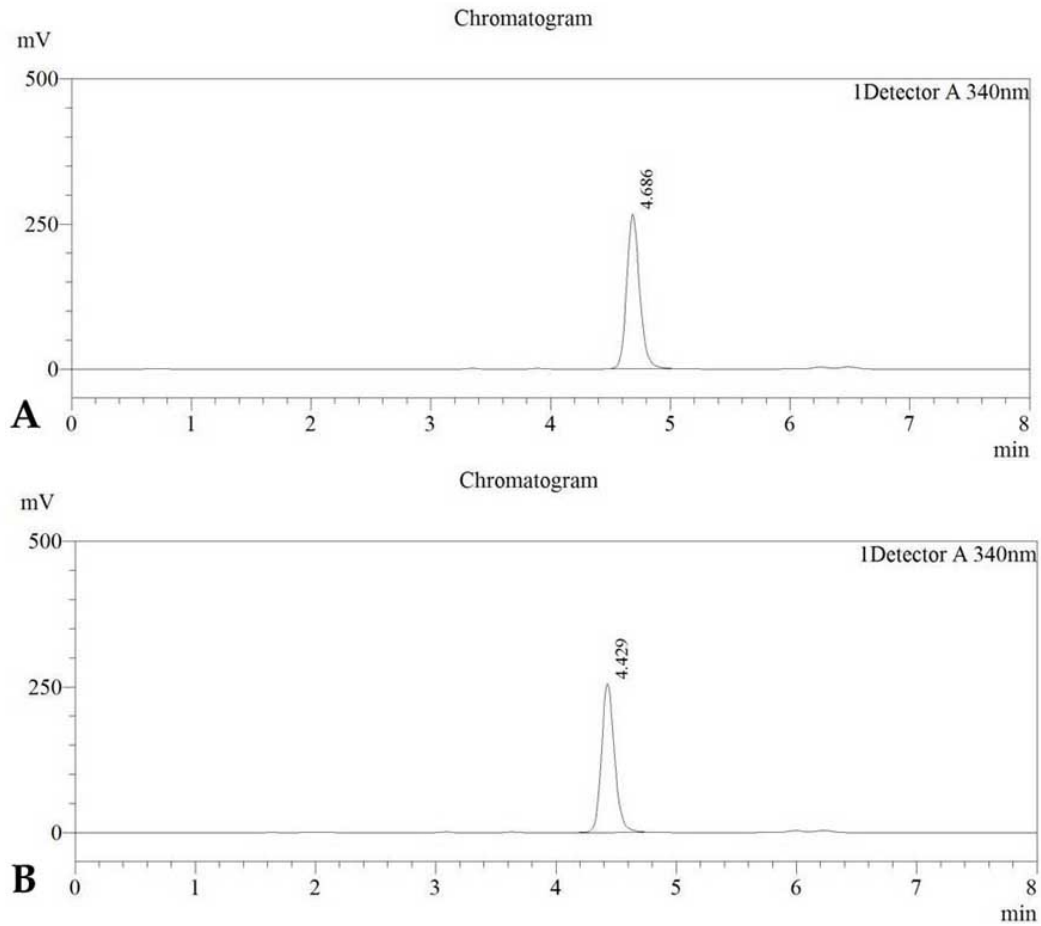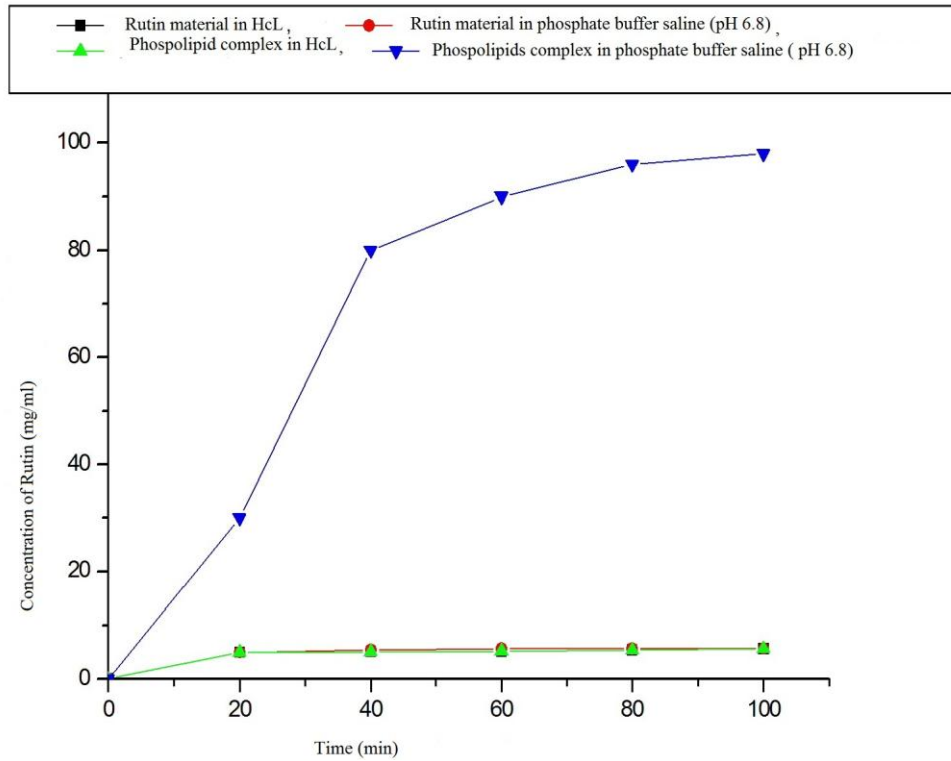
Fig. 3: HPLC Spectra of A) RT and B) RPC.

Fig. 4: Dissolution studies of different phytosome formulations.

Table 1: Apparent solubility of RT and RPC in n-octanol and water at 25 ºC.

Sample	Apparent solubility in n-octanol at 25ºC (µg/ml)			x̄ ± s	Apparent solubility in water at 25ºC (µg/ml)			x̄ ± s
	I	II	III		I	II	III	
Rutin (RT)	20.32	21.65	21.98	**21.32±0.507**	1.97	1.77	1.78	**1.846±0.662**
Physical mixture	36.56	35.11	36.00	**35.89±0.422**	8.45	7.39	8.11	**7.983±0.312**
Rutin phospholipid complex (RPC)	58.27	56.75	58.19	**57.74±0.493**	46.33	47.78	47.03	**47.05±0.418**

Table 2: Effect of RT and RPC on lipid profile in STZ induced diabetic rats.

Serum parameter	Normal control	Diabetic control	Glibenclamide	RT 50mg/kg	RT 100mg/kg	RPC 50mg/kg	RPC 100mg/kg
STG(mg/dl)	105.7±2.397	212.2±3.850	132.5±6.760***	188±5.050**	163.5±3.865*	192.8±2.392***	172.2±2.702***
STC	74.95±1.487	124.4±4.17	94.28±2.71***	108.7±3.54**	104.6±2.54**	108.5±3.211**	104.1±2.141***
HDL-c	30.51±1.184	21.62±0.600	41.30±0.608***	32.88±2.149**	37.26±1.242***	29.78±1.450***	33.89±1.430***
VLDL-c	21.14±0.479	42.44±0.716	26.49±1.352***	37.60±1.010**	32.70±0.772***	38.55±0.478*	34.54±1.478***
LDL_c	23.30±2.581	60.83±4.409	26.49±2.471***	40.33±2.043***	34.66±2.849***	40.16±3.728***	35.54±1.478***
TC/HDL-c ratio	2.470±0.123	5.776±0.246	2.287±0.087***	3.354±0.164***	2.820±0.097***	3.689±0.219***	3.094±0.112***
LDL-c/HDL-c ratio	0.780±0.108	2.805±0.227	0.644±0.067***	1.248±0.088***	0.950±0.081***	1.376±0.164***	1.061±0.072***

The values are expressed as mean ± SEM (n=6) *P< 0.05, **P< 0.01, ***P< 0.001as compared to diabetic control group.

Screening of RPC for Antidiabetic activity
Effect of RPC in normoglycemic rats
OGTT in normal rats

Administration of glucose produces a significant change in SG level of normal rats. Treatment with higher dose of RT (100 mg/kg) and RPC (50 mg/kg and100 mg/kg) and GLB (0.5mg/kg) significantly (P<0.01, P<0.001) improved the glucose tolerance whereas, treatment with lower dose of RT (50 mg/kg) did not significantly reduced the AUC$_{glucose}$ compared to the normal control group. The results are shown in Figure. 5 (A) and (B).

Effect of RPC on STZ-induced diabetes in rats
Single dose single day study

Acute hypoglycemic effect of RT and RPC was assessed in STZ- induced diabetic rats. A single dose of RT (50 and 100 mg/kg) and RPC (50 and 100 mg/kg) treatment exhibited reduction in SG levels at different time intervals compared to basal levels (0 h). Also administration of GLB showed significant (P<0.001) reduction in SG levels with maximum reduction (35.42%) at 4 h post GLB treatment compared to their basal levels, whereas, RT and RPC treated animals showed dose dependent percentage reduction in SG levels compared to their basal levels. (Figure 6)

Effect of RPC on STZ-induced diabetes in rats Multiple dose fifteen day study

Repeated administration of RT (50 and 100 mg/kg) and RPC (50 and 100 mg/kg) for 15days showed significantly (P<0.05, P<0.001) reduced levels of SG compared to the basal values (0 day). On 15ᵗʰ day tested doses of RT and RPC showed significantly (P<0.001) greater percentage reduction (34.22%, 41.21%, 32.67% and 39.09%) in glycemia compared to diabetic control. (Figure 7)

OGTT in diabetic rats

On 15ᵗʰ day of the treatment, oral administration of glucose (2 gm/kg) did not produce significant change in SG level of normal control rats and AUC for the 120 min interval was not altered. As depicted in Figure 8 (A), the diabetic rats exhibited marked elevation in fasting SG level (0 h) and showed significant impairment in glucose tolerance to exogenous administration of glucose compared to normal control rats.

Treatment of diabetic rats with RPC (50 mg/kg, 100 mg/kg) and GLB (0.5 mg/kg) significantly (P<0.001) improved glucose tolerance and marked fall in SG level over the period of 120 min compared to diabetic control group. Figure 8 (B), shows the integrated AUC$_{glucose}$ for the fraction. AUC$_{glucose}$ for RPC (50 mg/kg, 100 mg/kg) and GLB was found to be significant (P<0.001) over the period of 120 min. Collectively, RPC (50 mg/kg & 100 mg/kg) showed marked improvement in glucose tolerance.

Estimation of lipid parameter

Diabetic rats showed significantly (*P*<0.001) increased levels of STG, STC, VLDL-c and LDL-c levels, whereas HDL-c was decreased in diabetic rats compared to normal rats (Table 2). The markers of dyslipidemia such as Tc/HDL-c and LDL-c/HDL-c ratios were significantly elevated in diabetic group. Oral administration of different doses of rutin (50 & 100 mg/kg) and rutin phospholipid complex (50 & 100 mg/kg) for 15 days exhibited significant reduction (P<0.001) in all tested lipid parameters and restoring them to near normal values.

Histopathological examination

Figure 9 shows the histomorphological changes in pancreas of different groups of animals. Hitopathological examination of pancreas from normal control group showed normal pancreatic acini and islet of langerhans with normal cellularity (Figure 9 A) whereas, decreased in number and size of pancreatic islets, vacuolation, hydropic and necrotic cells, degranulation of cells (Figure 9 B).Both RT and RPC (Figure 9 D and E respectively) and GLB (Figure 9 C) markedly showed to amend the disrupted islets of langerhans of diabetic rats; islets architecture and integrity were improved. Also number and size of pancreatic islets were increased.
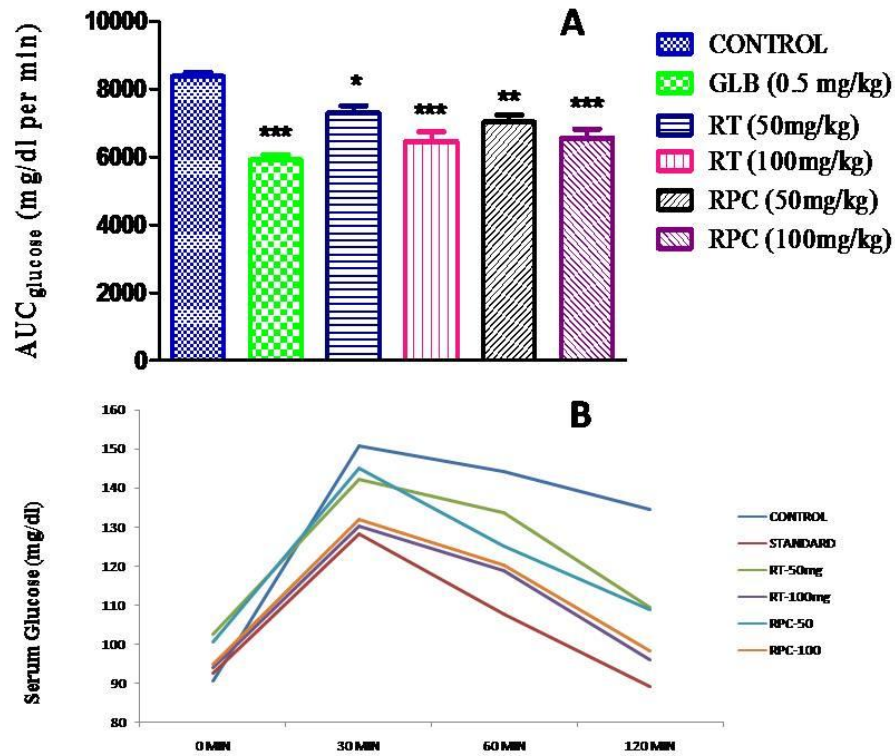
Fig. 5. A Effect of RPC in normoglycemic rats OGTT in normal rats, Area under curve for glucose (AUC glucose) values for 0-120 min post glucose load. Data represent the mean ± S.E.M., for n=6. *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$ as compared to control. **B)** Serum glucose level, SG levels were measured at 0, 30, 60 and 120 min after p.o. administration of glucose (2 gm/kg b.w)
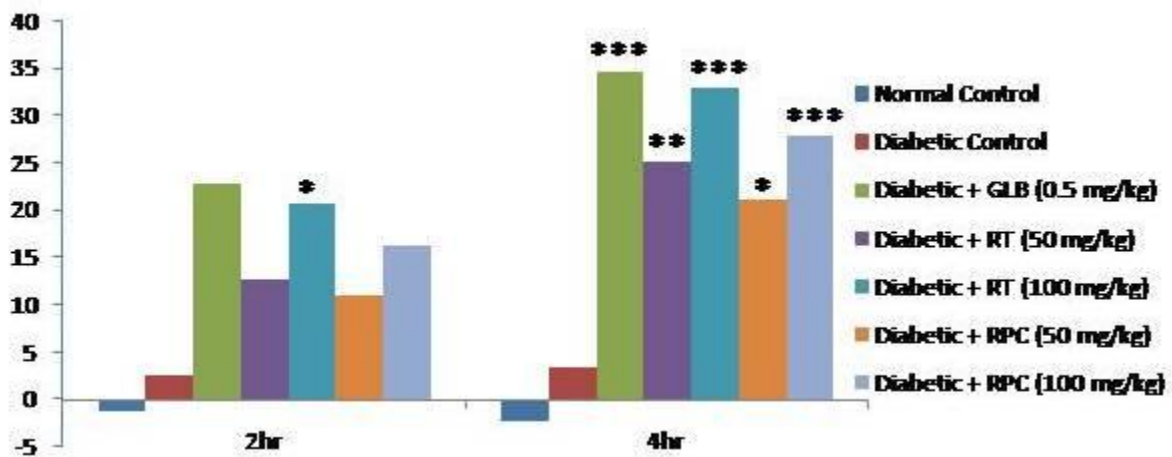

Fig. 6: Effect of RPC on STZ induced diabetic rats, Single dose single day study SG levels were measured at 0, 1, 2 and 4 hr after single oral administration of RT, RPC and GLB. Data are expressed as means ± S.E.M., for n = 6 rats per group. *$P<0.05$; **$P<0.01$; ***$P<0.001$, when compared to base values of the same group
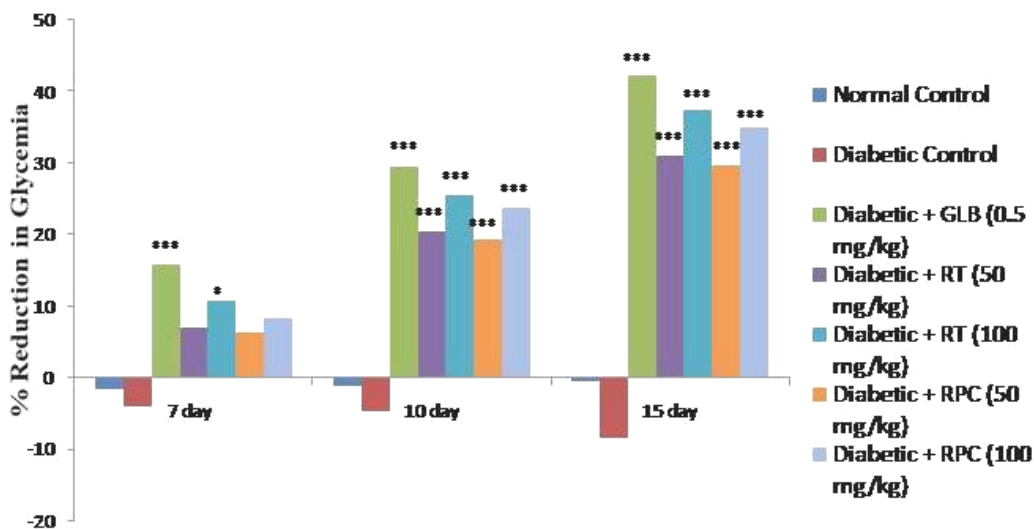
Fig. 7: Multiple dose fifteen day study, Bar graph represents the percentage reduction in glycemia with respect to the initial (0 day) level. Each value represents Mean ± S.E.M., for n=6.*P<0.05, **P<0.01, ***P<0.001 compared to diabetic control rats of the same time interval.
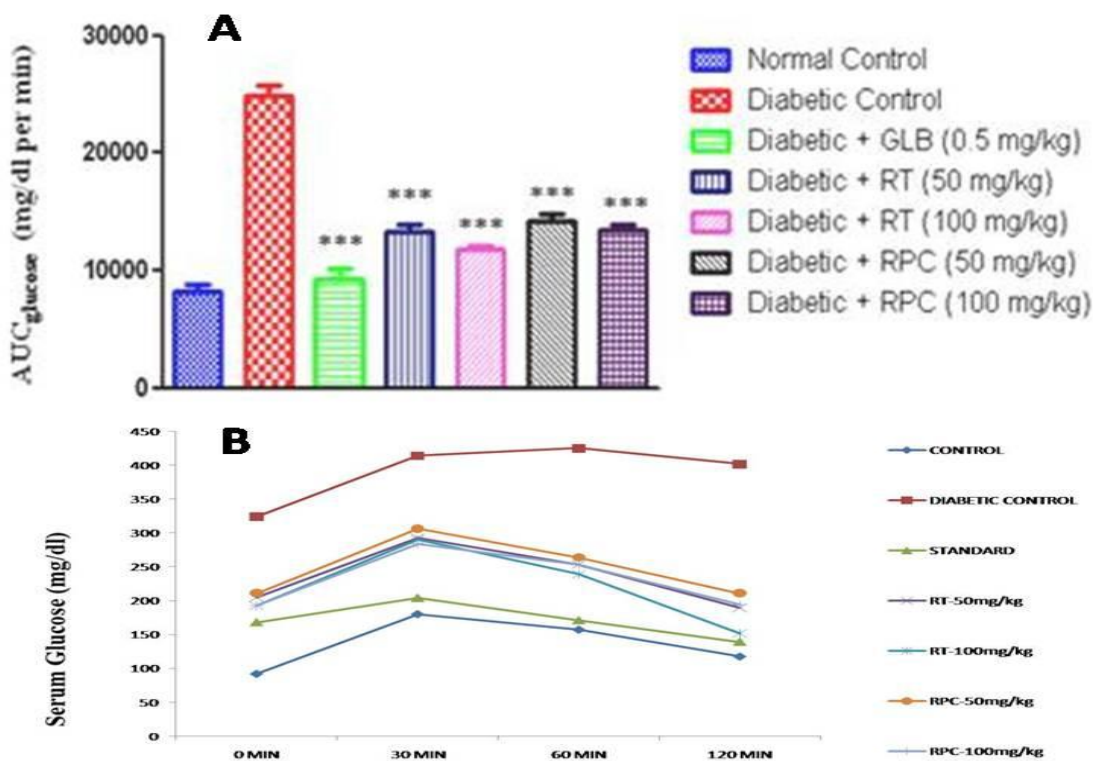

Fig. 8. A) OGTT in diabetic rats, AUC glucose values for 0-120 min post glucose load Data represents the mean ± S.E.M., for n=6. ***P < 0.001 as compared with diabetic Rats. **B)** SG levels were measured at 0, 30, 60 and 120 min after p.o. administration of glucose (2 gm/kg b.w)
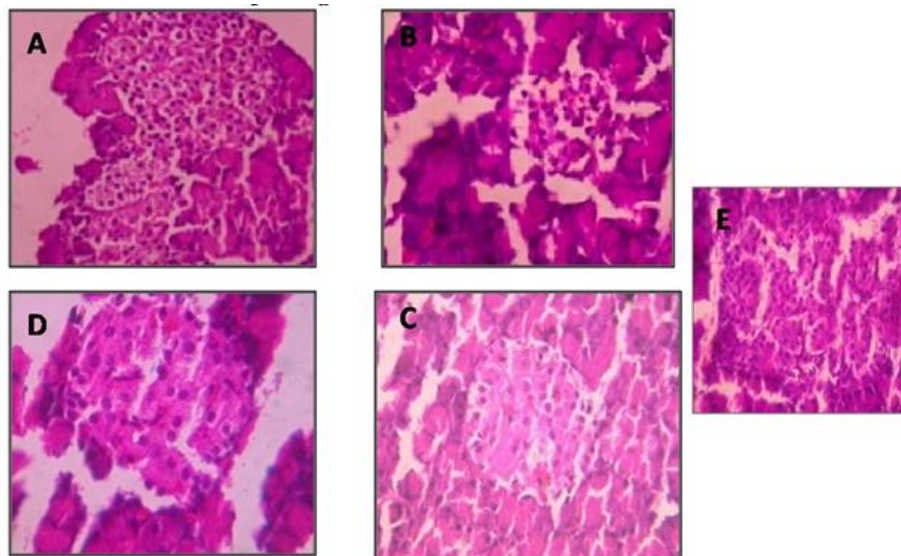
Fig. 9: Histopathological Microphotographs of rat pancreas after oral administration of RT & RPC A) Normal, B) Diabetic, C) Glibenclamide , D) Rutin, E) Rutin phospholipid complex.
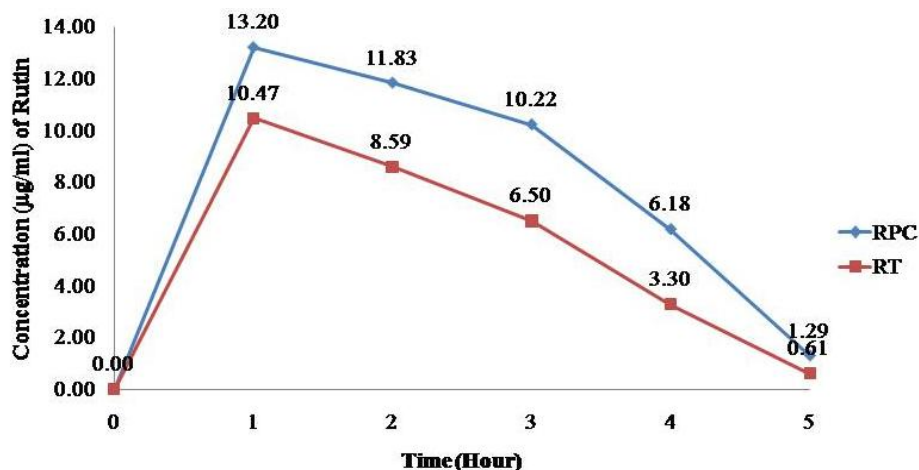

Fig. 10: Concentration of Rutin in rat serum, Serum concentration of RPC and RT in rats (n=6/groups).

Bioavailability experiments in rats

Figure 10 shows the results of serum concentration study of RT and RPC in rats. Peak serum concentration of 10.47 µg/mL attained rapidly within 1h after the administration of pure rutin. But in case of complex, the peak concentration 13.20 µg/mL appeared at same time and was much higher than pure rutin. The higher concentration was also maintained for longer time before being dropped down. The entrapment of RT in HSPC molecule may therefore decrease its molecular instability to improve its effective half life, which showed that RPC was in the stable form and prevented its degradation in liver.

DISCUSSION

The function of insulin is to maintain normal blood glucose levels either by suppression of glucose output from liver or by the stimulation of glucose uptake and its metabolism. Decrease in release of insulin or loss of insulin action at target tissues causes abnormal glucose and lipid metabolism. This results

in increased blood sugar level, the indication of diabetes. STZ – induced experimental diabetes is a valuable model for induction of type 1 diabetes. It is generally accepted that severe diabetes (SD) is similar to type 1 and mild diabetes (MD) is similar to type 2 diabetes (Matti *et al*., 2005). An oral glucose tolerance test is a more sensitive measure of early abnormalities in glucose regulation than fasting plasma glucose or glycosylated haemoglobin (Singleton *et al*., 2003). Impaired glucose tolerance reflects hepatic gluconeogenesis and reduced uptake of glucose from blood into skeletal muscle and adipose tissue following a meal (Robertson, 2007) and impaired glucose tolerance serves as a marker for the state of insulin resistance and predicts both large and small vessel vascular complications (Tominaga *et al.,* 1999).

Bio-flavanoids are having well-known multidirectional biological activities including antidiabetic efficacy. The biological properties of flavanoids are strictly related to their chemical structure and the choice of structural features allows the optimization of biological activities, lipophilicity, water solubility, and bioavailability. Further, it was also demonstrated that,

bioavailability of lipophilic drugs as solid dosage forms is low, which may be due to either slow or incomplete drug release in the lumen of the gastrointestinal tract (Mukherjee, 2003). The two main reasons for this are, Firstly, these are multiple ring molecules not quite small enough to be absorbed from the intestine into the blood by simple diffusion, nor does the intestinal lining actively absorb them, as occurs with some vitamins and minerals. Secondly, flavnoid molecules typically have poor miscibility with oils and others lipids (Thurapati *et al.,* 2011). In this case, improved drug concentration in the blood can be achieved by the use of delivery systems, which can enhance the rate and the extent of drug solubilizing into aqueous intestinal fluids. Therefore, a novel approach to increase therapeutic index of such compounds is essential for their better clinical utility. Phospholipids play a major role in the development of drug delivery systems. There are numerous advantages of phospholipids in addition to solubilizing property while considering them for a carrier system.

Development of valuable drug delivery system from natural resources is very much necessary because of beneficial role of herbal drug in the management of varied diseases. The present research is very much essential to explore the therapeutic efficacy of the natural molecules and to develop the proper delivery system to enhance the therapeutic potential of those molecules (Mukherjee, 2003). The application of novel drug delivery systems (NDDS) and polymer science especially the natural polymers started a new era in enhancing the clinical efficacy of the phytochemicals/extracts which are known as phytosomes.

In the present experiment we prepared rutin–phospholipid complex by a simple and reproducible method. The physicochemical investigations showed that rutin formed a complex with phospholipids. The complex has enhanced *n*-octanol solubility. The FTIR spectra revealed shifting of OH group to lower frequency in RPC spectra as compared to RT and either phospholipid, indicating the formation of strong hydrogen bond between hydroxyl groups of phospholipids and the RT in the phytosome form. The band of both long chain fatty acids in the phospholipid molecule of phytosome spectra is maintained unchanged, which shows that the long chain are not involved in phytosome formation.

DSC of phospholipids complex shows the endothermal peaks of drug and phospholipid are disappeared and the phase transition temperature is lower than the phase transition temperature of phospholipids, it was considered that rutin and phospholipids should have some interaction, such as hydrogen bonding or van der Waals force. The antidiabetic activity of the complex was significantly higher than pure rutin in all dose levels tested. The present data suggested that RT and RPC significantly reduced hyperglycemia in both single dose one day and multiple dose fifteen day diabetic studies. The efficacy of RPC is better than RT which is mediated by improving the glycemic control mechanisms (extra pancreatic) and increasing insulin secretion from remnant pancreatic b-cells in diabetic rats.

The RT and RPC supplementation also results in significant attenuation in STG, STC, VLDL-c and LDL-c. These effects may be due to low activity of cholesterol biosynthesis enzymes or low levels of lipolysis. Increased TC/HDL-c and LDL-c/HDL-c ratios are well known markers of dyslipidemia in STZ induced diabetic rats (Thurapati *et al*., 2005). RT and RPC administration reinstated dyslipidemic markers to near normal values.

The enhanced therapeutic efficacy of RT in terms of antidiabetic, obtained from RPC may be due to better absorption and bioavailability of the molecule. The exact mechanism behind the improved therapeutic efficacy of RPC requires further investigations of pharmacokinetic parameters to support the claim of better absorption and enhanced bioavailability.

CONFLICT OF INTEREST STATEMENT

We declare that we have no conflict of interest.

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