ISSN PRINT 2319 1775 Online 2320 7876
 UGC CARE Listed (Group -I) Journal Volume 11, Spl Iss 4,Dec 2022

FORMULATION AND DEVELOPMENT OF RUTIN PHYTOSOMAL COMPLEX FOR DIABETES

SanketMukeshKalmegh[1]*, Ravindra B. Laware[2]
[1]Research Scholar, Dept. of Pharmaceutical Sciences, Bhagwant University, Ajmer, Rajasthan, India.
[2]Dept. of Pharmaceutical Sciences, Bhagwant University, Ajmer, Rajasthan, India.
DOI:10.48047/IJFANS/V11/SPL4/134

Abstract:
Medicinal plants have been a major source of cure of human diseases since time immemorial. The demand for the plant derived drugs seems to increase in developing countries due to their medicinal value and economic procurement. Plants have been used in a wide variety of dosage form as diabetes is chronic and non-curable disease, regular treatment is needed.
Till now, no work has been carried out on preparation of rutin - phytosome (rutin-Phytosomal complex) for the treatment of diabetes. So, the present work aimed to formulation and development of rutin-phytosome (rutin-phospholipid complex) for diabetes and to compare in vitro and in vivo activity of rutin and rutin -phytosome (rutin - phospholipid complex).
Keyword: Herbal formulation, Development, Evaluation, Standardization and stability study of tablets, rutin - phospholipid complex, etc.

1. Introduction:

Diabetes is a principle cause of morbidity and mortality in human populations[1]. It is a syndrome characterized by hyperglycemia, polydipsia, and polyuria and causes complications to the eyes, kidneys, and nerves. It is also associated with an increased incidence of cardiovascular disease[2]. The clinical manifestations and development of diabetes often differ significantly between countries and also between racial groups within a country. For example, diabetes currently affects an estimated 15.1 million people in North America, 18.5 million in Europe, 51.4 million in Asia, and just under I million in Oceania[3]. It is estimated that globally, the number of people will rise from 151 million in the year 2000[4], to 221 million by the year 2010, and to 300 million by 2025[5].

What is diabetes?

Diabetes is a chronic metabolic disease characterized by the inability to maintain blood glucose concentrations within physiological limits. The study described in this thesis will focus on type 1 diabetes, a particular manifestation of diabetes mellitus. Type I diabetes is characterized by a loss of pancreatic B-cell function and an absolute insulin deficiency. Since insulin is the primary anabolic hormone that regulates blood glucose level, type 1 diabetics require a continuous supply of insulin for survival. Conventional therapy for type 1 diabetics involves insulin replacement through multiple daily injections (MDI) or a continuous subcutaneous insulin infusion (CSII) guided by daily blood glucose measurements.

Importance of glucose and insulin in diabetes

Glucose is one of the body's main sources of energy. In normal physiology, the body maintains blood glucose levels within a narrow range (70-130 mg/dl). Blood glucose is balanced between endogenous appearance from the liver (through glycogenolysis and gluconeogenesis) and kidneys, exogenous appearance from the intestines (following a meal), and utilization of glucose by all tissues. Two gross metabolic conditions exist. When fasting, the body relies primarily on glucose stored in the form of glycogen and fatty acids stored in

ISSN PRINT 2319 1775 Online 2320 7876
Research paper UGC CARE Listed (Group -I) Journal Volume 11, Spl Iss 4,Dec 2022

the form of triglycerides to fuel its metabolic needs. After a meal, glucose absorbed from the gut is used to replenish glycogen and fat stores diminished while fasting. The body regulates the processes that control the production and storage of glucose by secreting the endocrine hormone, insulin, from the pancreatic B-cells. Insulin facilitates anabolic metabolism throughout the body. An increase in insulin above basal concentrations (2-12 mU/1) will decrease the release of glucose from the liver and increase glucose uptake into insulin-receptive tissues. This has the net effect of decreasing endogenous blood glucose appearance[2]. There are many substances in the body that promote and inhibit insulin secretion, refining the detail to which the B-cells react to changes in the body's metabolic state. Glucose is by far the dominant stimulus for insulin secretion, establishing a direct relationship between insulin secretion and the blood glucose level in the body. An abnormally high resistance to insulin causes sustained hyperglycemia, especially following meals. A third class of diabetes, gestational diabetes, presents itself during pregnancy and is a health concern for the mother and the developing fetus.

2. Material and methods:

2.1 Preformulation Studies:

Preformulation is defined as phase of research and development process where PUSIcal, chemical and mechanical properties of a new drug substance are characterized i.e. and when combined with excipients, in order to develop stable, safe and effective dosage form.

In the preformulation study of new drug delivery systems, it is essential to have available knowledge of the physicochemical properties of the active component and excipients. Pharmaceuticals excipients are applied in dosage forms to facilitate administration and release the drug, as well as to protect it from the environment[6]. The excipients are considered inert, but incompatibilities (solid state interactions) between drug and excipients are commonly possible. The inadequate use of pharmaceutical excipients in sf oral dosage forms can cause serious biopharmaceuticals implications, modifying the release mechanism, absorption[7] and fast evaluation of possible incompatibility is facilitated using the analytical techniques, which offers significant advantages in saving both time and thermogravimetry derivative, thermogravimetry (TG/DTG) are extensively used in the substance. The differential scanning calorimetry (DSC). This thermo-analytical method allows evaluation of thermal behavior, decomposition kinetics, purity determination and investigation of interactions between drug and excipients according to appearance, shift or disappearance of peaks nor variations in the corresponding enthalpies of reactions[8]. In addition, Fourier transform infrared spectroscopy analysis (FTIR) was used to evaluate the compatibility of mixtures. Changes in the position, intensity or width of the vibrational bands can be observed in spectra when an interaction between drug and excipient occurs. Since our research involves the development of a delayed release multiparticle system containing rutin, the preformulation study searching for interactions is crucial to select appropriate excipients to ensure the final formulation quality. The multiparticle system due to its numerous technological and pharmacokinetics advantages in relation to the single pharmaceutical dosage, was the chosen one. The solvent evaporation method was used to obtain phytosometherefore it was indispensable to select proper excipients capable to provide adequate characteristics for phytosome production.

Fourier transform infrared (FTIR):

Prior to the development of the dosage forms the preformulation study was carried out. Hence infrared spectra of the physical mixture of the drug and the polymers CEsen were taken. The infra-red spectra of the drug (Indian Pharmacopoeia, 1996) and polymers were also taken. The application of infra-red spectroscopy lies more in the qualitative identification

ISSN PRINT 2319 1775 Online 2320 7876
 UGC CARE Listed (Group -I) Journal Volume 11, Spl Iss 4,Dec 2022

of substances either in pure form or in the mixtures and as a tool for establishment of the structure, since 1.R. is related to covalent bonds, the spectra can provide detailed information about the structure of molecular compounds. In order to establish this point, comparisons can be made between the spectrum of the substance and the drug. The above discussions imply that infra-red data is helpful to confirm the identity of the drug and to detect the interaction of the drug with the carriers. Formulation of phytosome: There are several methods which can be used to disperse drug and additives in formulation.

Solvent evaporation technique:

An amount of 10 g of soya lecithin was dissolved in 25 ml of chloroform. The mixture was refluxed under mechanical stirring and added drop wise to a solution of rutin prepared by adding methanol kept on a mechanical stirrer. After completion of the addition, the mixture was refluxed under stirring for 5-6 hour, then concentrated and finally dried under vacuum at 40°c for 48 hours. The resultant rutin phospholipid complex (RPC) was kept in an amber colored glass bottle flushed with nitrogen and stored in refrigerator.

For vesicular drug delivery (phytosome), add water at 35-38-degree C and sonicate (preferably probe sonicator) for 15 mint. This is method of preparation of phytosome by 1:1molar ratio. Two more batches of 1:1.08 &1.1.2 molar ratio were prepared by this method.

Evaluation of formulation:

- Surface morphology/ Visualization (SEM).
- Solubility study:
- Transition temperature (DSC).
- Entrapment efficiency.
- X-ray diffraction of formulation(XRD)
- In-vitro drug release study.
- Fourier transforms infrared (FTIR) study to check stability.
- Surface morphology:

Surface morphology was determined by scanning electron microscopy (SEM).

Procedure:

Morphology details of the specimens were determined by using a scanning electron microscope (SEM), Model JSM 35CF, JEOL, Japan. The samples were dried thoroughly in vacuum desiccator before mounting on brass specimen studies. The samples were mounted on specimen studies using double sided adhesive tape, and gold-palladium alloy of 120^0A was coated on the sample using sputter coating unit (Model ES 100 Polaron U.K.) in Argon ambient of 8-10 Pascal with plasma voltage about 20 MA. The sputtering was done for nearly 3 minutes to obtain uniform coating on the sample to enable good quality SEM images. The SEM was operated at low accelerating voltage of about 15 KV with load current of about 80 MA. The condenser lens position was maintained in between 4.4 5.1. The objectives lens aperture has a diameter of 240 microns and the working distance WD = 39 mm[9].

- Solubility Study:

Solubility of rutin, rutin -phospholipid complex, and rutin: lipid physical mixture was performed in various solvents like chloroform, Octanol, by adding excess of rutin and rutin-phospholipid complex to l0 ml of respective solvent in sealed glass containers at room temperature, the liquid was agitated for 24 hours on rotator shaker then centrifuged for 15. minutes to remove excessive rutin. The supernatant was filtered through membrane filter then I ml filtrate was mixed with 9 ml of methanol to prepare dilutions and these samples were measured at wavelength of at 258 nm and 348 nm by using UV spectrophotometer and the concentration was calculated by using calibration curve.

ISSN PRINT 2319 1775 Online 2320 7876
Research paper UGC CARE Listed (Group -I) Journal Volume 11, Spl Iss 4,Dec 2022

- Transition temperature:

The transition temperature of the vesicular lipid systems can be determined by differential scanning calorimetry (DSC).

- Drug Release Studies:

The vitro release of Rutin and rutin -phytosome was measured using dialysis method using lnhur and other impurities. Activated membrane was stored in 7.4 PBS until used. cellophane membrane. Activation of cellophane membrane was done in order to remove Rutin (20 mg) and rutin- phytosome (20 mg) was transferred to sample holder of two different diffusion cells where the receiving compartment contained 50 ml of distilled water. The whole set is placed on a magnetic stirrer. Adjusted to constant speed of 150 rpm at 25 °C. At predetermined time interval for 120 min, 1 ml of release medium was withdrawn for analysis and was compensated by same volume of fresh dissolution medium. Samples were measured at max 370 nm.

- Drug Entrapment Efficiency:

The various formulations of the phytosome were subjected for drug content analysis. Suspension of the various formulations was prepared by suspending complex (equivalent to 100 mg of pure rutin) in methanol. Each suspension was centrifuged for 40 min at 24°C to separate the free drug in the supernatant from the drug incorporated in the phytosome. Concentrations of rutin in the supernatant were determined by UV-visible spectrometry at 370 nm after suitable dilution. The amount of the drug incorporated in phytosome was calculated from the difference in drug concentrations between the supernatant and the original given concentrations. The entrapment efficiency was calculated according to the following

Equation:

$$\text{Percent Entrapment} = \frac{\text{Total drug- Diffused drug}}{\text{Total drug}} \text{ X100}$$

Each determination was made in triplicate.

- X-Ray Diffraction Studies:

Crystalline state in pure rutin, soya lecithin, and prepared rutin-phospholipid complex was evaluated in by powder x-ray. The obtained XRD spectra were compared with respect to degree of crystallinity.

- Spectroscopic evaluations:

To confirm the formation of a complex or to study the reciprocal interaction between the phytoconstituents and the phospholipids, the following spectroscopic methods are used.

ETIR: The formation of the complex can be also being confirmed by IR spectroscopy by comparing the spectrum of the complex with the spectrum of the individual components and their mechanical mixtures. FTIR spectroscopy is also a useful tool for the control of the stability of phytosome when micro-dispersed in water or when incorporated in very simple cosmetic gels. From a practical point of view, the stability can be confirmed by comparing the spectrum of the complex in solid form (phytosome) with the spectrum of its micro dispersion in water after lyophilization, at different time

Standardization of Formulation by HPTLC:

Standardization as defined herbal drug preparation to a defined content of constituent or group of substance with known therapeutic activity which can be used to standardize a biological effects and marker compounds. Herbal products represent complex biological mixture and achieving a reproducible pharmaceutical quality could be a very challenging task in standardization of herbal products, methodical approach is required for standardization of plant extracts as is done in contemporary medicine. Standardization of natural products like

ISSN PRINT 2319 1775 Online 2320 7876
Research paper UGC CARE Listed (Group -I) Journal Volume 11, Spl Iss 4,Dec 2022

polyherbal formulation is a complex task due to their heterogeneous composition, which is in the form of whole plant, plant parts or extracts obtained thereof. To ensure reproducible quality of herbal products, proper quality control of formulation is upmost essential. Thus the problems faced in the standardization of herbal formulations are numerous but one has to make a beginning somewhere. Keeping in mind that polyherbal formulation quality control generally necessitates a multidisciplinary approach which requires resources and expensive equipment. For identification of the crude drug in the polyherbal formulation it is best to possess the authentic reference standard of that particular crude drug[10]. Markers are compound (s) unique/characteristic to the plant in question and are preferably present in detectable amounts and can be easily isolated. The concentration of markers in a particular drug could be appropriately fixed and each batch of the drug produced should adhere to these limits. The quantification can be carried out by various methods like high performance thin layer chromatography (HPTLC). High performance liquid chromatography (HPLC), Gas chromatography (GC), etc.

HPTLC fingerprint is obtained at low cost and high speed and thus meets the need of a modern quality control method for polyherbal formulation[11]. HPTLC fingerprint serves a highly useful purpose in evaluating the changes in chemical composition during storage[12].

HPTLC is a valuable tool for the investigation of herbal products with respect to different aspects of their quality. HPTLC analysis is comparatively short and many samples can conveniently be compared side by side on the same plate. This is particularly important for screening and inspection / selection of raw materials and for process control during manufacturing. With HPTLC, the same analysis can be viewed using different wavelengths of light thereby providing a more complete profile of the plant and extreme flexibility of detection, the convenience of specific derivatization, and the possibility of multiple detections without repeating the chromatography. HPTLC results are not only reported as peak data but can also be presented and communicated as images[13]. For large composite samples (e.g., multiple samples of large batches of un-homogenized raw material) or samples of plant derived products (e.g., extracts), selectivity must also address the possibility of accidental or willful adulteration. This however is also a quantitative issue, concerning detection limits of unwanted components. In standardization of plant extracts the focus is given only on the discrimination of species because this is the area where HPTLC methods are most versatile.

HPTLC is an off-line method that offers enormous flexibility in the selection of parameters for the individual steps of the process[14]. Aside from the stationary and mobile phase, these parameters include the development conditions (chamber saturation, developing distance), derivatization (reagent concentration, time, heat), and waiting times between the subsequent steps. The method to be validated should define all parameters rigorously. We have adopted the concept of precision (repeatability, intermediate precision, reproducibility), which is commonly used only for quantitative methods, as a separate validation point. Precision helps to evaluate how predictably a fingerprint can be generated from a given botanical reference material.

Steps involved in HPTLC:

1. Selection of chromatographic layer
2. Sample and standard preparation
3. Layer prewashing
4. Layer preconditioning
5. Application of sample and standard
6. Chromatographic development
7. Detection of spot
8. Scanning

ISSN PRINT 2319 1775 Online 2320 7876
Research paper UGC CARE Listed (Group -I) Journal Volume 11, Spl Iss 4,Dec 2022

9. Documentation of chromatic plate
High Performance Thin Layer Chromatography:

Figure1 CamagLinomat V

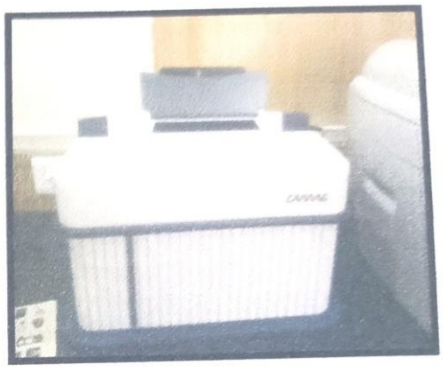


Figure 2: UV Chamber Figure automatic sample applicator

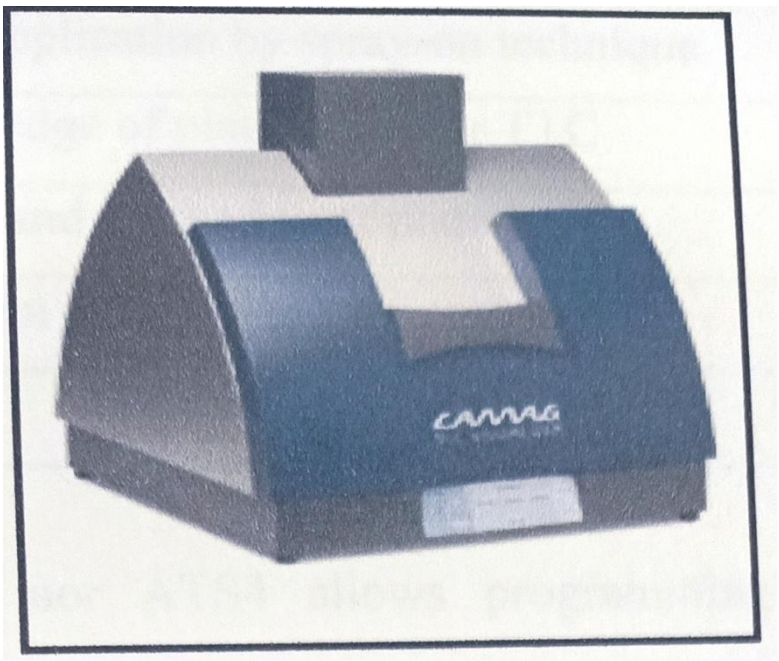


Figure 3: Camag TLC Visualizer

ISSN PRINT 2319 1775 Online 2320 7876
Research paper UGC CARE Listed (Group -I) Journal Volume 11, Spl Iss 4,Dec 2022

Chemicals and reagents used: Rutin standard was procured from SD Fine Chemical Ltd. Silica gel 60F254. TLC plates (20x10 cm, layer thickness 0.2 mm, E. Merck) was used as a stationary phase. All chemicals and reagents were of analytical grade and obtained from Qualigens Fine chemicals. Prepared formulation of rutin was used for analysis.

Instruments used:
The instruments used in the present study were Camag HPTLC system comprising of Camag Linomat V automatic sample applicator, Hamilton syringe (100 ul), Camag TLC scanner 3, Camag Win CATS software, Camag Twin trough chamber (20x10 cm).

Preparation of standard solution:
10 mg of each rutin was weighed separately transferred in 10 ml volumetric flasks. Drug was dissolved in 5 ml of methanol solvent by vigorous shaking and then volume was made up to mark with methanol to obtained final concentration of 1 mg/ml of each component.

Application of standard solution:
Linomat, Automatic TLC Sampler (ATS4), Samples are applied as bands by spray-on technique using following parameters:

Table 1: Parameters for spot application by spray-on technique

Distance from lower edge of plate in cm for TTC	08
Minimum distance from left and right edge of plate in mm	10
Minimum space in mm between bands /spot in mm	04
Band length in mm	08

Neither Linomate 5 nor ATS4 allows programming of distance between bands/spot. Therefore, distance between tracks (center to center) and band length must be chosen in order to meet the minimum distance requirements. For spot application volume and application speed have to be determined empirically.

Preparation of developing solvent:
Developing solvent consisting of more than one component are prepared by measuring the required volume of mobile phase and transferred into a solvent bottle of appropriate size. The bottle is closed with a lid and shaken to ensure proper mixing of the content.
For Rutin: Ethyl acetate: Formic acid: Glacial acetic acid: Water: methanol (25ml:2.7ml:2.7ml:6.5ml:2mlv/v) as mobile phase.

Development of plate:
Plates are developed in a saturated Twin Trough Chamber according to the following procedure: The appropriate volume (20 ml for 20x10 cm TTC) of developing solvent was prepared. The chamber was opened and correctly sized (20 x10 cm) piece of filter paper was placed in rear trough. The developing solvent was poured in chamber so that the filter paper thoroughly wetted and adheres to rear wall of TTC. The chamber was tilted on the side (about 45^0) so that all solvent volume in both troughs equalizes. Then the chamber was set on the bench, the lid was replaced and chamber was allowed to equilibrate for 5 min. The desired developing distance (60 mm from lower edge of the plate) was marked with a pencil on the right edge of the plate. Then the lid of the plate was slide off the side. The plate was inserted into front trough.
They should face the filter paper and the back of the plate is resting against front wall of TLC. The lid was replaced. The plate was developed to the mark. The lid was opened and the

ISSN PRINT 2319 1775 Online 2320 7876
 UGC CARE Listed (Group -I) Journal Volume 11, Spl Iss 4,Dec 2022

plate was removed. Then the plate was dried (vertically in the direction of chromatography) 5 min, in a stream of cold air. After each development remaining mobile phase and filter paper are discarded. Prior to being prepared for the next run the chamber was dried and, if necessary also cleaned.

Development of plates:
Each plate was scanned at UV 370 nm. Labeling plates: Each plate is given an individual identification number (ID), which will be written in pencil in the top right corner. The ID includes project number, dash, year, month, day, dash and a consecutive number each day.

Phytosome analysis for rutin phytosome:
2ml of complex (phytosome suspension) diluted up to 10 ml with methanol. The solution was sonicated for 15 min. Quantitative evaluation of rutin The quantitative evaluation was performed with the TLC Scanner 3 using WinCATS software. The analysis files are labeled to reflect the plate ID and any additional descriptive information if multiple evaluations under different condition are performed.

Method validation:
Method was validated and carried out as per the ICH guidelines. The parameters checked were precision, reproducibility, limit of detection, limit of quantification and recovery.

Method validation:
Method was validated and carried out as per the ICH guidelines. The parameters checked were precision, reproducibility, limit of detection, limit of quantification and recovery.
Calibration curves of rutin: A stock solution of rutin (1 mg/ml) was prepared in methanol. Different volumes of stock solution 2, 4, 6, 8, 10, 12, 14and 16 µl were spotted in duplicate on TLC plate to obtain concentrations of 2, 4, 6, 8, 10, 12, 14, 16 µg per spot of rutin. The data of peak area versus drug concentration were treated by linear least-square regression.

Precision:
The precision of an analytical procedure expresses the closeness of agreement (degree of scatter) between a series of measurements obtained from multiple sampling of the same homogeneous sample under the prescribed conditions. Precision may be considered at three levels: repeatability, intermediate precision and reproducibility.

Repeatability
Repeatability expresses the precision under the same operating conditions over a short interval of time. Repeatability is also termed intra-assay precision. Repeatability of sample application and measurement of peak area were carried out using six replicates of the same spot (6 µg for formulation per spot of rutin).

a. Intermediate precision
Intermediate precision expresses within-laboratories variations: different days, different analysts, different equipment, etc. Intermediate precision of sample application and measurement of peak area were carried out using six replicates of the same spot (6 µg for and 4µg for formulation per spot of rutin) and was expressed in terms of percent relative standard deviation (% R.S.D.) and standard error (S.E.). The samples were analyzed on same day.

b. Reproducibility
Reproducibility expresses the precision between laboratories (collaborative studies, usually applied to standardization of methodology). Repeatability of sample application and measurement of peak area were carried out using six replicates of the same spot (6 µg per

ISSN PRINT 2319 1775 Online 2320 7876
 UGC CARE Listed (Group -I) Journal Volume 11, Spl Iss 4,Dec 2022

spot of rutin). The intra- and inter-day variation for the determination of rutin was carried out at concentration levels of 6 µg per spot. As per ICH guideline reproducibility of sample application and measurement of peak area were carried out using sixteen replicates of the same spot (6 µg and 4ug for formulation per spot of rutin).

Limit of detection and limit of quantification:

Limit of detection

The detection limit (LOD) of an individual analytical procedure is the lowest amount of analyte in a sample which can be detected but not necessarily quantitated as an exact value. LOD was calculated using the following formula.

LOD = (3.3 x Standard deviation of the Y-intercept) / slope of calibration curve

ii. Based on visual evaluation

Visual evaluation may be used for non-instrumental methods but may also be used with instrumental methods.

The detection limit is determined by the analysis of samples with known concentrations of analyte and by establishing the minimum level at which the analyte can be reliably detected.

iii. Based on signal-to-noise

This approach can only be applied to analytical procedures which exhibit baseline noise. Determination of the signal-to-noise ratio is performed by comparing measured signals from samples with known low concentrations of analyte with those of blank samples and establishing the minimum concentration at which the analyte can be reliably detected. A signal-to-noise ratio between 3 or 2:1 is generally considered acceptable for estimating the detection limit.

b. Limit of quantification

The quantification limit (LOQ) of an individual analytical procedure is the lowest amount of analyte in a sample which can be quantitatively determined with suitable precision and accuracy. LOQ was calculated using the following formula.

LOQ = (10 x Standard deviation of the Y-intercept) / slope of calibration curve

i) Based on the standard deviation of the blank Measurement of the magnitude of analytical background response is performed analyzing an appropriate number of blank samples and calculating the standard deviation of these responses.

Linearity

The linearity of an analytical procedure is its ability (within a given range) to attain test results which are directly proportional to the concentration (amount) of analyte in the sample. Linearity should be evaluated by visual inspection of a plot of signals as a function of analyte concentration or content. If there is a linear relationship, Last results should be evaluated by appropriate statistical methods, for example, by calculation of a regression line by the method of least squares. In some cases, to obtain linearity between assays and sample concentrations, the test data may need to be subjected to a mathematical transformation prior to the regression analysis.

Data from the regression line itself may be helpful to provide mathematical estimates of the degree of linearity.

Recovery studies:

The accuracy of proposed method was evaluated by addition of standard drug solution to pre-analyzed microencapsulation sample solution at three different concentration level at 80, 100 and 120% of linearity of both the drug.

ISSN PRINT 2319 1775 Online 2320 7876
 UGC CARE Listed (Group -I) Journal Volume 11, Spl Iss 4,Dec 2022

Pharmacological screening:

A. In-vitro alpha-amylase activity

The a-amylase inhibition assay was adapted and modified from Giancarlo et al (2006). The Starch solution (0.5% w/v) was obtained by boiling and stirring 0.25 g of potato starch in 50 ml of deionized water for 15 min. The enzyme solution (0.5 unit/ml) was prepared by mixing 0.001 g of a-amylase (EC 3.2.1.1) in 100 ml of 20 mM sodium phosphate buffer (pH 6.9) containing 6.7 mM sodium chloride. The rutin phytosome were dissolved in DMSO to give concentrations from 10 to 100 mg/ml (10, 20, 40, 60, 80, 100 mg/ml). The color reagent was a solution containing 96 mM 3, 5-dinitrosalicylic acia (20 ml), 5.31 M sodium potassium tartarate in 2 M sodium hydroxide (8 ml) and deionized water (12 ml). 1 ml of rutin phytosome and 1 ml enzyme solution were mixed in a tube and incubated at 25°C for 30 min. To 1 ml of this mixture was added 1 ml of starch solution and the tube incubated at 25°C for 3 min. Then, 1 ml of the color reagent was added and the closed tube placed into an 85°C water bath. After 15 min, the reaction mixture was removed from the water bath and cooled thereafter, diluted with 9 ml railed water and the absorbance value determined at 540 nm in a Shimadzu Multispect- I501 spectrophotometer (Kyoto, Japan). Individual blanks were prepared for correcting he background absorbance. In this case, the color reagent solution was added prior to the addition of starch solution and then the tube placed into the water bath. The other procedures were carried out as above. Controls were conducted in an identical fashion replacing plant extract polyherbal combinations with 1 ml DMSOO. Acarbose solution (at the concentrations of 10, 20, 40, 60, 80, 100 ug/ml) was used as positive control. The inhibition percentage of a-amylase was assessed by the following formula:

$$I_{\alpha\text{-amylase}}\ \% = 100 \times (\triangle A_{Control} - \triangle A_{Sample}) / \triangle A_{Control}$$

$$\triangle A_{Sample} = A_{Test} - A_{Blank}$$

The $I_{\alpha\text{-amylase}}$% was plotted against the sample concentration and a logarithmic regression curve established in order to calculate the IC50 value (inhibitory concentration). This would represent the concentration of sample (ug/ml) necessary to decrease the absorbance of a-amylase by 50%.

B. In-vitro alpha-glucosidase activity

The a-glucosidase inhibition was determined using the modified version of the method according to Matsui et al. (1996). The a-glucosidase reaction mixture contained 2.9 mM p-nitrophenyl-a-D-glucopyranoside (PNPG), 0.25 ml of rutin phytosome l-III (varying concentrations) in DMSO and 0.6 U/ml a-glucosidase in sodium phosphate buffer, pH 6.9. Control tubes contained only DMSO, enzyme and substrate, while in positive controls acarbose replaced the rutin phytosome. Mixtures without enzyme, rutin phytosome (RPC) and acarbose served as blanks. The reaction mixtures were incubated at 25 °C for 5 min, after which the reaction was stopped by boiling for 2 min. roance of the resulting p-nitrophenol (PNPG) was determined at 405 nm using Spectrophotometer (Thermo Electron Corporation) and was considered directly determined as percentage of control as follows: proportional to the activity of the enzyme. Glucosidase activity inhibition was

% Glucosidase inhibition = 100% -%activity of test as percentage of control

$$\%\text{Activity of test} = \frac{\text{Corrected } A_{405} \text{ of test} \times 100\%}{A_{405} \text{ of controls}}$$

In order to eliminate background readings, the absorbance of the rutin without substrate and enzyme was subtracted from absorbance of the rutin phytosome and substrate mixture as follows:

Corrected A_{405} test samples = A_{405} rutin and substrate mixture -A_{40} rutin phytosome alone (background)

ISSN PRINT 2319 1775 Online 2320 7876
 UGC CARE Listed (Group -I) Journal Volume 11, Spl Iss 4,Dec 2022

The activity in controls (with a-glucosidase but without inhibitor) was considered to be 100%. Concentrations of polyherbal combinations resulting in 50% inhibition of enzyme activity (IC_{50} values) were determined graphically. Different plant species were compared on the basis of their IC_{50} values estimated from the dose response curves.

Experimental animals:

Wistar strain albino rats weighing between 195 ±15 g were obtained from the Serum Institute of India, Pune. The rats were housed in clean metallic cages and kept in a well ventilated room and allowed to acclimatize to the laboratory condition for one week before being used. They were fed with standard animal pellet and had free access to. water ad libitum, the animals were distributed randomly into six groups of six animals catch for antidiabetic study using streptozotocin-induced diabetic experiment. The otocol of the experiment (PRCOP/IAEC/2011-12/11) was approved by Institutional animal Ethics Committee (IAEC) of Pravara Rural College of Pharmacy, Loni anda were conducted in accordance with guidelines as per "Guide for the care and use of laboratory animal" and with permission from Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA).

Acute Toxicity Study:

Figure 4: Acute toxicity studies on rat

Procedure:

Healthy male Wistar rats, starved overnight (12 h), were divided into 42 groups of 6 each and were orally fed with increasing doses (50, 100, 250, 500, 1000, and 2500 me/kg) of combinations III, IV.V, VI and VII to determine the safe doses by up and down staircase method. The animals were observed continuously for one hour, then frequently for 4 hrs., and later at the end of 24 hrs. After administration of the drug, Irwin test was conducted, where the animals were observed for behavioral changes. Further, animals were observed daily for 30 days, and mortality was recorded (Ghosh et al., 1984). To know multiple dose toxicity of combinations, highest dose was fed once daily for 15 days and observed for incidences of mortality for a period of 30 days.

Pharmacological activity of herbal formulation:

A. In-vivo antidiabetic activity of herbal formulation in streptozocin induced diabetes-

Induction of diabetes mellitus

Diabetes was induced by a single intraperitoneal injection of freshly prepared streptozotocin (35 mg/kg, BW) in 0.1M citrate buffer (PH 4.5) to overnight fasted rats. The development of diabetes was confirmed after 48 hours of STZ injection, the animals with fasting blood glucose level more than 200 mg/dl were selected for the experimentation.

Experimental design

The Streptozocin-induced diabetic Wistar rats were randomly divided into six groups (n=6) as follows.

Group 1- Received normal saline p.o.

ISSN PRINT 2319 1775 Online 2320 7876
Research paper UGC CARE Listed (Group -I) Journal Volume 11, Spl Iss 4,Dec 2022

Group 2- Received Metformin (250 mg/kg, p.o.).
Group 3- Received plant extract. (250mg/kg, p.o.)
Group 4- Received standard rutin (50mg/kg, p.o.).
Group 5- Received standard rutin (100mg/kg, p.o.).
Group 6- Received rutin -phytosome (10mg/kg p.o.).
Group7- Received rutin-phytosome (25mg/kg p.o.).

Determination of blood glucose levels:
Blood samples were collected by cutting the tail-tip of the rats, for blood glucose determination at intervals of 0, 5, 10, 15, and 20 days. Determination of the blood glucose level was done by the glucose-oxidase principle using the ONE TOUCH Basic (Horizon) instrument and results were reported as mg/dl[15].

Body weight monitoring
Body weight was estimated on 0, 5, 10, and 20st day of experiment.

Biochemical estimation:
Blood glucose level (BGL), total cholesterol (TC), high density lipoprotein (HDL)-cholesterol, triglycerides (TG)[16,17,18] were estimated using standard kits of Bayers diagnostic Pvt. Ltd., India. Low density lipoprotein (LDL)-cholesterol was calculated he measurement by Friedwald formula. Glycosylated omoelobin was estimated using Excel diagnostics Pvt. Ltd., India. Body weight was determined gravimetrically.

Histopathology
On 20 days the animals were sacrificed, the pancreas of one animal from each group, was excised and stored in 10% formalin after washing with normal saline. Histopathological parameters were studied at Omega laboratory, Loni and, Satara, India. The tissue was washed, dehydrated with alcohol, cleared with xylene and paraffin blocks were made. Serial sections of 5 um thickness were cut using a rotary microtome. The sections were then deparaffinised with xylene and hydrated in descending grades of alcohol. The slides were then transferred to haematoxylin for 10 min, followed by rinsing with water. These were examined and later counterstained with esion, rinsed with water, dehydrated with ascending grades of alcohol, cleared with xylene and mounted.

Statistical analysis
The data was analyzed by ANOVA followed by Tukey-Kramar multiple comparison test. $p<0.05$ was considered statistically significant.

ISSN PRINT 2319 1775 Online 2320 7876
 UGC CARE Listed (Group -I) Journal Volume 11, Spl Iss 4,Dec 2022

3. RESULT AND DISCUSSION:

Thin Layer Chromatography

Thin layer chromatography technique was carried out for separation, isolation, and identification of constituents present in the aqueous extract of M. pudica leaves. The Rf value for steroidal compound from M. pudica was 0.47. The R; values for flavonoid compound from M. pudica was 0.38. The Rf values for tannins from M. pudica was 0.35. And for rutin the Ry value for M. pudica was 0.38.

Table 2: TLC of extract of M.pudica

Sr.no.	Chemical constituent	Mobile Phase	Visualization method	Color of spot	R_f
1	Steroids	Ethyl acetate: methanol: acetic acid (70:20:10)	Vanilin-Sulfuric acid.	Pink	0.47
2	Tannins	Acetic acid: ethanol: Hexane: Formic acid (1:3:5:1)	10% FeC13 solution	Bluish black	0.35
3	Flavonoid	Toluene: ethyl acetate (9:1)	Anisaldehyde-Sulfuric acid	Yellowish Green	0.38
4	Rutin	Ethyl acetate: formic acid :glacial acetic acid: water. (100:11:11:26)	Under VU 254 nm	Dark spot	0.38

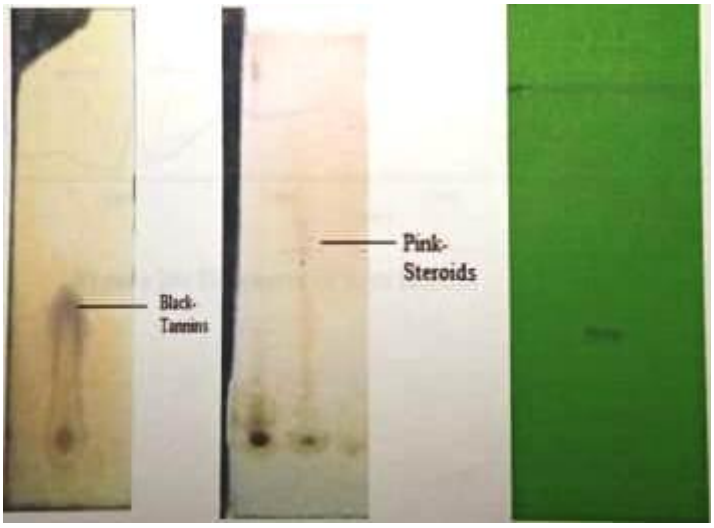


Figure 5: TLC for tannins. TLC of steroids. TLC of rutin

Preformulation Studies:

Compatibility Studies:

The application of infra-red spectroscopy lies more in the qualitative identification of substances either in pure form or in the mixtures and as a tool in establishment of the structure. Drug excipient compatibility studies were carried out prior to the preparation of formulation, to check for any compatibility related problems between drug and excipients used in the formulations. As no new bands or shift in characteristic peaks appeared, it indicates compatibility between drug and exeipients. IR spectra of all components in formulation are shown below:

ISSN PRINT 2319 1775 Online 2320 7876
Research paper UGC CARE Listed (Group -I) Journal Volume 11, Spl Iss 4,Dec 2022

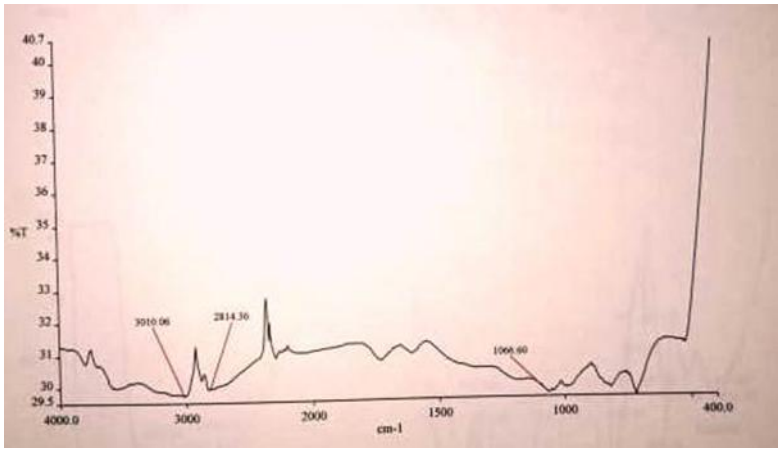


Figure 6: IR spectra of soya lecithin

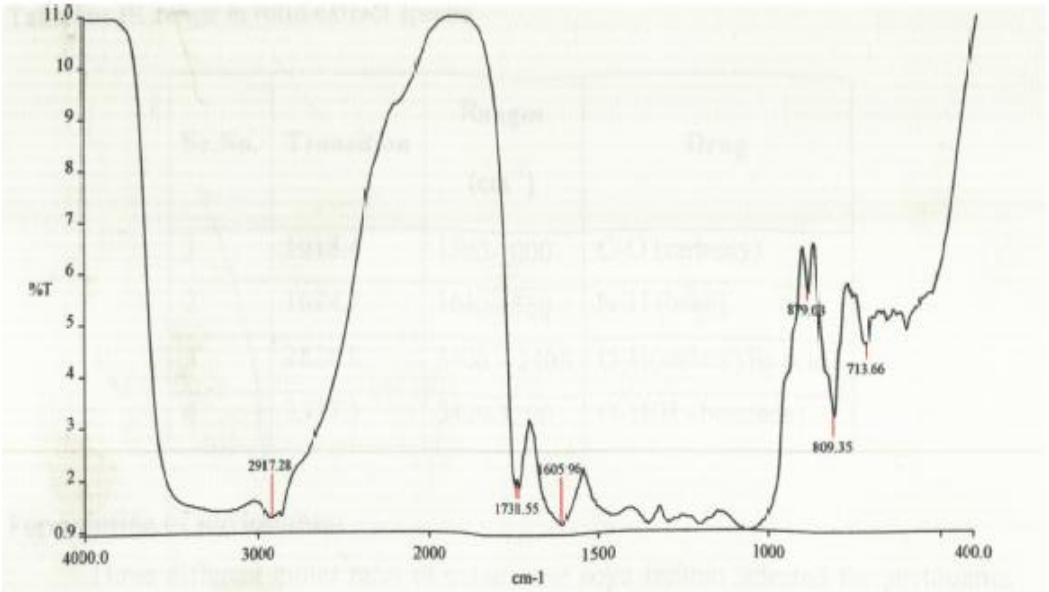


Figure21: IR spectra of rutin

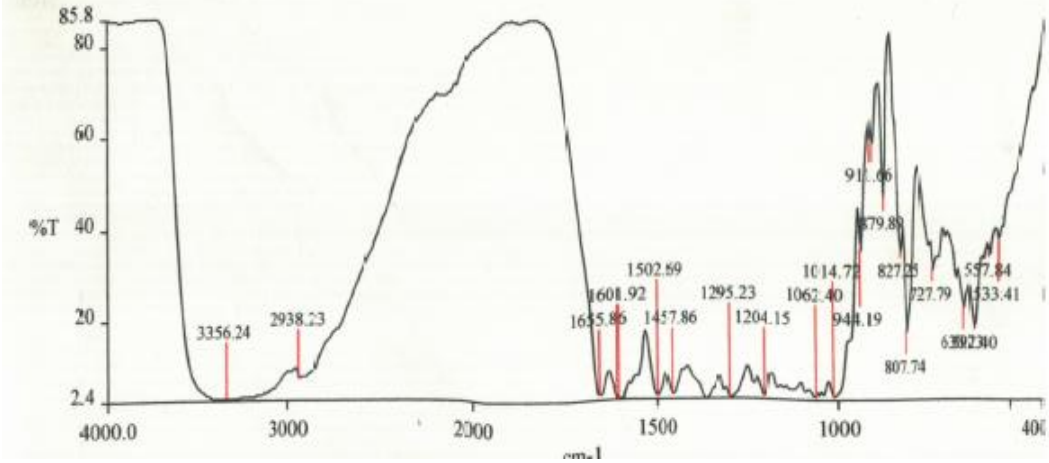


Figure 7 IR spectra of physical mixture of rution and soya lecithin

ISSN PRINT 2319 1775 Online 2320 7876
Research paper UGC CARE Listed (Group -I) Journal Volume 11, Spl Iss 4,Dec 2022

Table 3: IR range in rutin extract spectra

Sr.No.	Transition	Ranges (cm^{-1})	Drug
1	1018.4	1300-1000	C-O (carboxy)
2	1624.7	1640-1550	N-H(band)
3	28282.8	3400-2400	O-H(carboxylic acid)
4.	3377.5	3400-3200	O-H(H - bondede)

Formulation of phytosomes:

Three different molar ratio of extract and soya lecithin selected for phytosomes formulation in 1:0.8, 1:1, 1:1.2 proportion.

The standarde ratio of extract to soya lecithin given for the phytosomes formulation was 1:1. From the entraptment efficiency, the best formulation selected from the three different ratios. Entraptment efficiency for the formuation is given in following table.

Evaluation of formulation

I. Determination of surface morphology

Phytosome was examined by scanning electron microscope (SEM,1170 ESI) to observe the morphological changes occurred due to the formulation variation

Figure 8:SEM of rutin

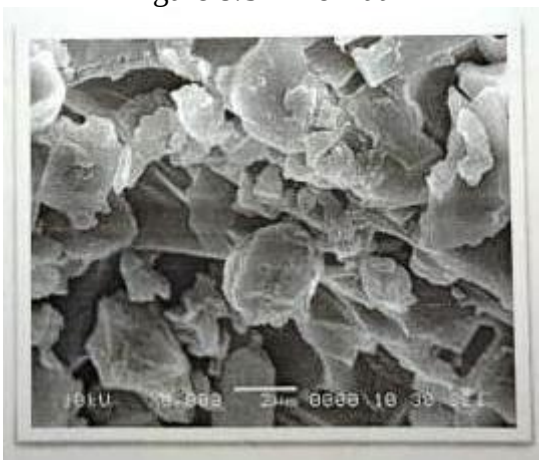


Figure 9:SEM of rutin phytosome (rutin – phospholipid complex)

From above figure23 it clearly shows crystalline nature of drug and figure 24 shows that after formulation it shows amorphous nature. This indicates complex formation between drug and phospholipid (phytosome formulation).

ISSN PRINT 2319 1775 Online 2320 7876
 UGC CARE Listed (Group -I) Journal Volume 11, Spl Iss 4,Dec 2022

II. Entrapment efficiency:

Table 4: Entrapment efficiency of various formulations.

Sr.No.	Formulation No.	Ratio of extract and soya lecithin	Entrapment efficiency (w/w)
1	F1	1:0.8	90.72
2	F2	1:1	94.55
3	F3	1:1.2	94.88

The best result was obtained for 1:1.2 ratio.

III. Solubility study:

Apparent solubility studies revealed that rutin was not soluble in n-Octanol and chloroform while rutin phospholipid complex(rutin-phytosome) was indicating that a reaction occurred between the rutin and phosphatidylcholine(soya lecithin) resulting in producing a lipophilic envelope, which allows the complexed rutin to dissolve in low polarity solvents like n-Octanol, chloroform can be from table 19 and 20.

Table 5: Calibration curve of rutin

Conc (µg/mL)	Absorbance
20	0.162
40	0.261
60	0.431
80	0.587
100	0.686

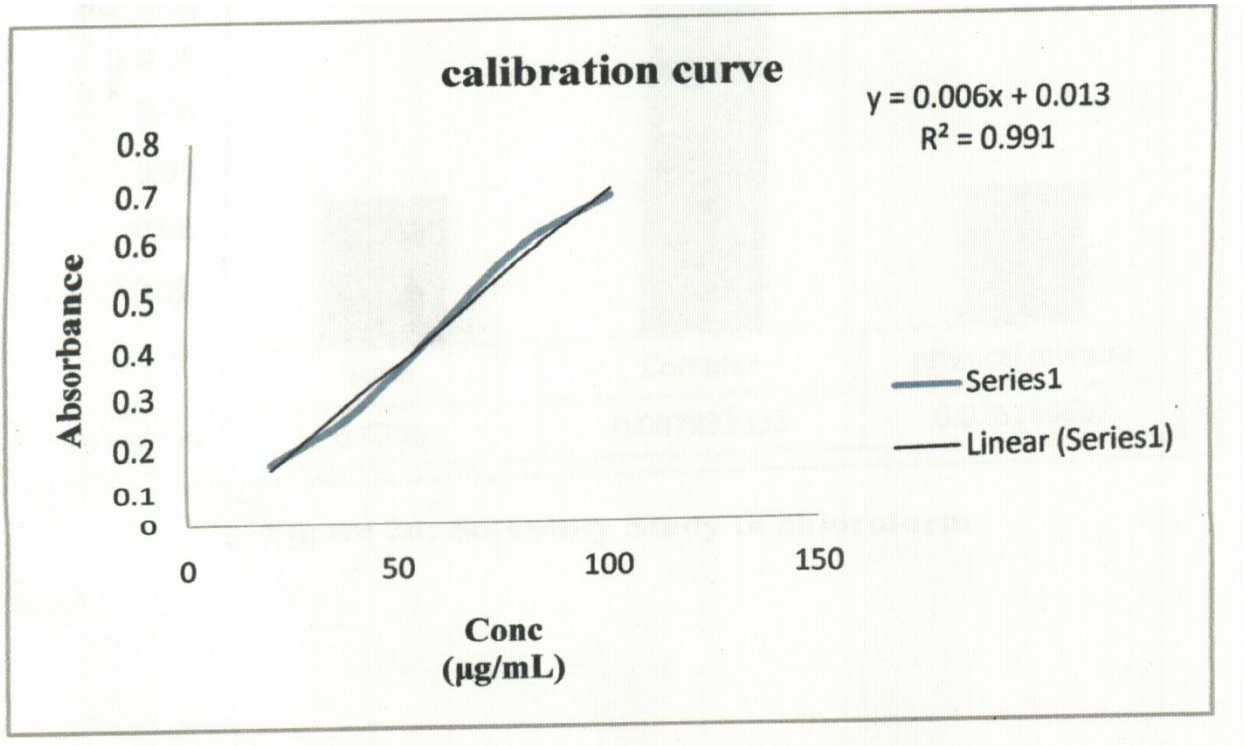


Figure 10: Calibration curve of rutin in methanol

ISSN PRINT 2319 1775 Online 2320 7876
 UGC CARE Listed (Group -I) Journal Volume 11, Spl Iss 4,Dec 2022

Table 6: solubility of rutin, complex & physical mixture in chloroform

Time (hr.)	Abs	Slop	Intercept	Ug/ml	Mg/ml
Rutin	0.166	0.006	0.013	25.5	0.0255
Complex	0.54	0.006	0.013	87.83333	0.087833
Physical Mixture	0.17	0.006	0.013	26.16667	0.026167

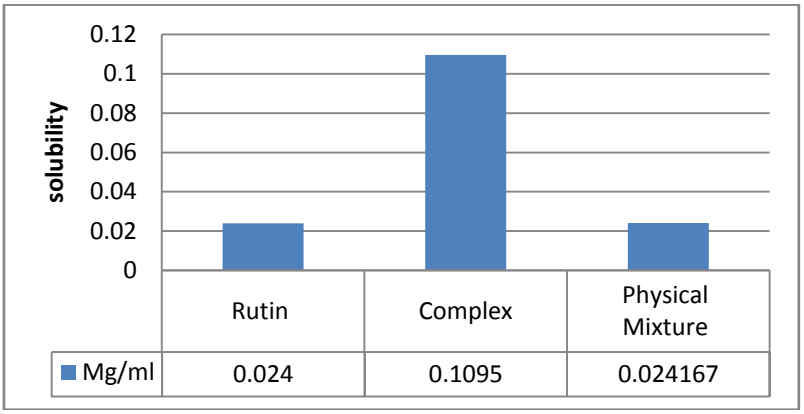


Figure 11: Solubility Study in chloroform

Table 7: Solubility of rutin, complex & physical mixture in n Octanol

Time (hr.)	Abs	Slop	Intercept	Ug/ml	Mg/ml
Rutin	0.157	0.006	0.013	24	0.024
Complex	0.67	0.006	0.013	109.5	0.1095
Physical Mixture	0.158	0.006	0.013	24.16667	0.024167

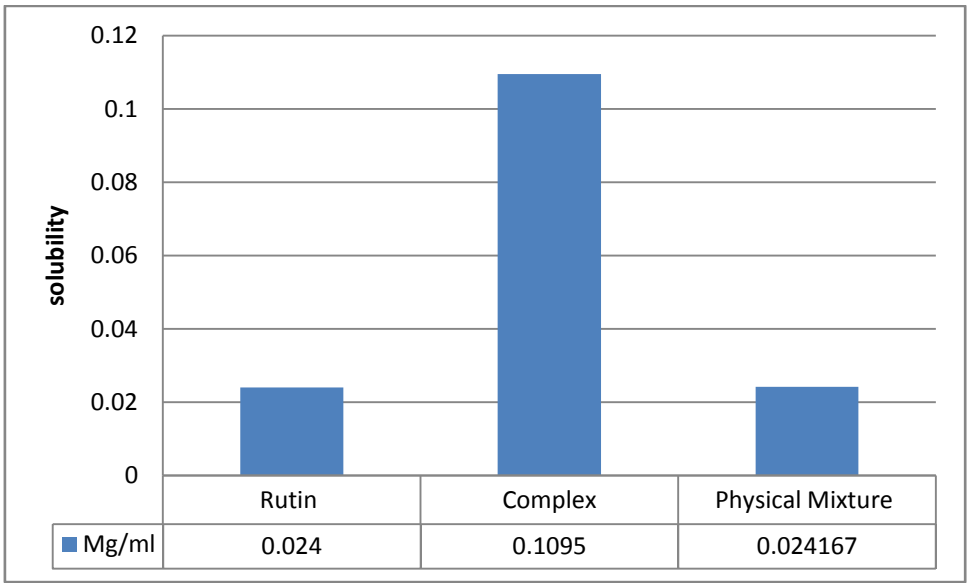


Figure 12: Solubility Study in n-Octanol

IV. In vitro drug release study:

Table 8: In vitro dissolution study in 7.4 phosphate buffer of rutin- phytosome.

Time	Abs	slope	intercept	ug/ml	ug/ml *5	ug/50ml	mg/ml	Release
0	0	0.006	0.013	0	0	0	0	0
15	0.035	0.006	0.013	3.666667	18.33333	916.6667	0.916667	5.522088
30	0.053	0.006	0.013	6.666667	33.33333	1666.667	1.666667	10.04016

ISSN PRINT 2319 1775 Online 2320 7876
Research paper UGC CARE Listed (Group -I) Journal Volume 11, Spl Iss 4,Dec 2022

45	0.078	0.006	0.013	10.83333	54.16667	2708.333	2.708333	16.31526
60	0.081	0.006	0.013	11.33333	56.66667	2833.333	2.833333	17.06827
75	0.103	0.006	0.0013	15	75	3750	3.75	22.59036
90	0.112	0.006	0.013	16.5	82.5	4125	4.125	24.8494
105	0.129	0.006	0.013	19.33333	96.66667	4833.333	4.833333	29.11647
120	0.149	0.006	0.013	22.66667	113.3333	5666.667	5.666667	34.13655
135	0.162	000.6	0.013	24.83333	124.1667	6208.333	6.208333	37.3996
150	0.179	0.006	0.013	27.66667	138.3333	6916.667	6.916667	41.66667
165	0.208	0.006	0.013	32.5	162.5	8125	8.125	48.94578
180	0.212	0.006	0.013	33.16667	165.8333	8291.667	8.291667	49.9498
195	0.242	0.006	0.013	38.16667	190.8333	9541.667	9.541667	57.47992
210	0.287	0.006	0.013	45.66667	228.3333	11416.67	11.41667	68.7751
225	0.307	0.006	0.013	49	245	12250	12.25	73.79518
240	0.321	0.006	0.013	51.33333	256.6667	12833.33	12.83333	77.30924
255	0.338	0.006	0.013	54.16667	270.8333	13541.67	13.54167	81.57631
270	0.364	0.006	0.013	58.5	292.5	14625	14.625	88.10241
285	0.376	0.006	0.013	60.5	302.5	15125	15.125	91.11446
300	0.387	0.006	0.013	62.33333	311.6667	15583.33	15.58333	93.8755

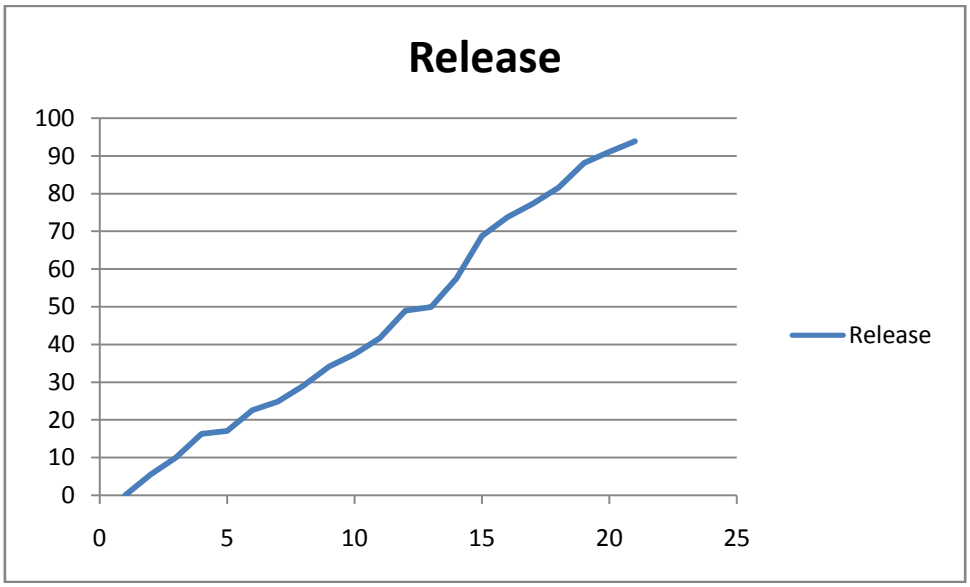
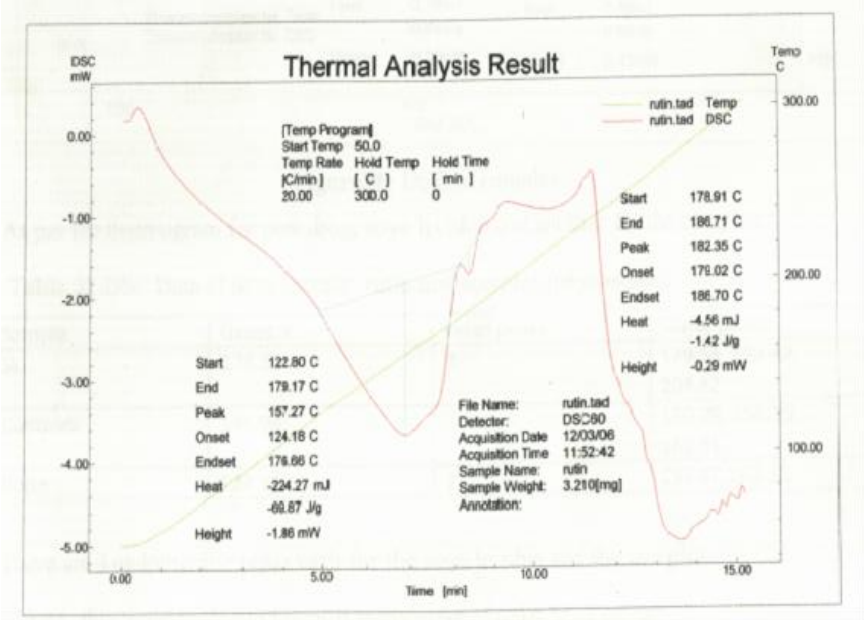


Figure 13: Graph of in vitro dissolution study in 7.4 phosphate buffer ofrutin-phytosome.

In vitro dissolution study clearly indicate that the phytosome showed extended release dissolution pattern. The phytosome showed release at the end of 5 hr. 93.87 % release. The dissolution study proved that phytosome showed extended release which not given by any conventional formulation.

ISSN PRINT 2319 1775 Online 2320 7876
Research paper UGC CARE Listed (Group -I) Journal Volume 11, Spl Iss 4,Dec 2022



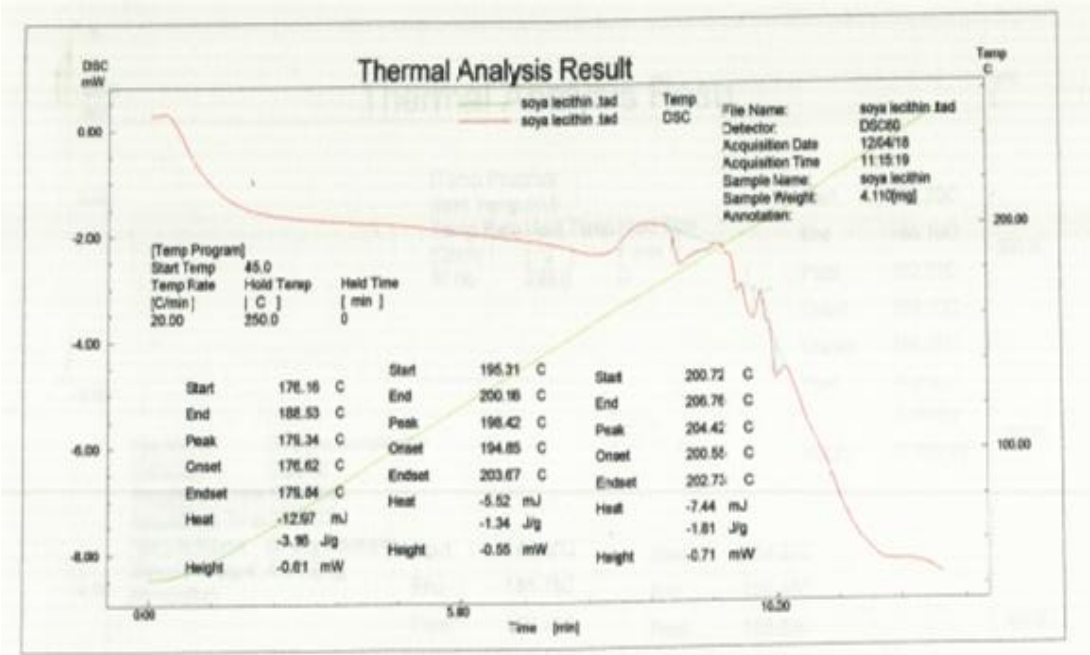
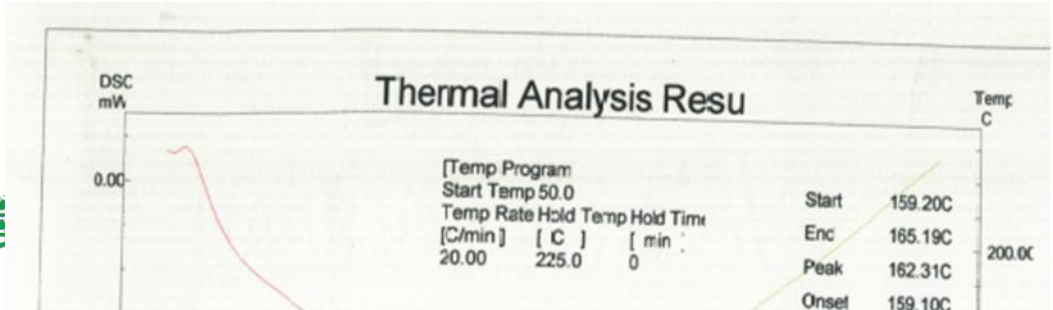


Figure 29: DSC of soya lecithin



ISSN PRINT 2319 1775 Online 2320 7876
 UGC CARE Listed (Group -I) Journal Volume 11, Spl Iss 4,Dec 2022

Figure 14: DSC of complex.

V. Differential Scanning Calorimetry

DSC is a fast and reliable method to screen drug- excipients compatibility and provides distinct information about the possible interactions. In DSC, an interaction is concluded by elimination of endothermic peaks, appearance of new peaks, change in peak shape and its onset, peak temperature, melting point and relative peak area or enthalpy. Figure 29, 30 and 31shows the DSC thermograms of a) pure rutin, b) pure soya lecithin, c) and RPC. Release. As per the thermogram for pure drug, soya lecithin and another for the complex.

Table 9:DSC Data of soya lecithin, rutin and complex (phytosome).

Sample	Onset°C	No of peaks	Major peaks
SL	176.62	3	179.34, 198.42, 204.42
Complex	146.94	3	150.29, 156.59, 162.31
Rutin	124.18	2	157.27,182.35

There are 3 endothermic peaks each for the soya lecithin and the complex.
179.34- this could be due to hot melt movement of polar head group.
198.42- may be as a result of phase transition from gel to liquid crystalline. Non polar hydrocarbon tail of SL might have melted at this point.
204.42- Third peak resulted perhaps due to melt occurred in 2 earlier phases.
From above table the peaks the complex has deviated from that of the pure drug, which suggests formation of complex. Also thermogram of complex when compared to SL reveals association of SL with the drug.

VI. X-Ray Diffraction Studies

It has been shown that polymorphic changes of the drug are important factors, which may affect the dissolution rate and bioavailability. It is therefore important to study the polymorphic changes of the drug.
X-ray diffraction pattern in Figure no 15reveals that soya lecithin was clearly in amorphous state.

ISSN PRINT 2319 1775 Online 2320 7876
Research paper UGC CARE Listed (Group -I) Journal Volume 11, Spl Iss 4,Dec 2022

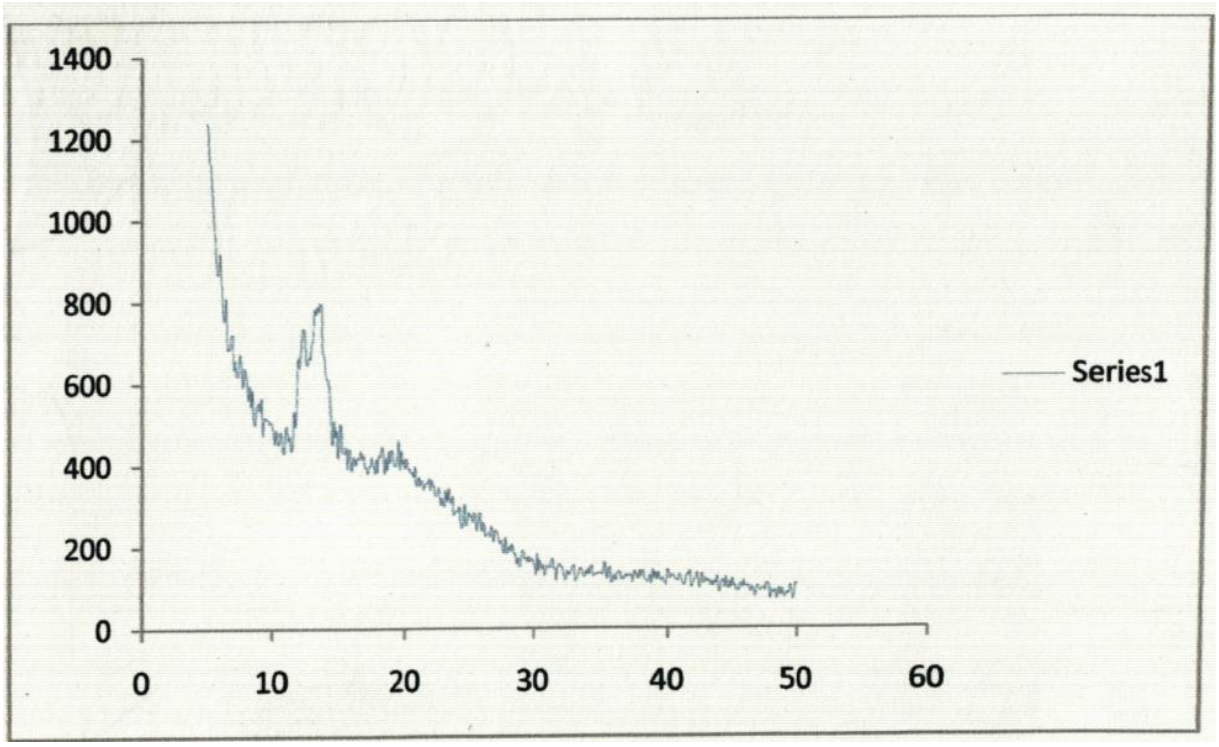


Figure 15: X-ray diffractogram of soya lecithin

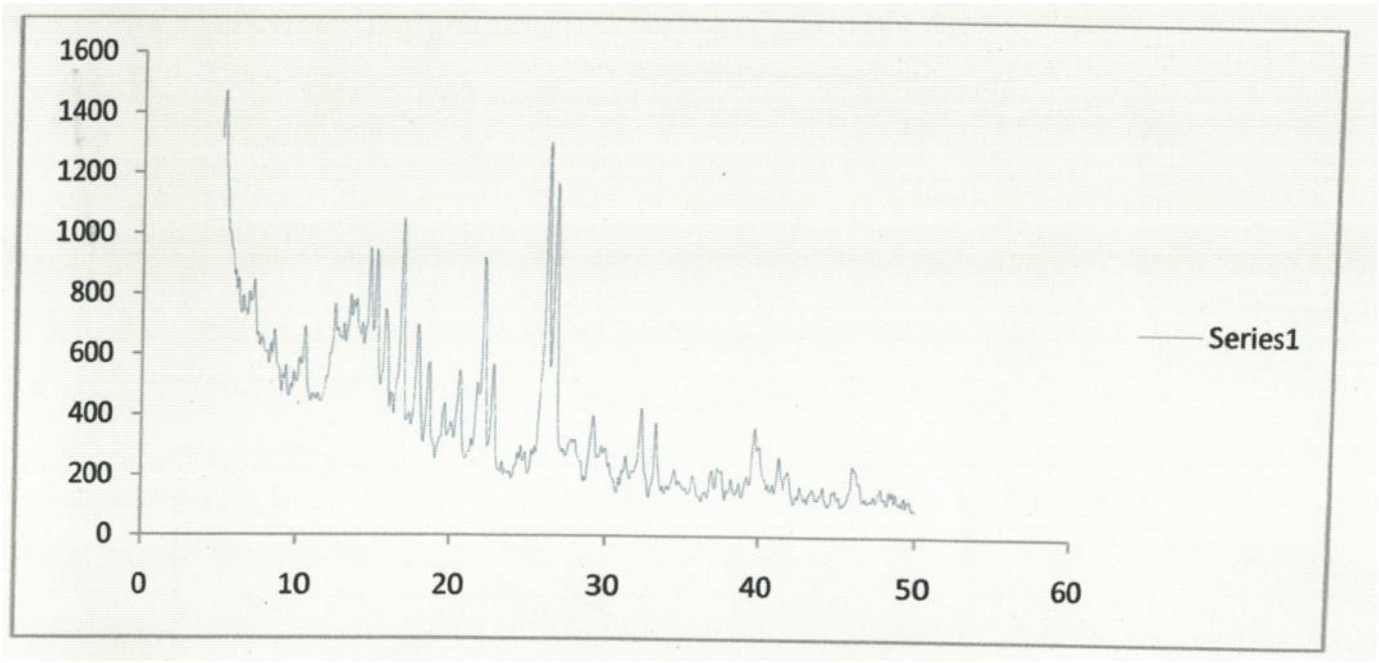


Figure 16: X-ray diffractogram of rutin

x-raydiffraction pattern in figure no 33 reveals that rutin was clearly in crystalline state. The crystalline nature of the drug was demonstrated by the characteristic XRD pattern with peaks appearing at 14.5, 14.9, 15.7, 16.8, 17.8, 18.6, 20.6, 22.1, 22.8, 26.3, 26.8, 29.2, 32.2 and 33.1 θ values

ISSN PRINT 2319 1775 Online 2320 7876
Research paper UGC CARE Listed (Group -I) Journal Volume 11, Spl Iss 4,Dec 2022

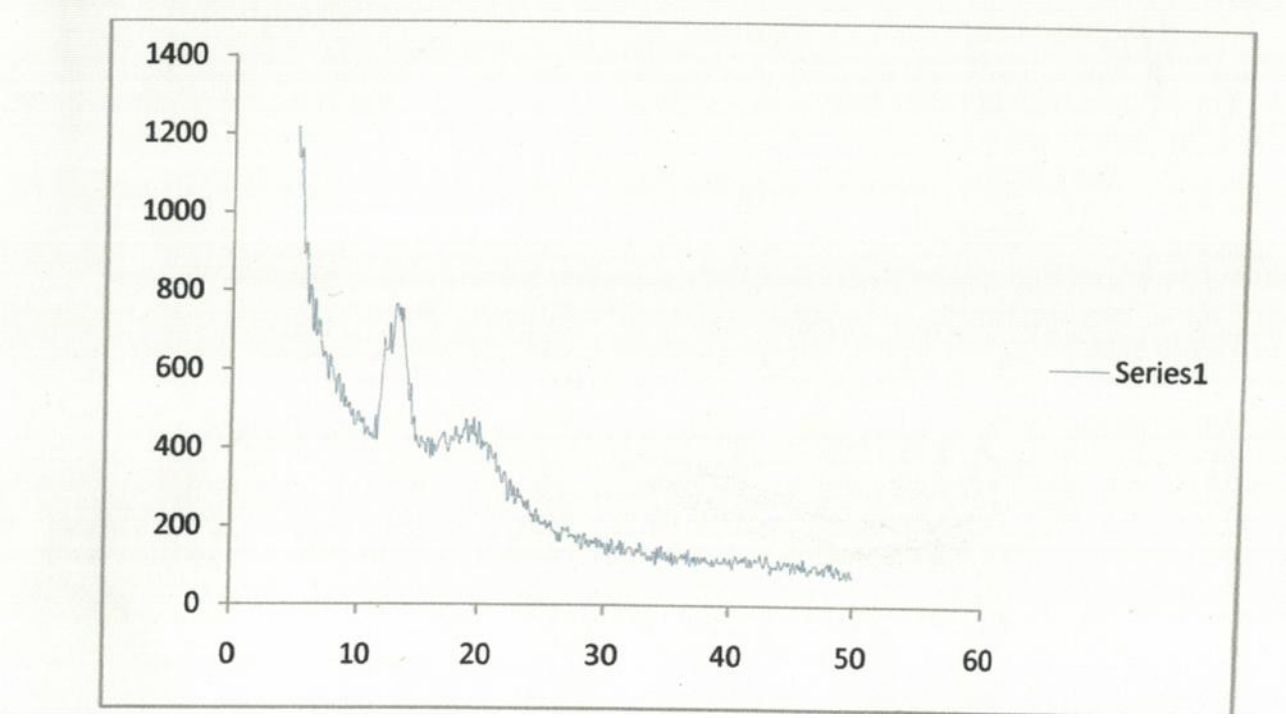


Figure no 17: X-ray demonstration of complex

Figure no 34 X-ray demonstration of that the loss of crystallinity was due to complex formation.

Such absence of rutin constructive reflections (specific peaks) in the complex X-ray diffractogram indicates that drug has almost entirely converted from crystalline to amorphous or complexed form. such lack of crystallinity in the complex system was understood to be as a result of complex between rutin and soya lecithin

This amorphization or complex of rutin in the phytosome system may contribute to the consequent improvement in the apparent solubility and therefore the dissolution rate of rutin.

Pharmacological evaluation of the dosage form using various in-vivo and in-vitro screening methods.

Phytosome formulation was prepared and evaluated for their pharmacological action in-vivo and in-vitro. Results found to be significant as compare to standard In-vitro alpha-amylase activity

Table 10: Absorbance for rutin, rutin-extract & phytosome of rutin on alpha-amylase inhibitory effects.

Concentration µg/ml	Absorbance				
	Acarabose	Rutin extract	Rutin	Rutin phytosome	Control
10	0.0600	0.756	0.661	0.625	1.125
20	0.585	0.686	0.638	0.552	
40	0.548	0.656	0.571	0.514	
60	0.497	0.635	0.511	0.500	
80	0.445	0.610	0.479	0.489	
100	0.385	0.581	0.447	0.480	

ISSN PRINT 2319 1775 Online 2320 7876
 UGC CARE Listed (Group -I) Journal Volume 11, Spl Iss 4,Dec 2022

Table 11: %inhibition of alpha amylase by rutin, rutin-extract & phytosome of rutin

Concentration µg/ml	%inhibition			
	Acarabose	Rutin extract	Rutin	Rutin phytosome
10	46.66	32.8	41.24	44.44
20	48	39.02	43.28	50.93
40	51	41.55	49	54.31
60	55.82	43.55	54.57	55.55
80	60.44	45.77	57.42	56.53
100	65.67	48.35	60.8	57.33

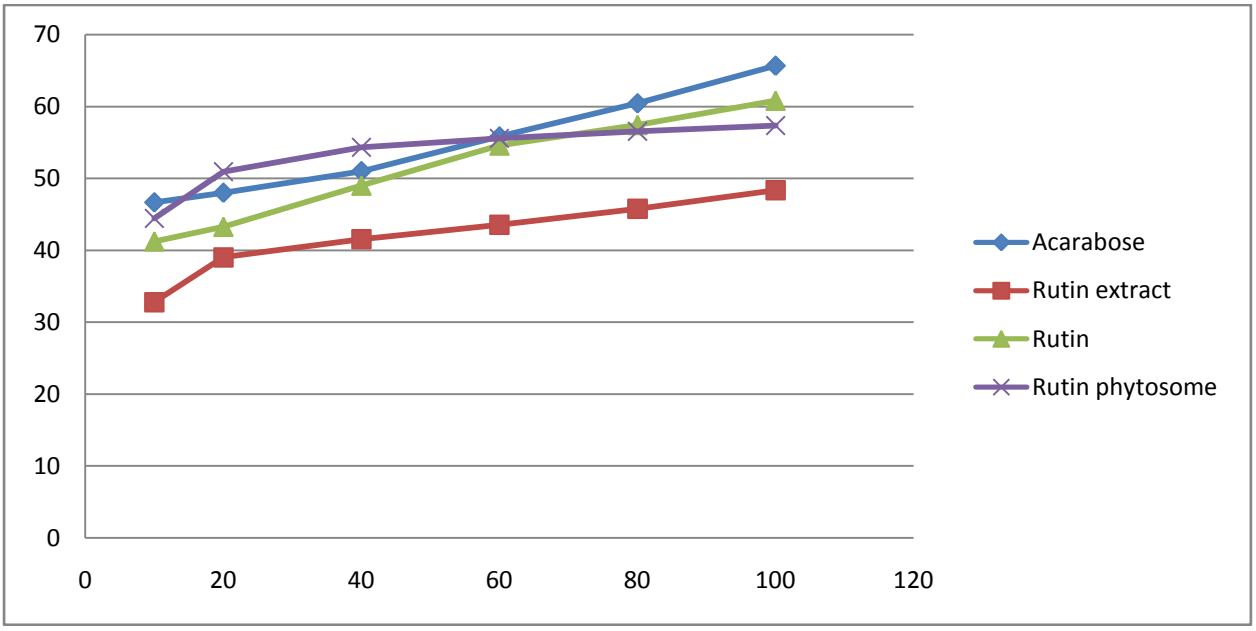


Figure 18: %inhibition of alpha amylase by rutin, rutin-extract &phytosome of rutin

Table 12: IC_{50} Value of rutin, rutin-extract & phytosome of rutin

Group	IC_{50} (µg/ml)
Acarabose	40±4.01
Rutin extract	110±2.323
Rutin	41.1±2.32
Rutin Phytosome	20.67±3

ISSN PRINT 2319 1775 Online 2320 7876
 UGC CARE Listed (Group -I) Journal Volume 11, Spl Iss 4,Dec 2022

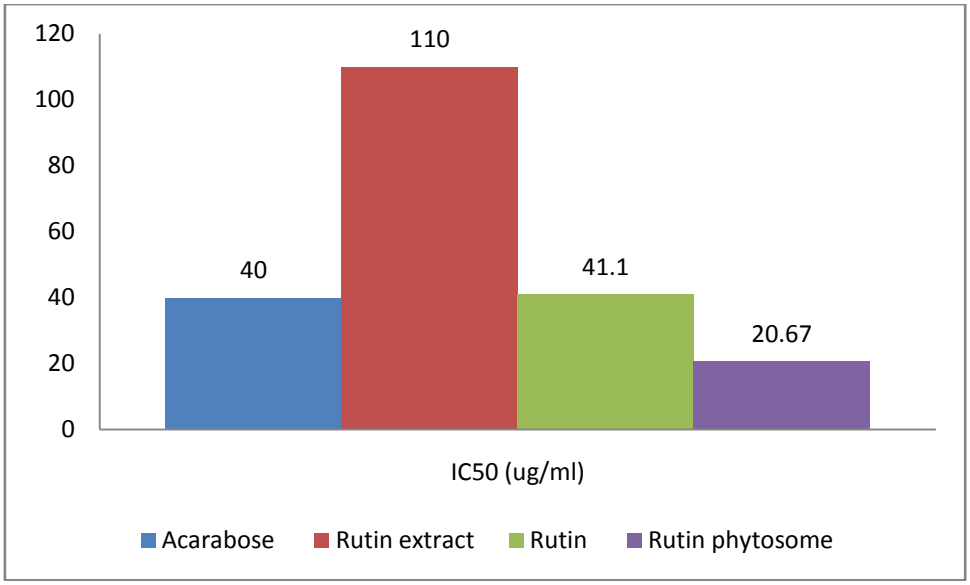


Figure 19: IC_{50} Value of rutin, rutin-extract & phytosome for alpha-amylase inhibitory effects

A. In-vitro a-glucosidase activity

Table 13: Absorbance of rutin extract, rutin& rutin- phytosome for alpha-glucosidase inhibitory effects,

Concentration (µg/ml)	Absorbance				
	Acarabose	Rutin	Rutin extract	Rutin phytosome	Control
50	0.485	0.671	0.515	0.505	1.138
100	0.278	0.531	0.354	0.328	
150	0.105	0.447	0.245	0.175	

Table 14: % inhibition of alpha-glucosidase by rutin, rutin -extract & phytosome of rutin

Concentration (µg/ml)	% Inhibition			
	Acarabose	Rutin	Rutin extract	phytosome
50	57.39	41.04	54.75	55.63
100	75.58	53.34	68.9	71.18
150	90.78	60.73	78.48	84.63

ISSN PRINT 2319 1775 Online 2320 7876
Research paper UGC CARE Listed (Group -I) Journal Volume 11, Spl Iss 4,Dec 2022

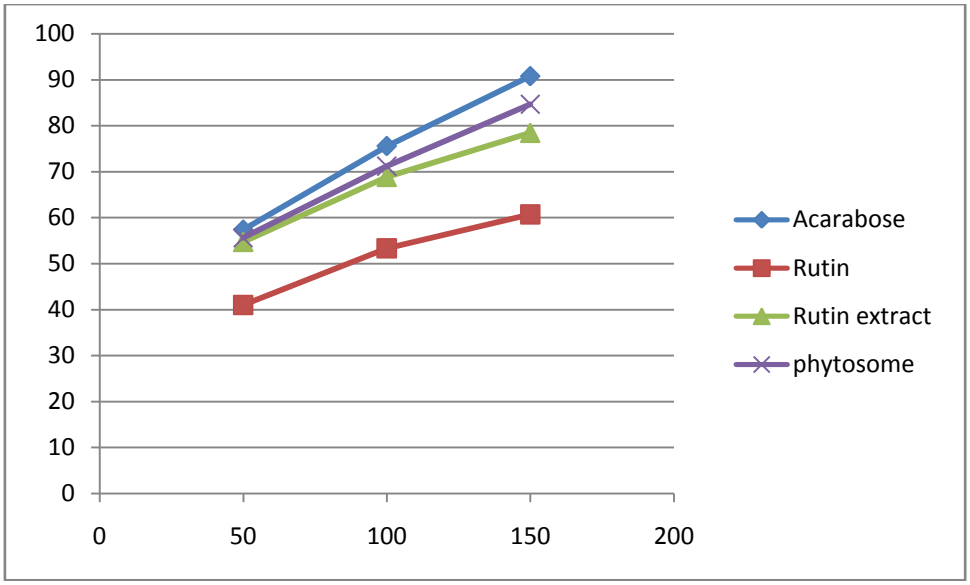


Figure 20: % inhibition of alpha-glucosidase by various polyherbal formulations.

Table 15: IC50 value of rutin, rutin -extract & phytosome for alpha-glucosidase inhibitory effects

Group	IC50 (µg/ml)
Acarabose	40±4.01
Rutin extract	90±3
Rutin	45±4.45
Phytosome	46±4.2

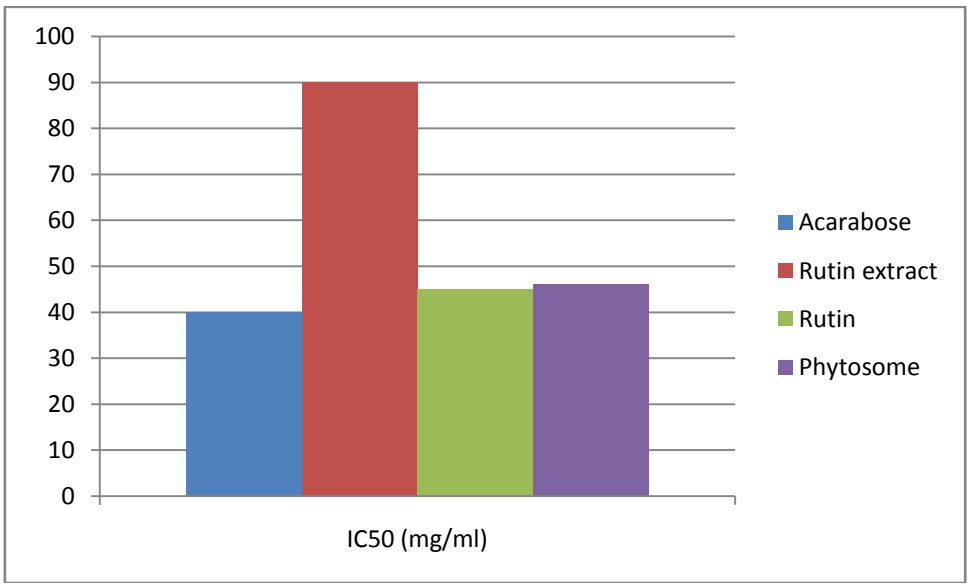


Figure 21: IC_{50} value of rutin, rutin extract and rutin-phytosome for alpha-glucosidase inhibitory effects

In-vivo streptozocin-induced diabetes in Wistar rats

I. Estimation of blood glucose level

ISSN PRINT 2319 1775 Online 2320 7876
 UGC CARE Listed (Group -I) Journal Volume 11, Spl Iss 4,Dec 2022

Table 16: Effect of various herbal combinations on streptozocin-induced diabetic Wistar rats.

Gr. No.	Group	Blood glucose level (mg/dL)				
		0^{th} day	5^{th} day	10^{th} Day	15^{th} day	20^{th} day
1	Normal control	108.89±3.80	111.64±2.8	111.80±3.5	114±2.16	109±1.2
II	Disease control (STZ)	574±6.73	615±5.0	438±8.2	571±11	555±10
III	Metformin	543±40.8	456±41.3	393*±15.1	238*±3.71	115*±10
IV	Rutin extract	556±15.2	362*±15.4	250*± 17.63	237* ±15.17	215*+14.78
V	Rutin 50 mg	456±18.2	444±17.6	241±11.28	198±11.58	140*±14.89
VI	Rutin 100mg	511±17.3	495±14.2	245+17.3	225*±17.3	125*±17.09
VII	Rutin phytosome10mg	515*+ 40.3	473*±15.4	413*±15.17	389*±11.58	225±10*
VIII	Rutin phytosome 25 mg	515*±40.8	498*±14.2	383*±14.78	277*±11.58	115*±10

Values are given as mean ± SEM for 6 rats in each group; experimental groups are compared with diabetic control. Values are statistically significant at *=P<0.05.

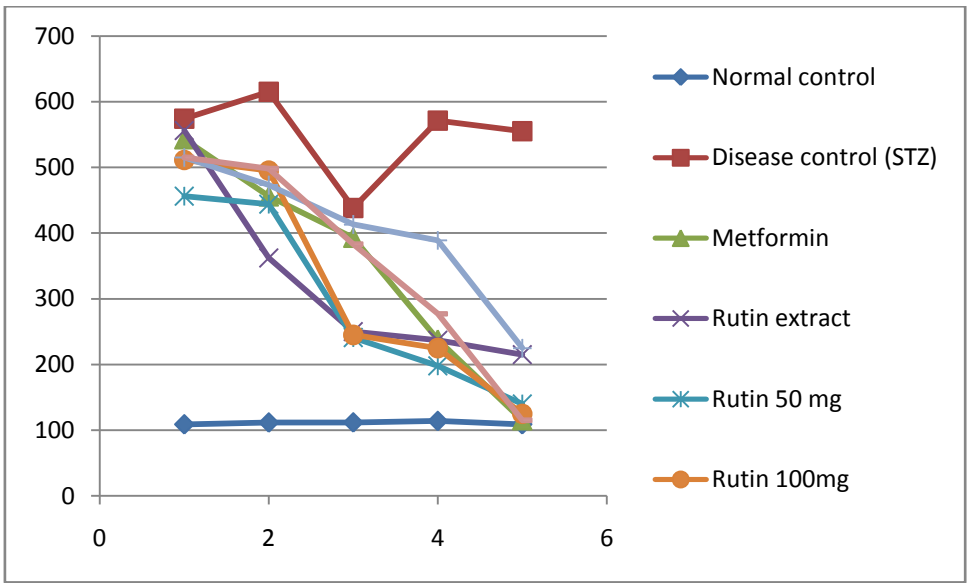


Figure 22: Effect of herbal combinations on streptozocin-induced diabetic Wistar rats.

II. Body weight monitoring

Table 17: Effect of various herbal combinations on body weight of streptozocin- induced diabetic Wistar rats.

Gr. No.	Group	Initial Body Weight	Weight (gm)				
			0^{th} day	5^{th} day	10^{th} day	15^{th} day	20^{th} day
I	Normal control	208±8.6	206±11.	204±6.8	210±5.3	215±	210±7.2

ISSN PRINT 2319 1775 Online 2320 7876
 UGC CARE Listed (Group -I) Journal Volume 11, Spl Iss 4,Dec 2022

			2		6	8.12	3
II	Disease control (STZ)	242+7.6	156+5.0	162±6.2	178±5.1	169±7.1	189±5.9 *
III	Metformin	210±5.5	164±5.9	168±5.1	172+5.4	180±2.63	201±8.2 *
VI	Rutin extract	234+6.1	165±4.4	171±8.1	179±9.0	198±11.4 *	213±9.1 *
V	Rutin 50 mg	242±4.6	162+8.7	170±8.1	185±2.9	191±5.1*	208±10. 8 *
VI	Rutin 100mg	234+13. 7	158±7.6	165±7.1	195±1.9	200±6.1	212±9.1
VII	Rutin phytosome10m g	244±10. 1	163±4.4 *	175+1.9 *	195±1.9 *	212+6.1*	222+9.1 *
VII I	Rutin Phytosome 25 mg	210±13. 7	154±5.0 *	163±7.1 *	179±6.1 *	185+6.1*	190±10*

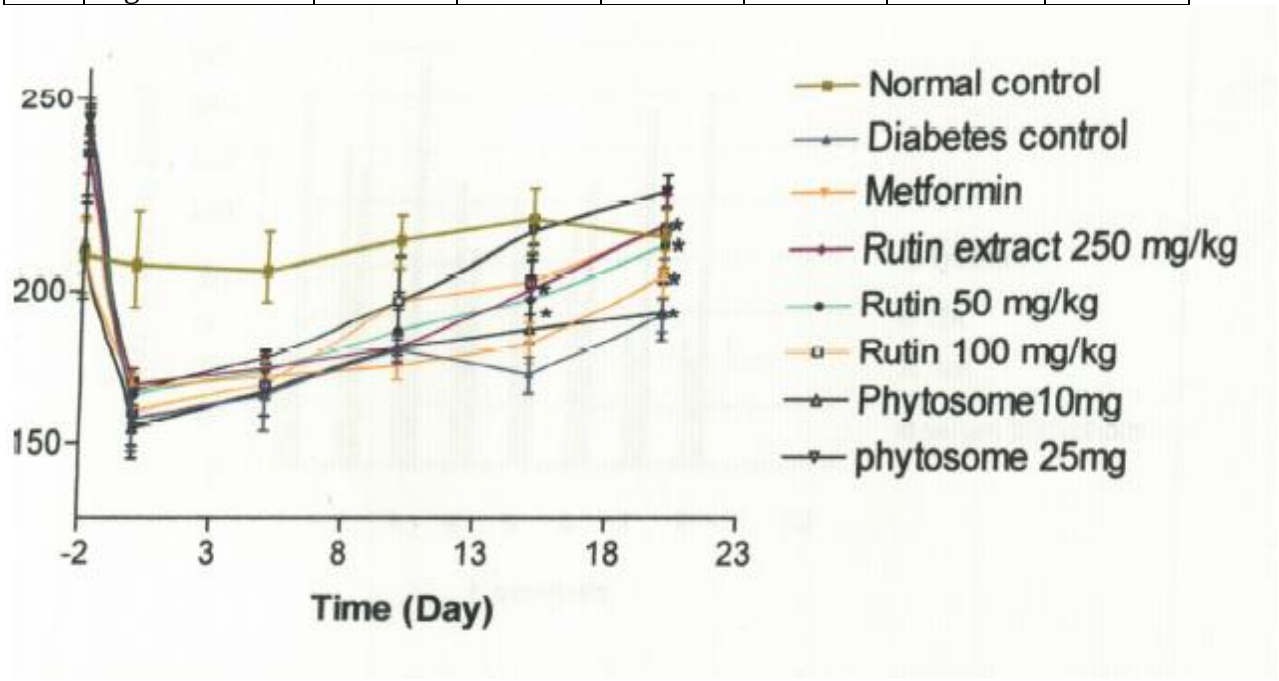


Figure 23: Effect of various herbal combinations on body weight of streptozocin- induced diabetic Wistar rats.

Estimation of serum lipid profiles and glycosylated haemoglobin in STZ induced diabetic rats.

Table 18: Effect of various herbal combinations on LDL, HDL, and serum triglyceride.

Treatment	LDL(mg/dl)	HDL(mg/dl)	Serum Triglycerides (mg/dl)
Control	49.52±5.0	43.99 ±2.6	145.0±3.5
Diabetic Control (ZIS)	121.1± 5.3	72±1.9	115.5 ±39
Metformin	54.54±5.2*	34.45±3.0*	153.4±2.9*
Rutin extract	107±0.6*	68±0.2*	85±0.4*

ISSN PRINT 2319 1775 Online 2320 7876
 UGC CARE Listed (Group -I) Journal Volume 11, Spl Iss 4,Dec 2022

Rutin 50 mg	100.6±0.7*	61±0.5	97±0.3
Rutin 100mg	91±0.7*	59±0.7	107±1.2
Rutin phytosome 10mg	53±0.5*	42±0.6*	134±0.2*
Rutin phytosome 25mg	51±0.7*	37+0.4*	141+0.6*

Values are given as mean ± SEM for 6 rats in each group; experimental groups are compared with diabetic control. Values are statistically significant at *=P<0.05.

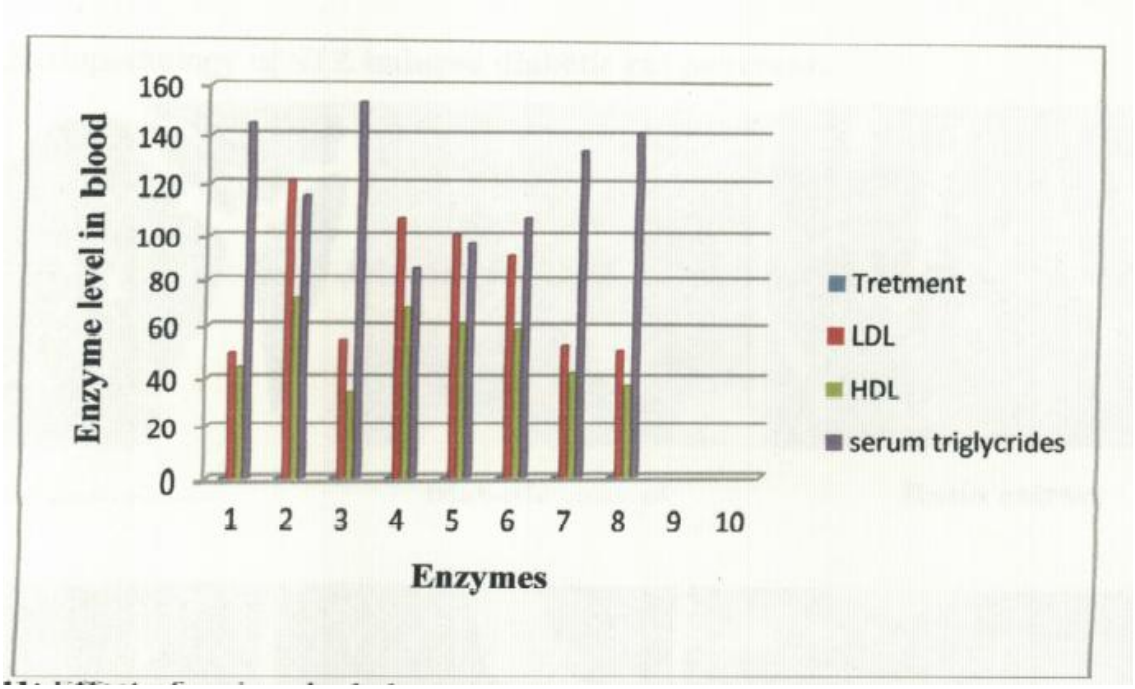


Figure 24: Effect of various herbal combinations on body weight of streptozocin- induced diabetic Wistar rats

III. Histopathology of STZ induced diabetic rat pancreas

Table 19: Histopathological observations of pancreas

Group	Necrosis of islets	Degeneration of acini	Leuvocytic infiltration	Reduction in size of islets
Group I	00	00	00	00
Group III	+++	+++	+++	+++
Group IV	++	++	++	++
Group V	++	++	++	++
Group VI	+	+	+	+

Note:
0: no abnormality detected
+: pathological changes up to less than 25 %
++: pathological changes up to less than 50 %
+++: pathological changes up to less 75 %

ISSN PRINT 2319 1775 Online 2320 7876
Research paper UGC CARE Listed (Group -I) Journal Volume 11, Spl Iss 4,Dec 2022

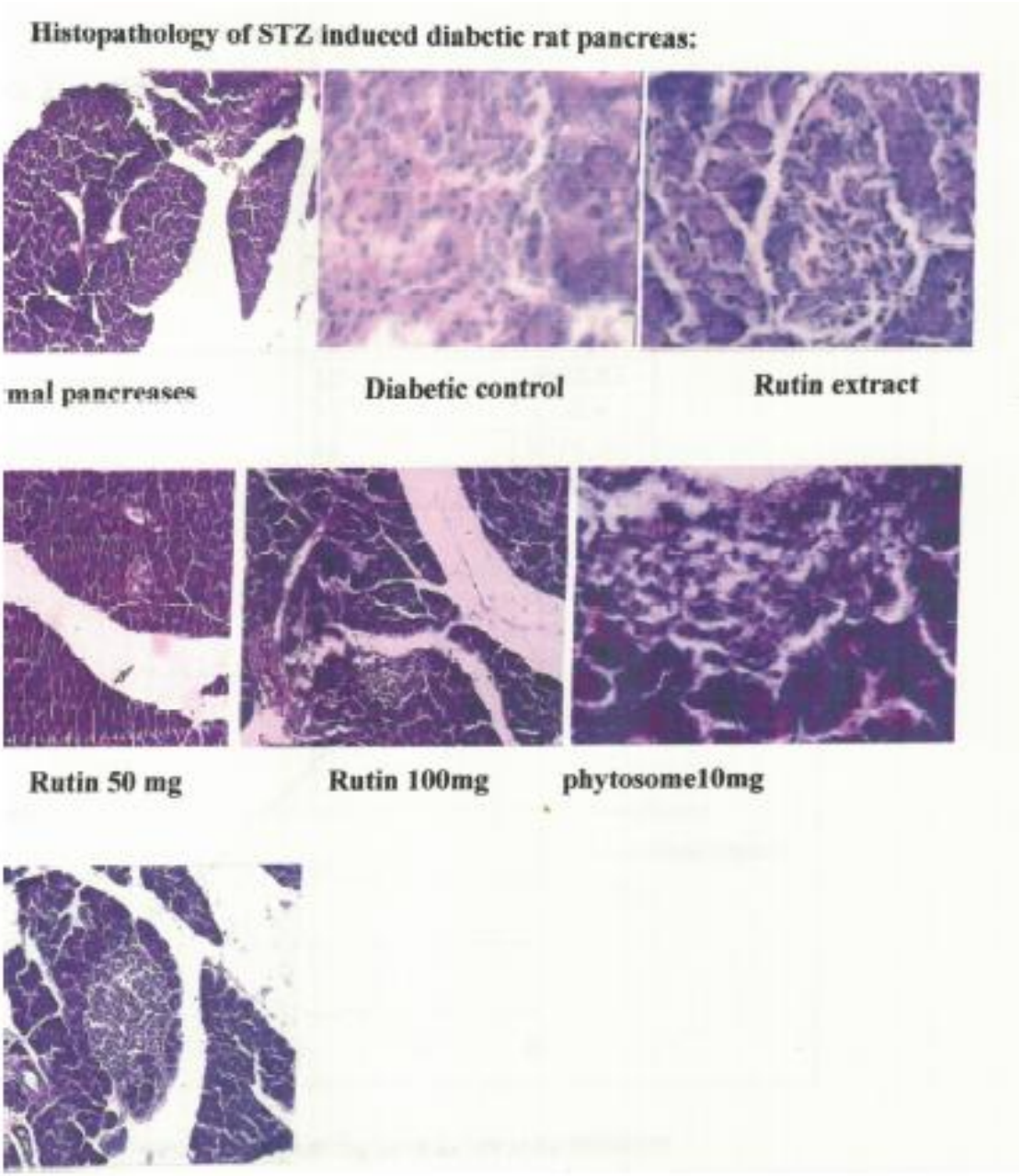


Figure 25 Effects of various herbal combination on histopathology of the pancreas

ISSN PRINT 2319 1775 Online 2320 7876
Research paper UGC CARE Listed (Group -I) Journal Volume 11, Spl Iss 4,Dec 2022

I Calibration Curve of rutin:

Table 20: Calibration curve showing area as per concentration

Concentration (µg/µl)	Area
2	397.76
4	2795.53
6	4193.3
8	5591.06
10	6988.83
12	8386.6
14	9784.36
16	11182.13

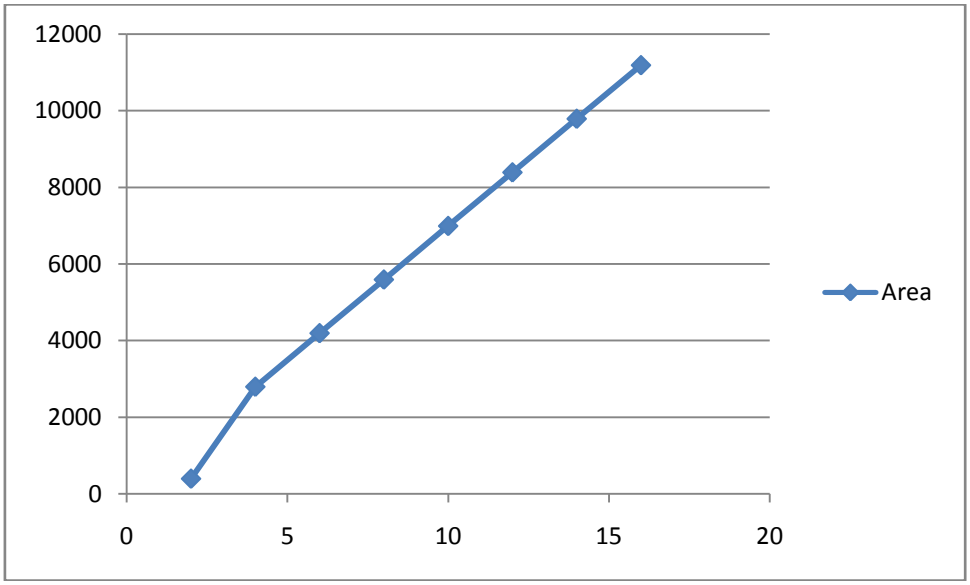


Figure 26: Calibration curve showing area as per concentration

I. Method validation

Table 21: Method validation parameters for the estimation of rutin by HPTLC

Parameter	Gymnemic acid
Linearity range (µg/spot)	2-16µg
Slope	164.6
Intercept	5.909
Coefficient of correlation	0.99954
Limit of Detection (LOD)	66.66ng/spot
Limit of Quantitation (LOQ)	200ng/spot

Table 22: Precision of HPTLC method for rutin

Parameters	Formulation	Rutin Mean area	%found	S.D.	% RSD	S. E
Repeatability	F (6 µg)	4077.9	94.58	2.84	0.06	0.024
Intermediate precision	F (6 µg)	4098.15	92.86	2.94	0.07	0.028
Reproducibility	F (6 µg)	4076.682	93.53	2.21	0.05	0.020

ISSN PRINT 2319 1775 Online 2320 7876
Research paper UGC CARE Listed (Group -I) Journal Volume 11, Spl Iss 4,Dec 2022

Determinations were carried out using six replicates of the same spot.

Table 23: Analysis of phytosome by HPTLC

Drugs	Rf	Amount found	%drug found
Rutin	0.37	10.00024 mg/20 mg of phytosome	100.024%

Determinations were carried out using six replicates of the same spot

Table 24: Analysis of phytosome by HPTLC

Drugs	Rf	Amount found	%drug found
Rutin	0.34	4.13	98.16%

Determinations were carried out using six replicates of the same spot

Table 25: Recovery studies of rutin

Level of % recovery	Standard added(mg/cap)	Total amount recovered(mg)	% recovery
80	8mg	17.24	94.58
100	10mg	18.57	92.86
120	12mg	20.58	93.53

Determinations were carried out using six replicates of the same spot.

Table 26: Statistical validation for rutin

Component	Mean	Standard Deviation	Coefficient of Variation	Standard Error
rutin	93.65	2.21	1.034	0.028

Determinations were carried out using six replicates of the same spot.

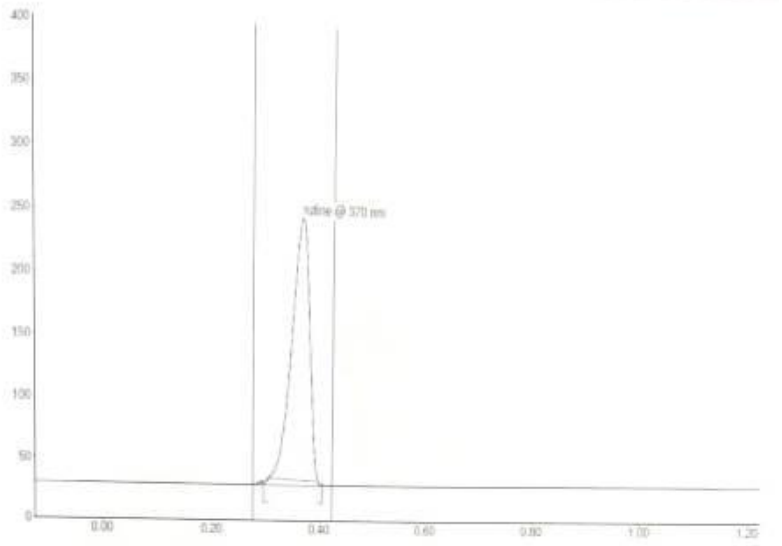


Figure 27: HPTLC chromatogram of phytosome formulation

ISSN PRINT 2319 1775 Online 2320 7876
 UGC CARE Listed (Group -I) Journal Volume 11, Spl Iss 4,Dec 2022

4. Conclusion:

From the obtained results, it can be concluded that:

❖ In TLC, extracts showed effective separation and presence of steroid, flavonoids, saponins, glycoside and alkaloid compounds.

❖ Selected plant extract was mixed together and were studied for their acute toxicity studies on rats. A prepared herbal formulation was found to be safe. No side effects were observed.

❖ Rutin extract, standard rutin and phytosome screened for antidiabetic activity by vivo, STZ-induced diabetes and in-vitro, alpha-amylase and alpha-glucosidase activity. Phytosome shows better result.

❖ Before preparation of dosage form, Rutin was checked for incompatibility with polymers, soya lecithin by studying FTIR of individual rutin and combination of rutin with soya lecithin. There was no interaction found between polymers and drug. All the polymers used are compatible with the drug under the proposed method of fabrication.

❖ Phytosome formulation was prepared by using solvent evaporation method.

❖ Evaluation parameters like Surface morphology (SEM) determines Smooth amorphous surface of formulation and crystalline nature of drug.

❖ An in-vitro dissolution study was also done in order to check controlled release of formulations. It shows extended release than conventional formulation

❖ Formulations formulation evaluated and standardize by HPTLC. was Quantification of formulation was done for one available markers rutin. The HPTLC method was also validated for proposed formulation.

5. References:

1. Steppan CM, Bailey ST, Bhat S. Brown EJ, Banerjee RR, Wright CM, Patel HR, Ahima RS, Stratton IM, Neil HA, YudkinJS (2000); Association of systolic blood pressure with macrovascular and microvascular complications of type 2 diabetes prospective observational study. British Medical Journal 321: 412-419.

2. Pickup JC, Williams G (1991); Textbook of diabetes Classification and diagnosis of diabetes mellitus and impaired glucose tolerance. Blackwell Scientific Publications, London, UK pp 37-44.

3. Kuhlmann J, Puls W (1996); Oral antidiabetics. 1" chapter, Springer-Verlag, Germany, Pp-25. Kukreja A, Maclaren NK, (1999). Autoimmunity and diabetes. J ClinEndocrinolMetab 84:4371-4378.

4. Amos A, McCarty D, Zimmet P (1997); The rising global burden of diabetes and its complications: estimates and projections to the year 2010. Diabet Med 14: S1-S85.

5. King H, Aubert RE, Herman WH (1998); Global burden of diabetes, 1995-2025: prevalence, numerical estimates and projections. Diabetes Care 21:1414-31.

6. Allen Jr, LV, Popovich NG, Ansel HC (2005); Ansel's Pharmaceutical Dosage Forms and Drug Delivery Systems, 8" Ed, Lippincott Williams & Wilkins: New York, pp.- 422-430.

7. Mura P, Faucci MT, Manderioli A, Bramanti G, Ceccarelli L (1998);Compatibility study between ibuproxam and pharmaceutical excipients using differential scanning calorimetry, hot-stage microscopy and scanning electron microscopy. J Pharm Biomed Anal 18: 151-63.

8. Oliveira GGG, FerrazHFG, Matos JSR (2005); Thermoanalytical study of glibenclamide and excipients.JTherm Anal Cal 79: 267 - 270.

9. Mukesh Chandra, Sharma and Smita Sharma(2010) Phytochemical and Pharmacological Screening of Combined Mimosa pudicaLinnandTridaxprocumbens for In vitro Antimicrobial Activity International Journal of Microbiological Research(3): 171-174.

10. Mukherjee PK (2003); Exploring Botanicals in Indian System of Medicine-Regulatory Perspectives. Clin Res reg affairs 20: 249-264.

ISSN PRINT 2319 1775 Online 2320 7876
 UGC CARE Listed (Group -I) Journal Volume 11, Spl Iss 4,Dec 2022

11. Y. Jianga, B. Tu, P. David and Y. Barbin, (2010);Recent analytical approaches in quality control of traditional Chinese medicines-A review, Anal. Chim. Acta., 657: 9-18.
12. X. Anandjiwala, J. Kalola and M. Rajani.(2006); Quantification of eugenol, luteolin, ursolic acid, and oleanolic acid in black (Krishna Tulasi) and green (Sri Tulasi) varieties of Ocimum sanctum Linn using high-performance thin-layer chromatography. J. AOAC. Int. 89: 1467-1474.
13. Reich E, Schibli A (2007); High performance thin layer chromatography for the analysis of medicinal plants, Thieme publishers, New York, 130,162.
14. Reich E, Schibli A (2004); A Standardized Approach to Modern High-Performance Thin- Layer Chromatography (HPTLC). J Planar Chromatogr17: 438-443.
15. Rheney CC, Kirk KK (2000); Performance of three blood glucose meters. Ann Pharmacother 34: 317-321
16. V. L. Suryavanshi, P. A. Sathe, M. M. Baing, Singh and S.Lakshmi(2007);Determination in Amaranthusspinosus Linn. of Rutin Whole Plant Powder by HPTLC.chromatographia 65.11-12.
17. Bergymeyer HU, Benut E (1963); Methods of Enzymatic Analysis, VerlagChemie GMBH WelhheimBerostr, Academic Press, New York, pp. 179-185.
18. Zlatkis A, Zak B, Boyle AJ (1953); A new method for the cholesterol. Journal of Laboratory and Clinical Medicine 41: 486-492.