



Preparation, therapeutic evaluation and pharmacokinetic study of quercetin-phospholipid complex in rats

Rashmi Vankudri¹, Prasanna Habbu^{*2}, Mahesh Hiremath², Pradeep Kumar M R³, Sudhir Iliger⁴, Chetan Savant⁵, Patil B.S²

¹Research scholar, PRIST University, Thanjavur, Tamilnadu, India.

²Department of Pharmacognosy and Phytochemistry, SET's College of Pharmacy, Dharwad 580002 Karnataka, India.

³Department of Pharmaceutical Chemistry, SET's College of Pharmacy, Dharwad 580002, Karnataka, India.

⁴Department of Pharmaceutics, SET's College of Pharmacy, Dharwad 580002, Karnataka, India.

⁵Department of Pharmacology, SET's College of Pharmacy, Dharwad 580002, Karnataka, India.

ABSTRACT

The dissolution and bioavailability studies are influenced by the biopharmaceutical properties and potency which together contribute to the clinical efficacy of the drugs. In the present study phyto-phospholipid complex was prepared in order to enhance the delivery of poorly soluble quercetin (QT). The preparation of quercetin-phospholipid complex (QPC) was done and investigated for various physico-Parameters using Fourier transform infrared spectroscopy (FTIR), differential scanning calorimetry (DSC), scanning electron microscopy (SEM), aqueous/n-octanol solubility and dissolution study. The investigation of antidiabetic activity by OGTT in normoglycemic and diabetic rats for QT and QPC at different time intervals was carried out along with the effect of QT and QPC (50 and 100 mg/kg b.w. p.o. respectively) in STZ induced diabetic rats for single day and fifteen days was studied. This is followed by estimation of serum glucose (SG) and lipid parameters. In order to support the above fact the histopathology studies of pancreatic tissue and bioavailability studies of QT & QPC were also studied. SEM photographs of QPC was found fluffy and porous with rough surface morphology. FTIR, DSC and SEM data confirmed the formation of phospholipid complex. The solubility of QT and QPC was improved in water/ n-octanol. The antidiabetic studies indicated that, the bioactivity of QT was maintained even after being complexed with the phospholipid. The SG levels were significantly reduced and also the altered lipid parameters were restored after the treatment of QPC (50 mg/kg b.w. p.o). Histopathological studies revealed that QPC also restored back the size of pancreatic islets and maintained the normal β -cells. Bioavailability and pharmacokinetic studies confirmed that QPC can produce better therapeutic effect in rats and stayed for longer period of time as compared to free QT through sustained release property followed by decreasing the rate of elimination.

Keywords: Quercetin phospholipid complex; dissolution studies; antidiabetic activity; bioavailability.

INTRODUCTION

Diabetes mellitus, a chronic metabolic disorder characterized by a deficiency of pancreas for insulin production or by peripheral insulin resistance which can be referred as a global epidemic disease. The Data of World Health Organization (WHO) estimated that this diabetes has affected 285 million people worldwide in 2010 and projections rise to 439 million in 2030 (Shaw JE *et al.*, 2010). Some disadvantages regarding the diabetes treatment was due to the adverse effects of hypoglycaemic drugs and insulin and the excessive cost of these medications can be mentioned as which stimu-

lated the search for new therapeutic agents that includes present safety, effectiveness and low cost. Nowadays, herbal preparations and/or their derivatives in traditional and complementary medicine to treat diabetes symptoms (Yeh GY *et al.*, 2003). The International Diabetes Federation (IDF) estimated the total number of people in India with diabetes to be around 61.3 million in 2011, projected to reach 101.2 million by 2030. India currently stands at number two in the list of top 10 countries (IDF Diabetes Atlas. 2011). The biologically active constituents of plants are polar or water soluble but these water soluble phytoconstituents like flavonoids, tannins, polyphenols etc which are poorly absorbed due to their large molecular size, which cannot be absorbed by passive diffusion or due to their poor lipid solubility, hence limiting their ability to transport across lipid rich biological membranes and results in their poor bioavailability (Kidd PM. 2009). To achieve better bioavailability and faster actions of herbal extracts/constituents, novel drug de-

* Corresponding Author

Email: prasherbs@yahoo.com

Contact: +91-9448224894 Fax: +91836-2467190

Received on: 25-06-2016

Revised on: 16-02-2017

Accepted on: 22-02-2017

livery systems such as phytosomes are used. Phytosomes are prepared when the standardized extract/active ingredient of herb are bound to the phospholipids on a molecular level (Dang Y. 2000). Due to the advantages of phytosomes it has become necessary to apply the phytosomes technology for standardized extracts and phytoconstituents to improve the bioavailability and better clinical efficacy.

Nanoparticle-based drug delivery systems promise the safety and efficacy of drugs. Bio-flavonoids are a group of phenolic secondary plant metabolites that are widespread in nature. The poor absorption of flavonoid nutrients is likely because of their multiple ring molecules not quite small enough to be absorbed from the intestine into the blood by simple diffusion and also due to poor miscibility with oils and other lipids. This severely limits their ability to pass across the lipid-rich outer membranes. Therefore, a novel approach to increase the therapeutic index of such compounds is essential for better clinical utility. It has been observed that complexation with certain biopolymers like hydrogenated soya phosphatidylcholine (HSPC) substantially improves the absorption and bioavailability of phytoconstituents (Tawheed *et al.*, 2012).

Quercetin (QT) a polyphenolic flavonoid compound mainly occurring in glycosidic forms, is a potent antioxidant found in vegetables and fruits which is capable of inducing hepatoprotection and also improving dyslipidemia. Also, it contains some phenolic hydroxyl groups that have strong antioxidant activity, functioning as a ROS scavenger itself. It possesses wide pharmacologic effects like antidiabetic (Ketan H and Annapurna A. 2014), anti-inflammatory, anti-proliferative, and anti-angiogenic activities. (Men K *et al.*, 2014) Considering the potential therapeutic activities of QT, it was identified as an ideal candidate for developing novel drug delivery systems. Quercetin was formulated into a novel self-emulsifying drug delivery system (SEDDS) to improve its oral bioavailability and antioxidant potential. (Jain S *et al.*, 2013) Quercetin is a potential chemotherapeutic drug with low solubility seriously limits its clinical use in enhancing cellular penetration of QT by sterol containing solid lipid nanoparticles (SLNs) which make bilayers fluent for targeting hepatocellular carcinoma cells. (Varshosaz J *et al.*, 2014) Characterization and biodistribution in vivo of quercetin-loaded cationic nanostructured lipid carriers was done to improve therapy efficacy by promoting the accumulation of hydrophobic bioactive compounds in tissues. (Lin L *et al.*, 2014). Furthermore, growing evidences has pointed to QT as a promoter of enhanced superoxide dismutase (SOD) and glutathione peroxidase (GSH-Px) activities. (Chi Yu Yang *et al.*, 2005). Recently, quercetin's effect in prevention and treatment of cancer was recognized. Nanoparticles-delivered quercetin has attracted many attentions for its enhanced anticancer potential and promising clinical application.

No data on bioavailability and pharmacokinetic is available for quercetin phytosome in the literature. However, the present investigations aims to prepare, characterise the QT-phospholipid complex and to investigate the antidiabetic activity of the complex along with bioavailability and pharmacokinetic studies.

MATERIALS AND METHODS

Materials

Standard QT was obtained from Himedia Laboratories Pvt. Ltd, Mumbai, 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) 5mg (Daonil) obtained from Aventis pharma Ltd. The other chemicals and reagents used were of analytical grade.

Preparation of quercetin phospholipid complex (QPC)

The 1gm of QT and 1gm HSPC were taken into a 100 mL round bottom flask and refluxed with 30 mL of dichloromethane at temperature 60°C for 3 h then the mixture is concentrated to 5-10 mL, 30 mL of n-hexane was added with continues stirring to get the precipitate which was filtered, collected and stored in vacuum desiccators for overnight. The dried precipitate is crushed in mortar and sieved in #100 mesh. Further powdered complex was placed in amber coloured bottle flushed with nitrogen and stored at room temperature (Prasanna H *et al.*, 2013).

Physicochemical properties of QPC

Microscopic view

Trinocular microscope (LX 300, LABOMED Inc, USA) was used for characterization of the complex. The quercetin 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 H *et al.*, 2013).

Scanning electron microscopy (SEM)

Scanning electron microscopy (JOEL JSM-6360 scanning microscope Japan) was used to determine the particle size and surface morphology. Samples were placed on an electron microscope and photographs of QT and QPC were taken by random scanning of the stub at 100x, 500x and 1000x magnifications (Prasanna H *et al.*, 2013).

Differential scanning calorimetry (DSC)

DSC thermograms were taken with TA Instruments, USA using DSC Q10 V24.4 version; Phospholipid, QT and QPC were placed in crimp cell and heated at 10° C/min from 0 to 300°C. In the atmosphere of nitrogen. Peak transition onset temperatures were recorded by means of an analyzer (Prasanna H *et al.*, 2013).

FTIR

FTIR (α -series, Bruker, Germany) spectral data were taken to ascertain the structure and chemical stability of QPC, phosphatidylcholine and QT. Samples were crushed and triturated with KBr to get pellets at 600 kg/cm² pressure. Spectral scanning was done in the range between 4000 and 500 cm⁻¹ (Prasanna H *et al.*, 2013).

Apparent solubility

Solubility determination of QT and phospholipid complex was carried out by adding excess of QT and phospholipids complex to 6 mL of water or *n*-octanol in sealed glass containers at 25°C. Each experiment was done in triplicate. The liquids were agitated and centrifuged to remove excessive QT (15-20 min, 4000 rpm). The supernatant was filtered through a 0.45 μ m membrane. The 1 mL filtrate was mixed with 9 mL of methanol and a 20 μ L aliquot of the resulting solution was injected into a HPLC and detected at wavelength of 220 nm, the concentration of QT was measured (Yanyu X *et al.*, 2006).

Determination of content of QT in QPC by HPLC

The QT in QPC content was determined using HPLC. About 50 mg of QT / QPC was placed into 50 mL volumetric flask. 35 mL of mobile phase (0.5% H₃PO₄ in water : Methanol (65:35)) was added and mixed using cyclomixer for 5 min, and sonicated to dissolve for 10 min. Final volume was made with methanol and a 20 μ L 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 μ m was kept at 25° C. The flow rate was 1.1 mL/min. Effluent was monitored at 220 nm (Maiti K *et al.*, 2007).

Dissolution studies

The dissolution studies were carried out using a dissolution test apparatus by paddle method. The dissolution flasks were immersed in a water bath at 37°C. The dissolution medium (pH 1.2 HCl or pH 6.8 phosphate buffer saline, 900 mL) was continuously stirred at 100 rpm. QPC which is equivalent to 185 mg of QT was added on the surface of the stirred dissolution medium at the beginning of the study. 10 mL samples were withdrawn at different time intervals and filtered using cellulose nitrate membrane, 10 mL fresh media were added into the flask. The 1 mL of above filtrate was mixed with 9 mL of HPLC grade methanol and a 20 μ L aliquot of the resulting solution was injected into a HPLC and wavelength was detected at a 220 nm, the concentration of QT was measured (Maiti K *et al.*, 2007).

Screening of QPC for antidiabetic activity.

Animals

Male Wistar rats weighing 150-200 g were used in the study. The animals were procured from Sri Venkateshwara Enterprises, Bangalore, India. Rats were

maintained in the animal house of SET's College of Pharmacy (SETCOP), Dharwad, India for experimental purpose. The animals were maintained under standard conditions of temperature (22 \pm 2°C), humidity (50 \pm 5 %) and 12h light-dark cycles. They were fed proper diet and water, *ad libitum*. The rats were housed individually in sanitized polypropylene cages containing sterile paddy husk as bedding. Animals were habituated to laboratory conditions for 48h prior to experiment in order to minimize non-specific stress. All the experimental studies conducted were approved by the Institutional Animal Ethical Committee (IAEC) of SETCOP, Dharwad.

QPC effect on normal rats

Oral glucose tolerance (OGTT) in normoglycemic rats

The Oral glucose tolerance was performed in 18 h overnight-fasted normal rats and they were divided into six groups of six each and treated as follows:

Group 1: Normal control (NC) received distilled 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 QT (50 mg/kg, p.o.)

Group 4: NC rats treated with QT (100 mg/kg, p.o.)

Group 5: NC rats treated with QPC (50mg/kg, p.o.)

Group 6: NC rats treated with QPC (100 mg/kg, p.o.)

The Rats were fasted for 18 h and were administered with different doses of vehicle, QPC, QT and glibenclamide (GLB) orally. 30 min later glucose (2 gm/kg in distilled water) was administered orally. Blood samples were collected 0, 30, 60 and 120 min afterwards. Serum glucose (SG) levels were estimated using glucometer (SD Check Gold Blood Glucose Meter, Standard Diagnostics, Korea). The obtained results are expressed as integrated area under curve for glucose (AUC_{glucose}) by trapezoid rule (Shirwaikar A and Rajendran K. 2006).

QT and QPC effect on streptozotocin -induced diabetic rats

Induction of diabetes mellitus (DM)

Diabetes was induced by single i.v administration of STZ (45 mg/kg). STZ was solubilised in ice-cold citrate buffer (pH-4.5) and immediately injected within few minutes to avoid degradation. Rats were provided with 10% glucose solution after 6 h of STZ administration for the next 24 h to avoid hypoglycemia as STZ induces fatal hypoglycemia as a result of which increased pancreatic insulin release into the bloodstream. The blood samples were collected after 48 h, and SG levels were estimated to confirm the diabetes. Animals with blood glucose level more than 250 mg/dL were used for the experiment (Kumar GPS *et al.*, 2006). Diabetic rats were divided into different groups.

Single-dose one-day study

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

Group 1: Normal control (NC) received distilled water (10 mL/kg, p.o.)

Group 2: Diabetic control (DC) received distilled 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 QT (50 mg/kg, p.o.)

Group 5: DC rats treated with QT (100 mg/kg, p.o.)

Group 6: DC rats treated with QPC (50 mg/kg, p.o.)

Group 7: DC rats treated with QPC (100 mg/kg, p.o.)

Blood samples were collected at 0, 2 and 4 h after QT, QPC and GLB administration. SG was estimated by using glucometer. Percent reduction in glycemia was calculated using below formula with respect to the initial (0h) level.

$$\text{Percentage reduction in glycemia} = \left[\frac{(Gi - Gt)}{Gi} \right] \times 100$$

Where Gi-initial glycemia and Gt-glycemia at 2 and 4 h.

Multiple-dose fifteen-day study

The diabetic rats were treated with respective doses of QT, QPC and GLB for fifteen consecutive days and SG were measured as explained above.

Group 1: Normal control (NC) received distilled water (10 mL/kg, p.o.)

Group 2: Diabetic control (DC) received distilled 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 QT (50 mg/kg, p.o.)

Group 5: DC rats treated with QT (100 mg/kg, p.o.)

Group 6: DC rats treated with QPC (50 mg/kg, p.o.)

Group 7: DC rats treated with QPC (100 mg/kg, p.o.)

Estimation of biochemical parameters

Blood samples were collected from retro orbital plexus at the last day of treatment. Serum was separated using centrifuge and analysed spectrophotometrically for triglycerides (TG), total cholesterol (TC), HDL-cholesterol (HDL-c), VLDL-c, LDL-c, TC/HDL-c & LDL-c/HDL-c, using standard diagnostic reagent kit (ERBA diagnostics Mannheim GMBH, Germany) (Kumar GPS *et al.*, 2006).

Histopathological Studies

Pancreas of individual animal were dissected, stored in 10% buffered neutral formalin, and fixed in bovine solution. Further, the sections were embedded in paraffin using standard microtechnique. Simultaneously sections were stained with alum-haematoxylin and eosin, were observed photomicroscopically for pathological changes in pancreatic tissues (Fiedewald WT *et al.*, 1072).

Bioavailability experiments in rats

Serum sample preparation

For bioavailability study rats were divided into two groups of six in each group and fasted for 12 h. QT 20 mg/kg and QPC equivalents to 20 mg/kg were suspended into distilled water using Tween 20 and administered to rats orally. The rats were anesthetized by ether and 500 μ L blood was taken from retro-orbital plexus in heparinised tubes at an interval of 0, 1, 2, 3, 4, 5 h and was centrifuged at 3000 rpm for 10 min and plasma was separated. 10 μ L of above plasma was taken into 10 mL volumetric flask, 15 mL of methanol was added and centrifuged at 5000 rpm for 10 min. The supernatant liquid was collected and used for the estimation of QT in plasma by HPLC (Galigher AE and Kozloff EN, 1971).

Estimation of QT content in serum

The content of QT in serum was determined by HPLC (Shimadzu LC-2010, Japan) system. The stationary phase, Hypersil ODS, C-18 250mm x 4.6mm, 5 μ m and was kept at 25°C. The mobile phase used was a mixture of 0.5% H₃PO₄ in water : Methanol (65:35). The flow rate was adjusted at 1.5 mL/min. Effluent was measured at 220nm and peaks were observed.

Extraction of QT from serum and preparation of sample

1 ml of serum sample was taken into a 10 ml volumetric flask, maintained to room temperature and 5 ml methanol was added to it. The mixture was continuously shaken and heated at 60-70°C for 20-30 min. The volume was made up to 10 ml with methanol and solution was transferred to centrifuge tube and centrifuged at 5000 rpm for 10 min and supernatant was collected. The supernatant was filtered and 10 μ L was subjected to HPLC analysis.

Preparation of standard solution

Standard QT was dissolved in 10ml methanol in volumetric flask and 0.1 ml of this solution was taken further it was serially diluted to obtain a final concentration.

Validation of extraction and quantification method

QT from rat serum was collected completely by extraction process and obtained standard curves were linear ($r = 0.9913$) ranging from 0.10- 2.0 μ g/ml. Minimum detection level of QT was found to be 50ng/ml. The

extraction and quantification of QT from rat serum was done by performing rate recovery experiments. Three different concentrations i.e. high, middle and low, were selected for extraction as well as quantification of QT from the ranges as mentioned. The recovery rates of QT from above mentioned concentrations ranges were 82.18, 85.23 and 84.37%. The inter-days were 3.67, 2.54 and 3.29% respectively and intra-days relative standard deviations were 4.16, 2.90 and 3.42% respectively.

Pharmacokinetic parameters

The pharmacokinetic parameters of QPC were obtained from computer designed program "WINNONLIN-4.1" and the parameters were compared to that of free QT. Maximum concentration (C_{max}) and time to reach maximum concentration (T_{max}) are the values obtained directly from the concentration-time curve. Area under the concentration-time curve (AUC_{0-t_n} and AUC_{0-t_d}), elimination half life ($t_{1/2el}$), elimination rate constant (K_{el}), clearance (cl) and volume of distribution (V_d) were determined. Relative bioavailability was calculated as ratio of total amount of drug absorbed from the QPC to the total amount of drug absorbed from standard quercetin using the formula:

$$F = \frac{\text{Total amount of drug absorbed from quercetin - Phospholipid complex (Amax complex)}}{\text{Total amount of drug absorbed from quercetin}} \times 100$$

$$F = \frac{(V_d \times K_{el} \times AUC_{0-t_d})_{\text{complex}}}{(V_d \times K_{el} \times AUC_{0-t_d})_{\text{quercetin}}} \times 100$$

Statistical evaluation

The data were expressed as Mean \pm S.E.M. Statistical comparisons were performed by one-way ANOVA followed by Tukey's post-test. For serum concentration study, animal data were analyzed by Student "t" test. $P < 0.05$ were considered to be significant.

RESULTS

Preparation of QPC

Quercetin phospholipid complex was prepared and content of QT in the phospholipids complex was found to be 70.12 % w/w. The formation of QPC is depicted in Figure. 1

Physicochemical properties of QPC.

Microscopic view and scanning electron microscopy.

Under the microscopy of QPC the presence of spherical structures of the complex were observed which showed that the QT particles are associated along with the phospholipid forming the complexes with non-uniform size and which was seen to be intercalated in the lipid layer. The surface morphology of phospholipids complex as examined by SEM is shown in Figure.2 we could see that QT was uniformly distributed in

phospholipids under different magnifications and forming the fluffy spherical structure of irregular shape.

Differential scanning calorimetry (DSC)

Figure 3 shows the DSC thermograms of standard Quercetin (a), quercetin phospholipid complex (b). QT shows an endothermic peak at 90.51°C, QPC showed a broad endothermic peak at 168.84°C. The above interaction may be due to 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. Because of increased temperature, HSPC was melted and QT was dissolved in the HSPC which partly formed phospholipid complex and explained through the theory of preparation by melt-out method.

FTIR

FTIR indicated that strong hydrogen bonding was formed between hydroxyl groups of the phospholipids and the quercetin constituent in the phytosomes form and hydroxyl group was shifted to a lower frequency in complex.

Solubility studies

The solubility of QT, physical mixture and phospholipids complex in water or n-octanol was carried out and the data shows that solubility of QPC in n-octanol was more than in water. The results are depicted in table 1.

Determination of content of QT in QPC by HPLC

Figure 4a and 4b shows the HPLC chromatograms of pure QT and QPC respectively. Content of QT in the complex was estimated by HPLC and was found to be 70.12% w/w.

Dissolution studies

Fig. 5 shows the dissolution profile of QT from QPC and QT in HCl (pH 1.2) and phosphate buffer saline (pH 6.8) respectively. The dissolution of QT from phospholipid complex in pH 6.8 phosphate buffer saline was not complete until 60 min; the amount was about 122.4 mg, hence at about 30 min, the dissolution in 0.1 N HCl was complete and the amount was only 5.3 mg. The dissolution procedures are greatly influenced by the pH of media, and the dissolution amount of QT was increased with increase in pH of media.

Effect of QPC in normoglycemic rats

OGTT in normal rats

Glucose administration produces a significant change in SG level of normal rats. Treatment with higher dose of QT (100 mg/kg) and QPC (50 mg/kg and 100 mg/kg) and GLB (0.5mg/kg) significantly ($P < 0.01$, $P < 0.001$) improved the glucose tolerance whereas, treatment with lower dose of QT (50 mg/kg) did not significantly reduced the AUC_{glucose} compared to the normal control group. The results are shown in Figure 6a.

Effect of QT and QPC on STZ-induced diabetes in rats

Single dose one day study

A single dose of QT and QPC (50 and 100mg/kg) treatment exhibited reduction in SG levels at different time intervals compared to basal levels (0hr). Administration of GLB showed significant reduction in SG levels with maximum reduction at 4 hr compared to basal levels. QT and QPC treated animals showed dose dependent percentage reduction in SG levels compared to basal levels. (Figure.7)

Effect of QPC on STZ-induced diabetes in rats multiple dose fifteen day study

Repeated administration of QT and QPC (50 and 100 mg/kg) showed significant reduction levels of SG compared to basal levels (0 day). On 15th day, tested doses of QT and QPC (50 and 100 mg/kg) showed significant ($P < 0.01, P < 0.001$) greater % reduction in glycemia as compared to diabetic control. (Figure-8)

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 quercetin (50 & 100 mg/kg) and quercetin 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 morphological changes in pancreatic cells of different groups of treated animals. The normal control group showed normal islet of langerhans and pancreatic acini with normal cellular structure (Fig-9a). In the diabetic group reduced number of pancreatic islets, disturbed cellular structure, increased vacuolation, hydropic, necrotic cells and also cellular degranulation followed by invasion of connective tissues were detected (Fig-9b). QT and QPC (50 and 100 mg/kg) and standard drug GLB (Glibenclamide) markedly succeeded in amending the ruptured islets of langerhans of diabetic rats and also improved islets architecture, integrity, number and size of pancreatic islets were observed (Fig-9c, d and e).

Concentration of quercetin in rat serum

Figure 10: shows the serum concentration study of QT and QPC in rats. Peak serum concentration of 11.93 $\mu\text{g/ml}$ attained rapidly within 1h after the administration of pure quercetin. But in case of complex, the peak concentration 14.16 $\mu\text{g/ml}$ appeared at same time and was much higher than pure quercetin and was maintained for longer time.

Pharmacokinetic parameters

Table 3 shows the main pharmacokinetic parameters of QPC and QT in rats. C_{max} and T_{max} was increased in case of the complex. The elimination half life of QT was increased in the complex form with phospholipids and the clearance of the QT molecule in complex was also lowered. The complex stayed for a longer period of time in body with a higher relative bioavailability of 112.32 %.

DISCUSSION

Phytosomes are prepared with the standardized extract/active ingredient of herb which are bound to the phospholipids on a molecular level. These are the novel compounds comprising of lipophilic complexes of components of various plant active ingredients with phospholipids. Phytosomes are the advanced technology for herbal products which are having better absorption and as a result showed results better than conventional herbal extracts. Flavonoids are a large group of polyphenolic secondary metabolites in plants showing wide range of pharmacological properties. Bioavailability of quercetin aglycone is an important factor for *in-vivo* activity. When QT was administered orally it lead to poor absorption, In order to overcome this limitation a herbal formulation was developed in combination with phospholipid (HSPC) which has better absorption and utilization when administered orally.

In the present study QPC was prepared and its physicochemical properties were evaluated. The phytosome complex was prepared with equal quantities of QT and phospholipid which was stable compared with other variables. The microscopic view with Trinocular as well as SEM indicated that the presence of structures like vesicles consisting of HSPC and QT which were intercalated in the lipid layers. During the surface morphology of the QPC at various magnifications, it was observed that the QT particles are associated with the HSPC forming complexes with non uniform particles, the QT particles were not clearly seen due to the of the complex formation. The DSC and FT-IR studies QPC and QT showed that QT and phospholipids combined and formed supramolecular structures by non covalent bonding, such as hydrogen bonding or van der Waals forces. After the combination of QT and the HSPC polarity parts, the carbon-hydrogen chain in phospholipid could turn freely and enwrap the phospholipid molecule polarity parts, which made the sequence decrease between phospholipid aliphatic hydrocarbon chains, made the second endothermal peak of phospholipid disappear and depressed the phase transition temperature. The *In vitro* dissolution rate of QPC increased significantly. QT and QPC (100 mg/kg) significantly reduced serum glucose level in STZ-induced diabetic rats may be due to potentiating the insulin effect by increasing either the pancreatic secretion of insulin from the existing β -cells or by its release from the bound form. The content of QT present in the complex, as

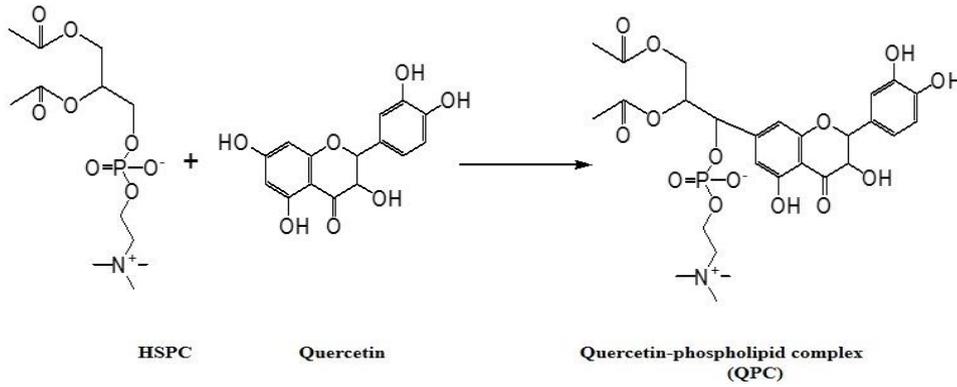


Figure 1: Probable scheme for formation of Quercetin-phospholipid complex.

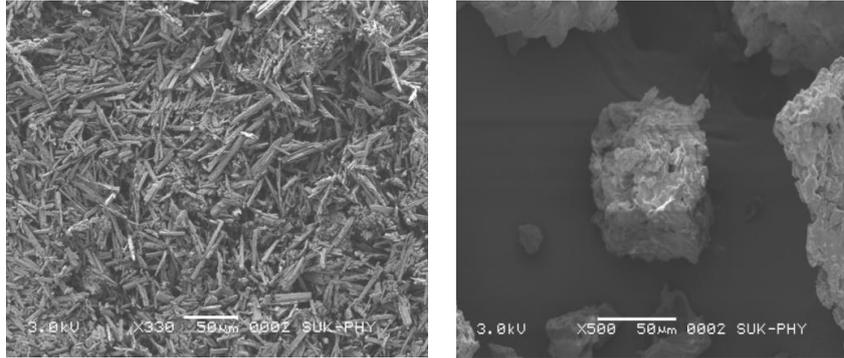


Figure 2: Surface morphology of QT and QPC at a) 330X b) 500X

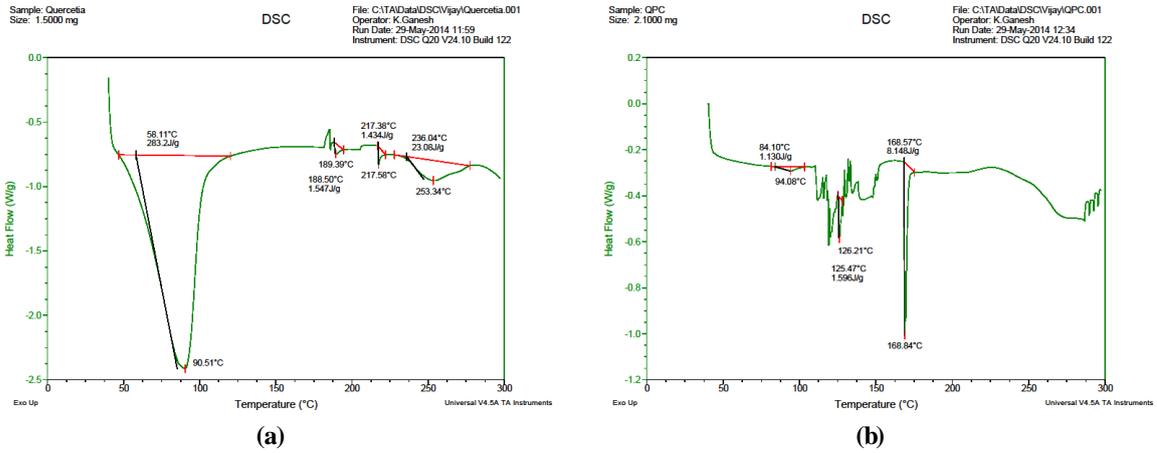


Figure 3: Differential scanning calorimetry (DSC) a. Quercetin b. Quercetin phospholipid complex

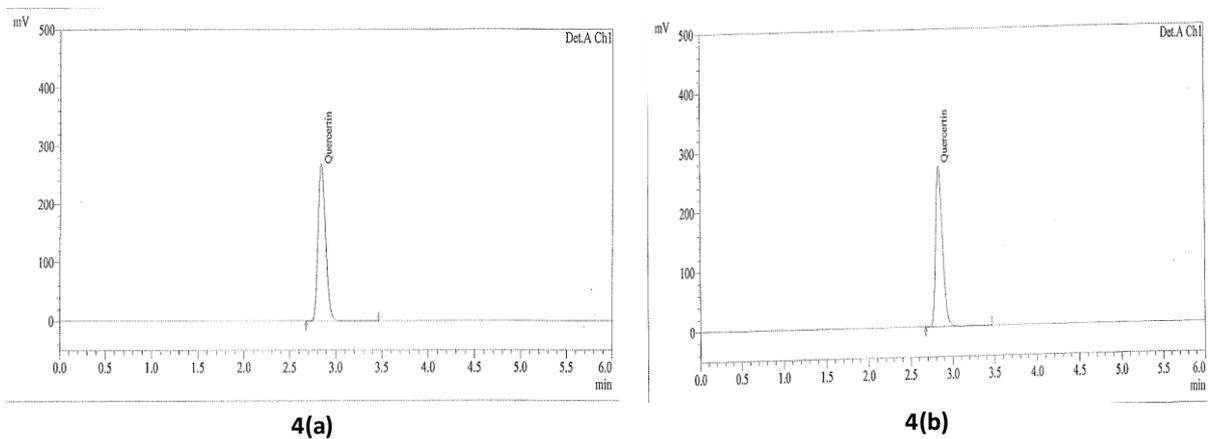


Figure 4: 4a and 4b. Determination of content of QT in QPC by HPLC

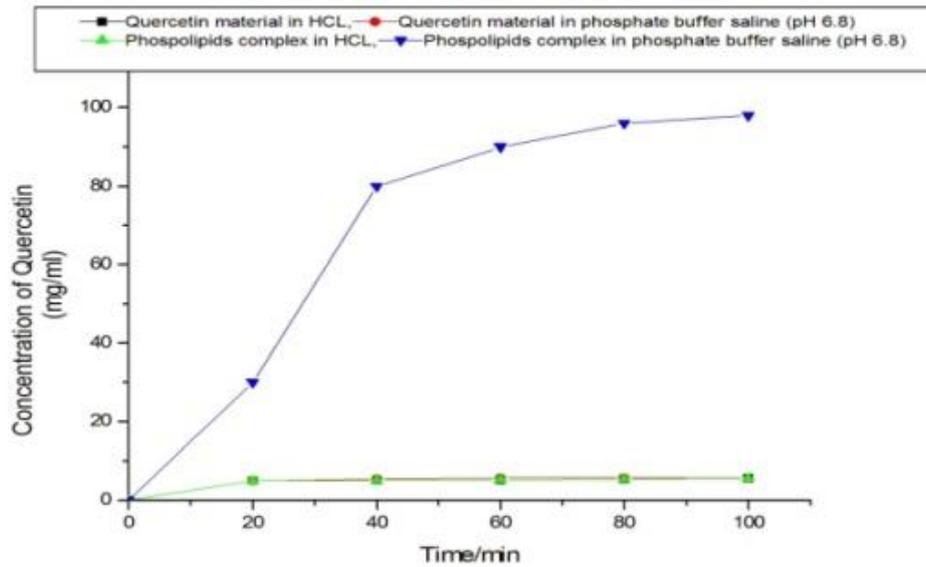


Figure 5: Dissolution profile of QT and QPC

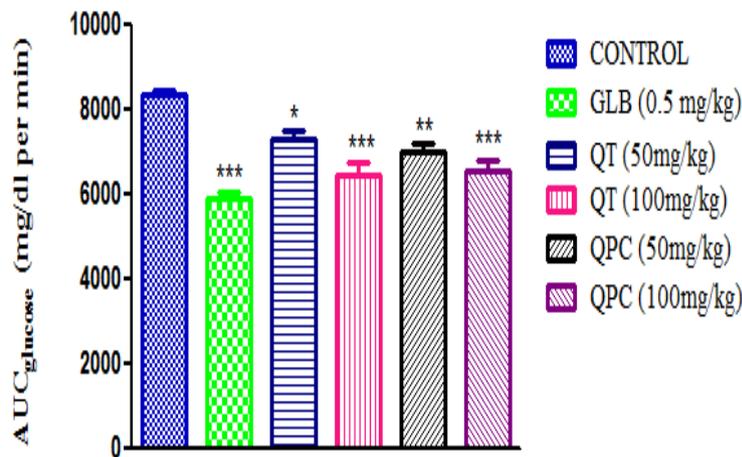


Figure 6: Effect of QT and QPC in normoglycemic rats (OGTT in normal rats)

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 with normal rats.

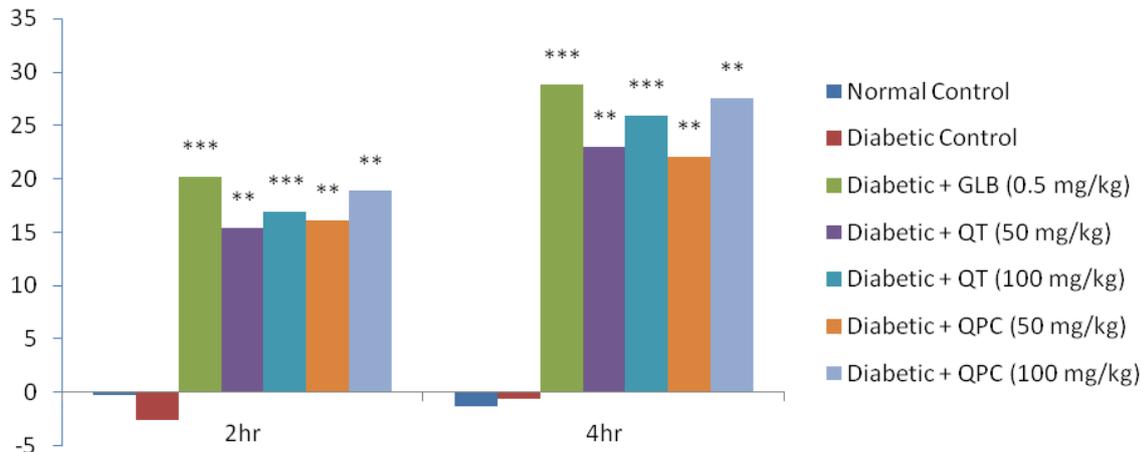


Figure 7: Effect of QPC on STZ-induced diabetes in rats (Single dose One day study)

SG levels were measured at 0,1,2, and 4 hr after single administration of QT and QPC and GLB. Data are expressed as mean ± S.E.M., for n=6. * P < 0.05; ** P < 0.01; *** P < 0.001 as compared with normal rats.

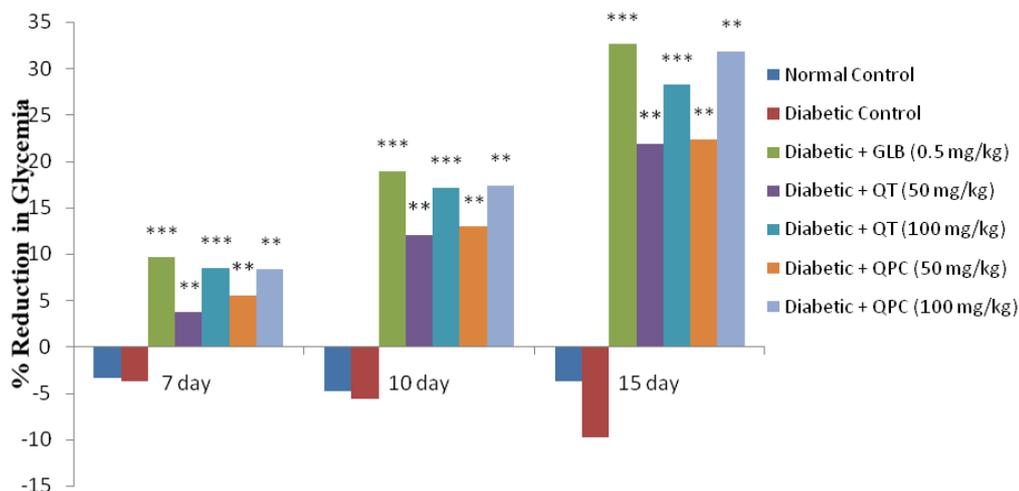


Figure 8: Effect of QT and QPC on STZ-induced diabetes in rats (Multiple dose fifteen day study)
 Bar graph represents the percentage reduction in glycemia with respect to the initial (0 day) level. Each value represents mean ± S.E.M., for n=6. * P < 0.05; ** P < 0.01; *** P < 0.001 as compared with normal rats.

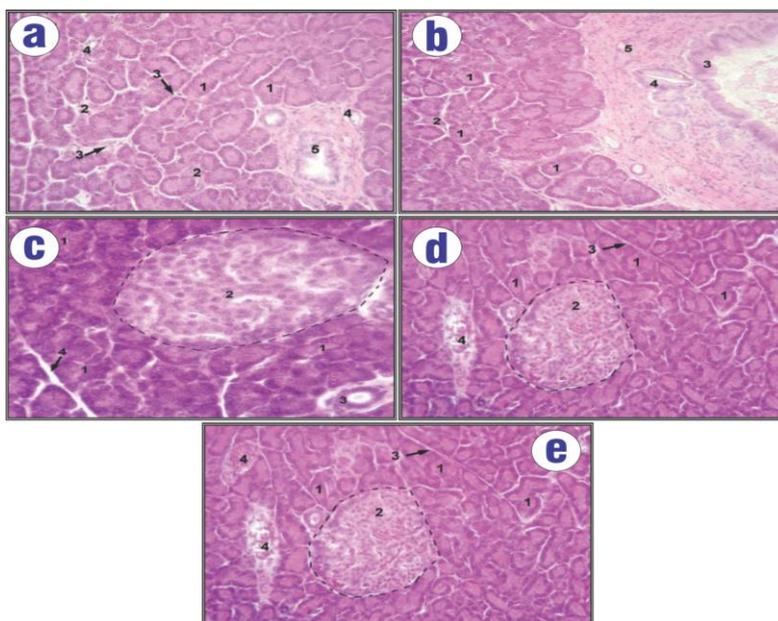


Figure 9: Effect of QT and QPC on histopathological changes in rat pancreatic tissue
 a) Normal control group 1--Normal acinus, 2--Increased number of islet of Langerhans, 3--Interlobular connective tissue, 4--Intralobular duct, 5--Interlobular duct. b) Diabetic control group 1--Glandular acinus are observed, 2--Decreased number of islet of Langerhans, vacuolation, 3--Main duct showing degranulation, 4--Interlobular duct with vacuoles, 5-- Invasion of connective tissue. c) Diabetic control with GLB treated group 1-- Normal acinus, 2-- Increasing in the number of islet of Langerhans, 3--Normal intralobular duct, 4-- Interlobular connective tissue without invasion. d) Diabetic control with QT treated group 1--Normal acinus were observed, 2-- Moderate increase in islet of Langerhans, 3--Increased interlobular connective tissues, 4-- Moderate increase in blood vessels. e) Diabetic control with QPC treated group 1--Normal acinus were observed, 2--Moderate increase in islet of Langerhans, 3-- Increased interlobular connective tissues, 4-- Moderate increase in blood vessels.

Table 1: Apparent solubility of QT and QPC in water and n-octanol at 25° C

Samples	Solubility in water µg/ml	Solubility in n-octanol µg/ml
Quercetin	8.33±0.23	4.08 ± 0.28
Quercetin phospholipid Complex	26.67 ± 1.34	45.99±2.32
Physical mixture	12.40 ±0.52	8.87± 0.31

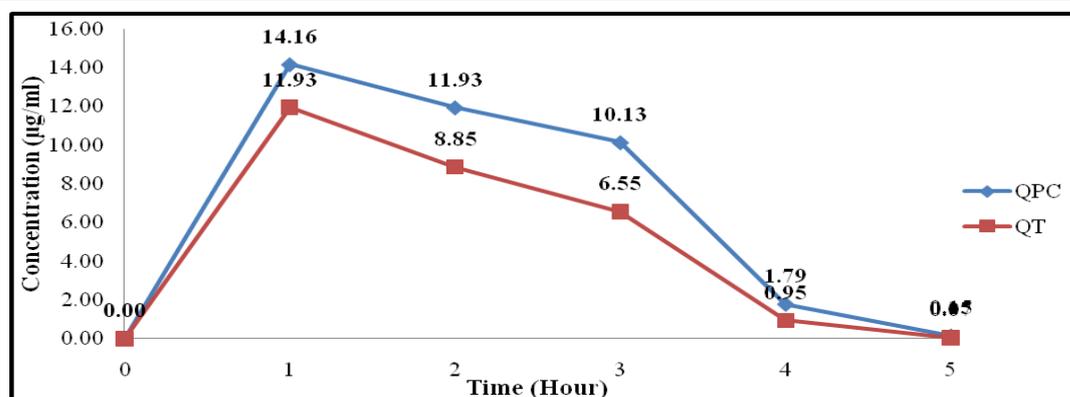
Table 2: Effect of QT and QPC on lipid profile in STZ induced diabetic rats

Serum parameter	Normal control	Diabetic control	Glibenclamide	QT 50mg/kg	QT 100mg/kg	QPC 50mg/kg	QPC 100mg/kg
STG (mg/dl)	101.7± 2.428	220.2± 3.861	143.5± 6.619***	194± 5.019**	173.5± 3.996*	215.8± 2.408***	182.2±2.688***
STC	69.86± 1.512	112.4± 3.16	85.98± 1.98***	105.74± 4.91**	98.8± 1.89**	99.45± 3.145**	102.5±2.126***
HDL-c	32.49± 1.208	19.95± 1.007	38.15± 0.535**	31.48± 1.247**	36.94± 1.457**	30.14± 1.487*	34.45±1.846**
VLDL-c	22.53± 0.328	41.54± 0.547	25.65± 1.494***	3684± 1.149**	31.74± 0.456***	38.92± 0.623*	35.02±1.142***
LDL_c	22.45± 1.948	62.19± 3.142	27.19± 2.659***	41.65± 2.169***	35.74± 2.642***	42.85± 4.052***	36.19±1.326***
TC/HDL-c ratio	1.947± 0.236	6.235± 0.189	2.486± 0.087***	2.849± 0.289**	3.597± 0.116*	2.275± 0.186***	2.262±0.236***
LDL-c/HDL-c ratio	0.986± 0.097	2.749± 0.426	0.576± 0.054***	1.924± 0.072**	1.873± 0.069**	1.402± 0.249***	1.115±0.081***

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

Table 3: Pharmacokinetic parameters of QT (20mg/kg, p.o) and QPC (equivalent to 20mg/kg, p.o) in rats (n =6).

Pharmacokinetic parameters	Quercetin	Quercetin phospholipid complex
C _{max} (µg/ml)	11.93	14.16
T _{max} (h)	0.59	1.01
Area under the concentration -time curve(AUC _{0-t_n}) (µg/ml h)	2.95	5.05
Area under the concentration -time curve(AUC _{0-t_d}) (ml ⁻¹ h)	2.63	6.41
Elimination half life (t _{1/2el}) (h)	1.58	1.72
Elimination rate constant (K _{el}) (h ⁻¹)	0.43	0.24
Clearance (cl) (l h ⁻¹)	86.12	42.71
Volume of distribution (V _d) (l)	212.02	96.56

**Figure 10: Bioavailability studies of QT and QPC in rats**

estimated by HPLC, revealed that quercetin content in the complex was 70.12%. Bioavailability study revealed that the QT standard and phospholipid complex orally administered to rats, the complex showed enhanced oral bioavailability of QT *in vivo* for rats and stayed in the blood for a longer period, which make the complex achieved higher bioavailability of QT. Release of QT from the complex was mainly due to phospholipids which plays a major role in drug delivery technology and thereby produce a sustained release effect. The release of QT was almost completed within 5 h whereas the complex extended the release upto 24 h. Hence

the result produced by the complex may be due to a combined effect of sustained release property of the quercetin complex and protective effect of phospholipids on QT. The evident for the experimental result was complexation, which played a major role in sustained release of quercetin from the complex.

CONCLUSION

An attempt has been made in the present study to prepare QPC and evaluate its physicochemical properties. The FTIR and DSC curves showed that there are formations of weak hydrogen bonding between the

phospholipids and quercetin such as van-der Waals force and the entrapment efficiency of complex was up 50%. Anti diabetic activity was carried out in STZ induced rats for QT and QPC (50 mg/kg and 100 mg/kg) where QT and QPC (100 mg/kg) has shown a significant hypoglycaemic effect. The bioavailability studies revealed that the complex has better absorption compared to quercetin alone. The present study showed that QPC can produce better therapeutic effect in rats for longer period of time as compared to the molecule by sustained release property and thereby decreasing the rate of elimination.

ACKNOWLEDGEMENTS

Authors are thankful to Rajiv Gandhi University of Health Sciences, Bangalore for financial support to carry out this work. Authors also thank President, Soniya education trust, and Principal SET's college of pharmacy, Dharwad for providing necessary facilities to carry out this research.

CONFLICT OF INTEREST

We declare that we have no conflict of interest.

REFERENCES

Chi Yu Yang, Su Lan Hsiu, Kuo-Ching Wen, et al. Bioavailability and metabolic pharmacokinetics of Rutin and Quercetin in Rats. *J of food and drug analysis*, 13(3), 2005, 244-250.

Dang Yi. UPC code-0300540111783, New product concept. 2000.

Friedewald WT, Levy RI, Fredrickson DS. Estimation of low-density lipoprotein cholesterol in plasma, without use of the preparative centrifuge. *Clin Chem*, 18, 1972, 499-502.

Galigher AE, Kozloff EN. Essentials of practical micro-technique, 2nd edn. Lea and Febiger, Philadelphia, 1971, 77-79.

IDF Diabetes Atlas, 5th edition. International Diabetes Federation, Brussels, Belgium, 2011.

Jain S, Jain AK, Pohekar M et.al. Novel self-emulsifying formulation of quercetin for improved in vivo antioxidant potential: implications for drug-induced cardiotoxicity and nephrotoxicity. *Free radical Biology and Medicine*, 65, 2013, 117-130.

Ketan Hatware and Annapurna A. The effect of quercetin on blood glucose levels of normal and streptozotocin induced diabetic (type I & type II) rats. *Int J. I of pharmaceutical, chemical and biological sciences*, 4(3), 2014, 613-619

Kidd PM. Bioavailability and activity of phytosome complexes from botanical polyphenols: the silymarin, curcumin, green tea, and grape seed extracts. *Altern Med Rev.*, 14(3), 2009, 226-46.

Kumar GPS, Arulselvan P, Kumar DS, et al. Anti-Diabetic activity of Fruits of *Terminalia chebula* on Streptozotocin Induced Diabetic Rats. *J Health Sci*, 52(3), 2006, 283-91.

Liu L, Tang Y, Gao C. et.al. Characterization and biodistribution in vivo of quercetin-loaded cationic nanostructured lipid carriers. *Colloids Surf B Biointerfaces*. 115, 2014, 125-31.

Maiti K, Mukherjee K, Gantait A, et al. Curcumin-phospholipid complex: therapeutic evaluation and pharmacokinetic study in rats. *Int J Pharm*, 330 (1-2), 2007, 55-163.

Men K, Duan X, Wei XW et.al Nanoparticle delivered quercetin for cancer therapy. *Anticancer agents Med.chem.*, 14(6), 2014, 826-32.

Prasanna H, Ramesh K, Rashmi V. Preparation and evaluation of Bocopa- Phospholipid complex for anti-amnesic activity. *Drug invention today*, 5, 2013, 13-21.

Shaw JE, Sicree RA, Zimmet PZ 2010. Global estimates of the prevalence of diabetes for 2010 and 2030. *Diabetes Res Clin Pract.*, 87, 2010, 4-14.

Shirwaikar A, Rajendran K. Effect of aqueous bark extract of *Garuga pinnata Roxb.* in streptozotocin-nicotinamide induced type-II diabetes mellitus. *J Ethnopharmacol*, 112, 2006, 1-6.

Tawheed A, Suman VB. A Review on Phytosome technology as a novel approach to improve the bioavailability of nutraceuticals. *Int J. of Adv. in Res. & Tech.*, 1(3), 2012, 1-15.

Varshosaz J, Jafarian A, Salehi G et.al. Comparing different sterol containing solid lipid nanoparticles for targeted delivery of quercetin in hepatocellular carcinoma. *J Liposome Res.*, 24(3), 2014, 191-203.

Yanyu X, Yunmei S, Zhipeng C, et al. The preparation of silybin-phospholipid complex and the study on its pharmacokinetics in rats. *Int J Pharm*, 307(1), 2006, 77-82.

Yeh GY, Eisenberg DM, Kaptchuk TJ, Phillips RS. Systematic review of herbs and dietary supplements for glycemic control in diabetes. *Diabetes Care*, 26, 2003, 1277- 1294.