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# Preparation, Characterization, And Pharmacokinetic Interactions Study of Green Synthesized Silver Nanoparticles of *Pterocarpus Marsupium* with Antidiabetic Drug

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## ABSTRACT

*In recent years, the greenway of metal nanoparticles i.e., biosynthesized silver nanoparticles play an essential role nowadays due to their less toxic, economic, environmentally friendly. The objective of the study is to investigate the antidiabetic effect of green synthesized silver nanoparticles from aqueous extract of Pterocarpus marsupium and to study pharmacokinetic interaction with the antidiabetic drug. The current study was pursued using the green synthesis of silver nanoparticles and was prepared using the Pterocarpus marsupium Roxb. Bark since this method helps bark extract (Pterocarpus marsupium) to serve as a reductant and a stabilizing agent for the synthesis of AgNPs. These nanoparticles were characterized and evaluated by various tests such as visual examination, UV visible spectral analysis, FTIR spectroscopy, drug entrapment efficacy, determination of particle size and zeta potential, SEM analysis, in-vitro antidiabetic study, in-vitro drug release kinetics study. Literature surveys regarding diabetes mellitus have revealed that patient-administered complementary or alternative medicine like herbal medicine along with synthetic drugs has led to the herb-drug interaction. Hence this research work has been carried out to find any pharmacokinetic interaction between green synthesized Pterocarpus marsupium silver nanoparticles with metformin and glimepiride using the equilibrium dialysis method. The study was carried out by assessing the percentage of protein binding using metformin and glimepiride individually and in combination with green synthesized silver nanoparticles of Pterocarpus marsupium. The results show that the percentage protein binding of metformin and glimepiride individually and in combination with silver nanoparticles were found to decrease with the increase in time of contact. The concentration of metformin and glimepiride was found to get altered at different time intervals. From the Scatchard plot, the number of binding sites and affinity constant was also calculated and reported.*

***Keywords:*** *green synthesis, silver nanoparticles, Pterocarpus marsupium, anti-diabetic, pharmacokinetic interaction, equilibrium dialysis method*

## INTRODUCTION

Nanomaterials are small-sized nanospheres with sizes ranging between 1 and 1000 nm**[1].** The commonly used metals for nanoparticle synthesis are cadmium (Cd), aluminum (Al), copper (Cu), cobalt (Co), gold (Au), iron (Fe), lead (Pb), silver (Ag), and zinc (Zn). **[2]** Silver nanoparticles (AgNPs) are one of the most fascinating nanomaterials among several metallic nanoparticles that are involved in biomedical applications, particularly in nanomedicine. The biological production of silver nanoparticles mainly involves the use of microorganisms and plant sources, and it has been reported that nanoparticle production methods based on microorganisms and plants are safe, less cost, and are relatively less harmful to the

environment than chemical synthesis**.** The green synthesis approach is the choice of the solvent medium (preferably water), an eco-friendly reducing agent, and nontoxic material for the stabilization of the nanoparticles.[3]

There are three main types of diabetes mellitus: i.e., Type 1 DM, Type 2 DM, Gestational diabetes.[4] Diabetes is a major global public health problem currently affecting 463 million individuals having diabetes worldwide and is projected to affect 700 million by 2045. In India, 77 million people currently have diabetes and this number is expected to almost double to 134 million by 2045. A study revealed a varying range of complementary and alternative medicine (CAM) use rates among diabetic patients in India (67.7%). Many patients with chronic diseases such as diabetes mellitus are reliant on CAM use because of its perceived efficacy, low cost, and safety.[5] CAM can affect the management of diabetes by either herb-drug interaction with the use of herbal remedies or indirectly by affecting medication adherence when using herbal or any other CAM types. [6]

The protein-bound drug is a large complex that cannot easily cross cell membranes and therefore has a restricted distribution and it is pharmacologically inactive. In contrast, the free or unbound drug crosses cell membranes and is therapeutically active.[7] When the clinical significance of the fraction of drug bound is considered it is important to know whether the study was performed using pharmacological or therapeutic plasma drug concentrations. The fraction of drug bound can change with the dose of drug administered and plasma drug concentration. [8]

A drug interaction results when the effects of a drug are altered in some way by the presence of another drug, food, or by environmental exposure. A drug interaction can cause involves one drug which alters the pharmacokinetics of another medical drug. Drug interaction mechanisms can be broadly divided into two groups i.e., Pharmacodynamic interactions, Pharmacokinetic interactions. A pharmacokinetic drug interaction is where one drug alters the rate or extent of any of the four basic pharmacokinetic processes such as absorption, distribution, metabolism, or excretion (ADME) of a second drug. This type of interaction is measured by a change in one or more of the kinetic parameters, such as the half-life amount of drug excreted in the urine, maximum serum concentration, area under the concentration-time curve, etc. [9]

Herbal medicines are often used by patients in conjunction with their conventional allopathic medicines. Herb-drug interactions are capable of modulating the pharmacokinetic and pharmacodynamic profiles of drugs.[10]

## MATERIALS AND METHODS

### Chemical Reagents

Silver Nitrate, α- amylase enzyme, Bovine Serum Albumin, Disodium hydrogen phosphate, Sodium chloride, Potassium dihydrogen phosphate, Methanol

### Plant material collection and processing

The bark of *Pterocarpus marsupium* had been collected, identified and authenticated by Dr. M. U. Sharief, Scientist ‘E’, Botanical Survey of India, Tamil Nadu Agricultural University (TNAU), Coimbatore, India. The collected bark was shade dried & it was grounded into fine powder with the help of an electronic blender. Then the powder obtained was stored in a well-closed container and kept in a dry place.

## Preparation of Aqueous Extract of *Pterocarpus marsupium* ROXB

50g of the bark powder of *Pterocarpus marsupium* was stirred with 500 mL of deionized water and kept at 65 °C for 30mins. Then the extracts were filtered by using Whatman No. 1 filter paper after cooling to room temperature. The extract was stored at 4 °C for future use. [11]

## PREFORMULATION STUDY

### Preliminary Qualitative Phytochemical Analysis

Chemical tests were carried out using the extract of *Pterocarpus marsupium* for the presence of various phytochemical constituents like tannins, phenolics**,** saponins, flavonoids, alkaloids. etc.

### UV- Visible Spectral Analysis of *Pterocarpus Marsupium* Roxb Bark Extract

1ml of *Pterocarpus marsupium* Roxb. bark extract was taken in a 10 ml standard flask and diluted with distilled water. Uv visible spectra were taken in the range of 200- 400 nm using phosphate buffer at pH 7.4 as blank. [12]

### FTIR SPECTROSCOPY OF *Pterocarpus marsupium* ROXB BARK

50 mg each of dried *Pterocarpus marsupium* Roxb bark and wood were mixed with 100 mg of spectral grade KBr and pressed into a disc under hydraulic pressure. Then FTIR spectra were recorded in the 4000- 400 cm-1 range. [13]

### GREEN SYNTHESIS OF SILVER NANOPARTICLES

An aliquot (1ml, 2ml,3ml, 4ml, 5ml) of aqueous plant extract sample was separately added to 10 ml of 1mM aqueous $AgNO_3$. To drive nanoparticle formation the reaction mixtures were kept in a magnetic stirrer with constant stirring at 120 rpm. Color change of the reaction mixtures was monitored to determine silver nanoparticle formation which is indicated by a colloidal brown color. [14]

### CHARACTERIZATION OF SYNTHESIZED *Pterocarpus marsupium* ROXB SILVER NANOPARTICLES

Characterization of *Pterocarpus marsupium* Roxb. Silver nanoparticles are important in order to evaluate the functional aspects of the synthesized particles. Characterization is performed using a variety of analytical techniques, including UV-vis spectroscopy, Fourier transform infrared spectroscopy (FTIR), scanning electron microscopy (SEM), particle size measurement, stability from zeta potential, and drug entrapment efficacy for elemental analysis. [15,16, 17]

### *In-vitro* methods

### α- Amylase inhibitory effect

pancreatic alpha-amylase is one of the important enzymes, that act as catalysts in the reaction which involves the hydrolysis of the alpha-1,4 glycosidic linkages of the starch, amylopectin, amylose, glycogen, and numerous maltodextrins and is responsible for starch digestion. The other important enzyme is alpha-glucosidase or maltase which catalyzes the final step of the digestive process of carbohydrates, mainly starch by acting upon 1,4-alpha bonds and producing glucose as the final product. The large molecules like starch cannot cross the blood-brain barrier as glucose has to reach the brain thus; to overcome this problem alpha-

amylase cleaves the large starch molecules into smaller fragments of sugars in order to cross the blood-brain barrier. If there will be an excess conversion of starch to sugars, it will increase the sugar level in the blood, then the role of insulin will come into action by ordering cells to metabolize the excess sugar moieties and store them as energy sources *i.e.* glycogen. This cycle is endlessly happening in a healthy person. But in some cases, due to excess activity of amylase enzyme and insulin deficiency or resistance to insulin, a level of blood glucose arises which might result in hyperglycemia. To control hyperglycemia studies on the inhibition of amylase enzyme activity are being studied. [18]

About 1.0 ml of reagent B (starch solution) was mixed with 1.0 ml of increasing concentration of drug (2.5 - 160 µg/ml). To this 1 ml of reagent F (enzyme solution) was added and left to react for 3 minutes at 25°C. After this 1.0 ml of calorimetric reagent E was added. The contents were heated for 10 to 15 minutes in a boiling water bath. The generation of maltose was quantified by the reduction of 3,5-nitro salicylic acid to 3-amino 5-nitro salicylic acid. This reaction, corresponding to color change from orange to red was measured at 540 nm against the reagent blank. Acarbose was used as the standard drug. The percentage of inhibition was determined by using the following formula:

**Inhibition activity%=Abs(control)-Abs(extract)÷Abs(control)×100**

Acarbose is an antidiabetic drug used to treat type 2 diabetes mellitus. The positive control used for this assay is **acarbose** which works by slowing the action of certain chemicals that break down food to release glucose into your blood. Slowing food digestion helps keep blood glucose from rising very high after meals. [19]

## *INVITRO* DRUG RELEASE STUDY

The antidiabetic drug *Pterocarpus marsupium-loaded* silver nanoparticles (300 mg) were suspended in 10 mL of phosphate-buffered saline (PBS) in a dialysis bag. The dialysis bag was sealed and then slowly shaken in 90 mL of PBS at 37°C in a 250-mL beaker and kept in a magnetic stirrer at an rpm of 170. Aliquots of the solution outside the dialysis membrane (2 mL) were replaced with 2 mL of PBS at various times intervals and tested at 427 nm by UV Spectrophotometer. The change of the concentrations of the drug with respect to different time intervals was obtained from curves of the absorbance A versus concentration C of *Pterocarpus marsupium* silver nanoparticles in PBS based on Lambert-Beer law. **[20]** In order to understand the mechanism of drug release, in vitro drug release data were treated with kinetic models such as zero-order (Time vs Cumulative percentage drug release), first-order (Time vs log cumulative percentage), and Higuchi model (Square root of time vs Cumulative percentage release) and Korsmeyer-Peppas model (Log time vs log cumulative percentage).

## PHARMACOKINETIC INTERACTION STUDY OF GREEN SYNTHESIZED NANOPARTICLES AND ANTIDIABETIC DRUGS

### Estimation of Metformin Using UV Spectrophotometry

Stock solution of 100 µg/ml and a standard curve was prepared to plot absorbance measured at 233 nm against concentration. **[22]**

## ESTIMATION OF GLIMEPIRIDE USING UV SPECTROPHOTOMETRY

Stock solution of 100 µg/ml and a standard curve was prepared to plot absorbance measured at 229 nm against concentration.**[23]**

**DETERMINATION OF PROTEIN BINDING OF METFORMIN AND GLIMEPIRIDE USING EQUILIBRIUM DIALYSIS METHOD [23,24]**

Equilibrium dialysis is one of the methods used for the determination of protein binding of any compound. This method was used for the determination of protein binding of green synthesized nanoparticles and its 1:1 mixture with metformin and glimepiride. The dialysis membrane was activated and then dialysis was performed.

Samples were taken at 10, 30, 60, 120, 240,480 mts till equilibrium was reached and absorbance of metformin and glimepiride were measured at 233 nm & 229 nm respectively. The concentration was then determined. [26]

**INTERACTION STUDY OF METFORMIN WITH *Pterocarpus marsupium* SILVER NANOPARTICLES**

The interaction study on metformin is carried out by adopting the equilibrium dialysis method. In this type of interaction, study metformin is considered as the standard and *Pterocarpus marsupium* silver nanoparticles are added *i.e.*, a 1:1 mixture of metformin and silver nanoparticle and absorbance was measured at 233 nm at different time intervals.

**INTERACTION STUDY OF GLIMEPIRIDE WITH *Pterocarpus marsupium* SILVER NANOPARTICLES**

The interaction study on glimepiride is carried out by adopting the equilibrium dialysis method. In this type of interaction, study glimepiride is considered as the standard and *Pterocarpus marsupium* silver nanoparticles are added *i.e.*, a 1:1 mixture of glimepiride and silver nanoparticles and absorbance was measured at 229 nm at different time intervals.

**DETERMINATION OF PROTEIN BINDING PERCENTAGE**

Initially, a known amount of drug was taken in the buffer compartment. The protein was added to the plasma compartment (dialysis bag). Then, the concentration of drug present in the buffer (outside of this compartment) after equilibrium was measured. This measurement gave the total amount of drug that remains in the buffer compartment. Thus, we can get a sum of free drugs and plasma-bound drugs at equilibrium.

The percentage of protein binding (F) of the drug is calculated using the following equation,

$$F = \frac{conc\ of\ bound\ drug}{conc\ of\ drug\ after\ equilibrium\ with\ the\ membrane} X\ 100$$

**Determination of the number of binding sites and the affinity constants**

The number of binding sites and affinity constants of green synthesized nanoparticles and their 1:1 mixture with metformin and glimepiride were calculated by the Scatchard method. The curve was produced by plotting r/[D] versus r, where r is the ratio between the molar concentration of the bound drug and the molar concentration of protein i.e.,

$\frac{[B]-[A]}{[Protein]}$B-[A][Protein]

and D is the concentration of the unbound drug i.e., [A]. The curve thus obtained is called a Scatchard plot. The Scatchard plot when extrapolated on Y axis, gave an intercept nK, the

intersection on the X-axis representing n, and the slope of line AB being k. Here, k is the affinity constant and n is the number of binding sites of protein binding. **[27]**

# RESULTS AND DISCUSSION

## Preformulation Studies

### Physical Characteristics

*Pterocarpus marsupium* was checked for its color, odor, and texture. It is a light-yellow colored powder in appearance and has a pleasant odor. Phytochemical screening confirmed the presence of Phyto-constituents like tannins, phenolics**,** saponins, flavonoids, starch, steroids and alkaloids. **[28, 29]**

### GREEN SYNTHESIS OF *Pterocarpus marsupium* ROXB SILVER NANOPARTICLES

An aliquot (5ml) of aqueous plant extract sample was taken and added 10 ml of 1mM aqueous AgNO3 dropwise and kept in a magnetic stirrer with constant stirring at 120 rpm. Color change of the reaction mixtures was monitored to determine silver nanoparticles formation which is indicated by a colloidal brown color.

### CHARACTERIZATION OF SYNTHESIZED *Pterocarpus marsupium* ROXB SILVER NANOPARTICLES

### VISUAL EXAMINATION

The addition of plant extract of *Pterocarpus marsupium* into the beakers containing an aqueous solution of silver nitrate led to the change in the color of the solution from yellowish to reddish-brown color. This formation indicates that silver ions in the reaction medium have been converted to elemental silver having the size of a nanometric range. F1, F2, F3, F4, F5 formulations of *Pterocarpus marsupium* silver nanoparticles were prepared by the green synthesis method. In the addition of different concentrations (1 to 5 mL) of bark extracts to aqueous silver nitrate solution keeping its concentration 10 mL (1 mM) constant, the color of the solution changed from light yellow to colloidal brown indicating formation of silver nanoparticles. Hence the formulation F5 was chosen for further evaluation studies because of the formation of colloidal brown color.

### UV Visible spectral analysis of *Pterocarpus marsupium* silver nanoparticles

UV-Visible spectral analysis characterizes the formation and completion of silver nanoparticles by using UV-Visible spectrophotometer. The reduction of silver ions in solution was monitored by periodic sampling of aliquots at the time interval of 30 min, 210 min and 24 hr were taken by using distilled water as blank from the wavelength of 200-800 nm and are as shown in Fig. 1 .

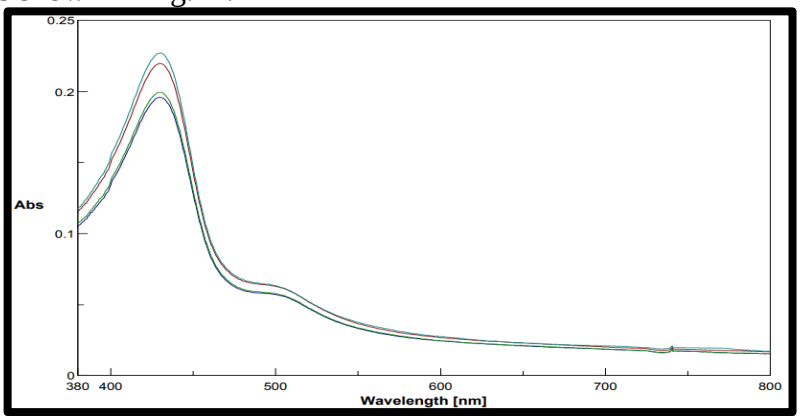


*Fig. 1. UV- visible absorption spectra of Pterocarpus marsupium Roxb. silver*

*nanoparticles at different time intervals*

The reduction of silver ions to silver nanoparticles was indicated by a color change from yellow to brown color and it was reflected in spectral data obtained by using a UV-Visible spectrophotometer. The color is characteristic of the surface plasmon resonance (SPR) of silver nanoparticles. It shows an absorption peak around 429 nm which is specific for *Pterocarpus marsupium* bark silver nanoparticles.

## FTIR SPECTROSCOPY OF *Pterocarpus marsupium* SILVER NANOPARTICLES

FTIR helps to identify the different functional groups in the compounds which cause the conversion of silver ions to silver nanoparticles and is also used for capping or stabilization of silver nanoparticles. FTIR spectra of the *Pterocarpus marsupium* -AgNPs was recorded to identify functional groups.

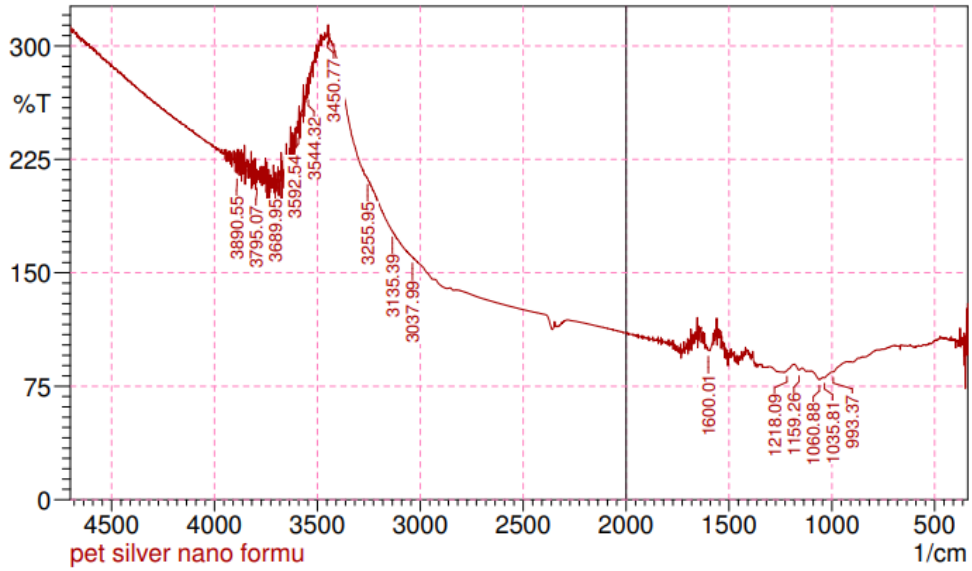


*Fig. 2 FTIR spectrum of Pterocarpus marsupium silver nanoparticles*

From the above spectral analysis depicted in Fig.2 , it was concluded that the drug and excipient don't have any incompatibility. since the peaks present in the drug can also be visualized in the drug and excipient spectra. Hence these excipients can be chosen for the preparation of silver nanoparticles for further analysis. The presence of hydroxyl, carboxyl and phenolic was confirmed.

## DRUG ENTRAPMENT EFFICIENCY

Drug entrapment efficacy is a measure of drug loading capacity of the system. Drug entrapment can be determined from the supernatant liquid of *Pterocarpus marsupium* bark silver nanoparticle after centrifugation by UV spectrophotometry at 429 nm. The % entrapment of drug Pterocarpus *marsupium Roxb.* silver nanoparticles were found to be 72% for F1, 78% for F2, 81% for F3, 86% for F4, 95% for F5.

Hence, the highest amount of drug was entrapped in the formulation F5 which is 95% and it was observed to be a colloidal brown color which indicated the formation of *Pterocarpus marsupium* silver nanoparticles. Based on drug entrapment results, the formulation F5 was chosen for further evaluation studies as it possessed high drug entrapment capacity as compared to other formulations.

## SCANNING ELECTRON MICROSCOPY

A SEM employed to analyze the surface morphology and size details of the silver nanoparticles. The SEM images were taken in different magnification such as 3000X, 5000X, 15000X and shown in Fig.3,4,5 respectively.

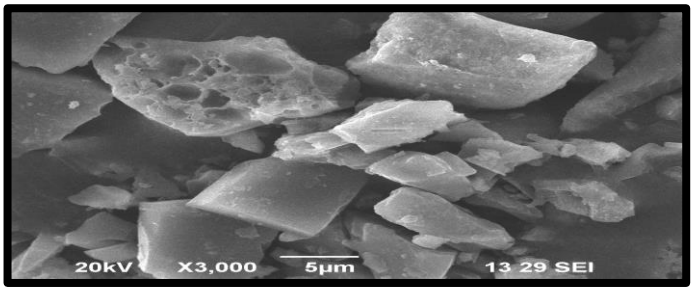


*Fig.3 SEM analysis of Pterocarpus marsupium Roxb. Silver nanoparticle of 3000X magnification*

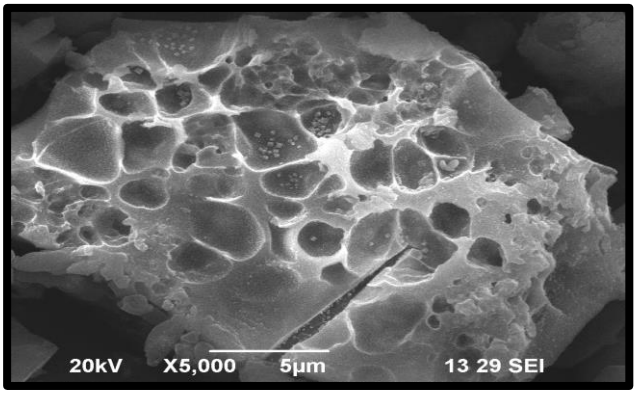


*Fig. 4 SEM analysis of Pterocarpus marsupium Roxb. Silver nanoparticle of 5000X magnification*

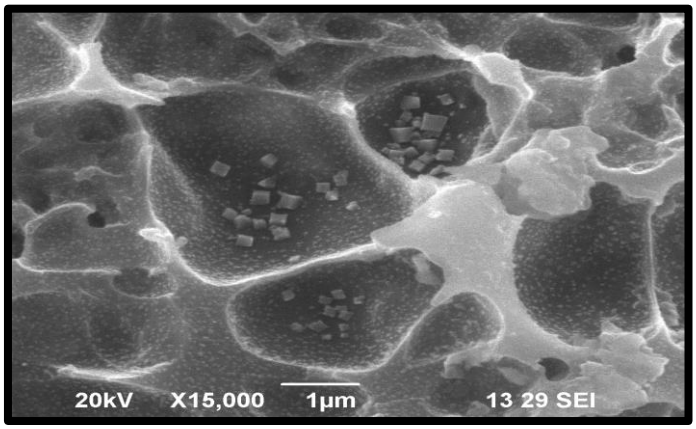


*Fig. 5 SEM analysis of Pterocarpus marsupium Roxb. silver nanoparticle of 15000X magnification*

The SEM micrographs of *Pterocarpus marsupium* silver nanoparticles showed that the nanoparticles synthesized were polydisperse spherically shaped and highly distributed with aggregation. The SEM image showing silver nanoparticles synthesized using *Pterocarpus marsupium* aqueous extract of bark confirmed the development of silver nanostructures.

## PARTICLE SIZE MEASUREMENT

The mean particle size (z-average), polydispersity index (PI) of *Pterocarpus marsupium* silver nanoparticles were determined by dynamic light scattering technique using a zeta size analyzer (Malvern Instruments). The study revealed average particle size (z-average) is found to be 36.44nm. Particle size analysis showed the presence of nanoparticles with polydispersity indices PDI value 0.825 with intercept 0.781. it is presented in the Fig.6.

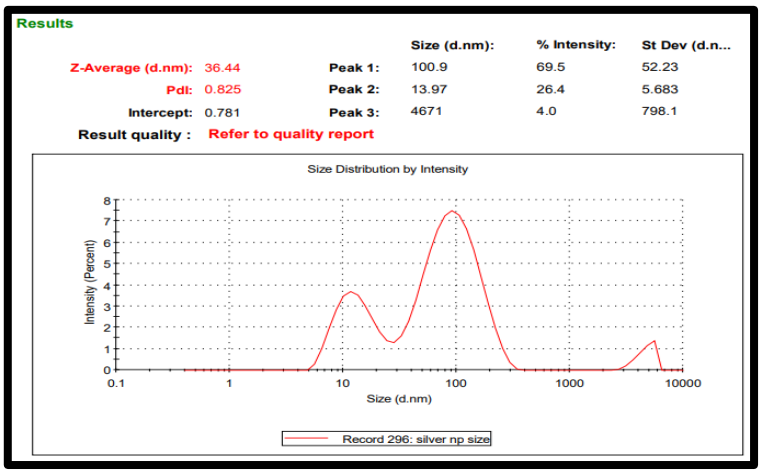


*Fig.6 Particle size measurement of Pterocarpus marsupium silver nanoparticles*

## ZETA POTENTIAL.

Zeta Potential was determined using the Malvern zeta-sizer instrument. The surface charge of the particles and stability of the solution was characterized by zeta potential. For *Pterocarpus marsupium* silver nanoparticles zeta potential was found to be -22.6 mV with peak area 100 intensity. These values indicate that the formulated *Pterocarpus marsupium* silver nanoparticles are stable. Zeta potential distribution of silver nanoparticles are depicted in the Fig.7.

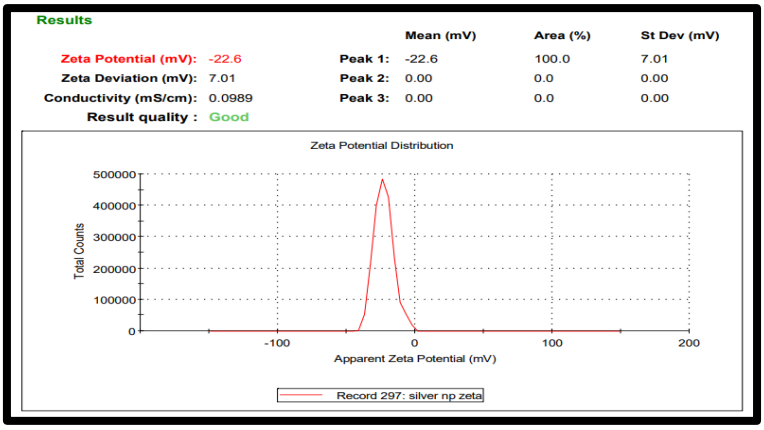


*Fig. 7 Determination of zeta potential of Pterocarpus marsupium silver nanoparticles*

## IN VITRO DRUG RELEASE STUDY

The in vitro release of drug from the nanoparticle was measured in phosphate buffer (pH 7.4), using dialysis bag diffusion method. Amount of drugs released at different time intervals (1h,2h,3h,4h,5h and 24h) were observed.

The invitro drug release was fitted into different kinetic models consisting of zero order, first order, Higuchi model and Korsmeyer – Peppas model. The various profiles were evaluated by the correlation coefficient ($R^2$). The highest degree of correlation coefficient determines the suitable mathematical model that follows drug release kinetics.

## DRUG RELEASE DATA FITTED TO VARIOUS KINETIC MODELS

### Zero order

Time vs Cumulative percentage release were plotted and shown in Fig.14 From the graph $R^2$ values were found to be 0.8966 as depicted in Fig.8.

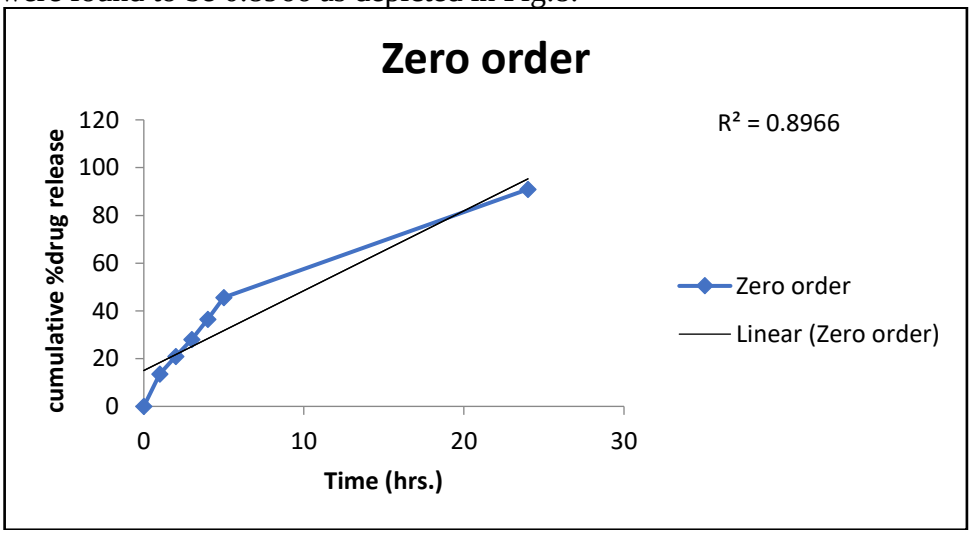


*Fig. 8 Zero order plot*

### First order

Time vs log cumulative percentage were plotted and shown in Fig.15 From the graph $R^2$ value found to be 0.3223 as depicted in Fig.9.

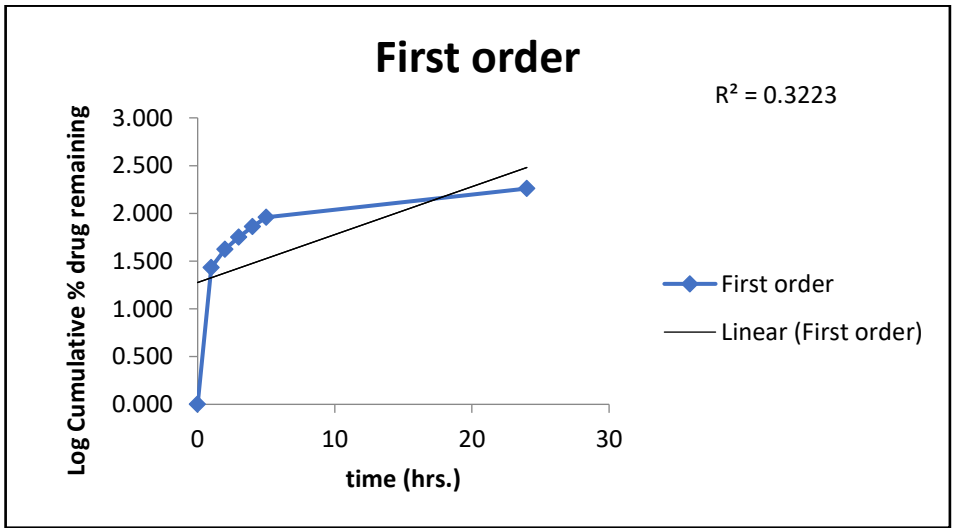


*Fig.9 First order plot*

### Higuchi's plot

Square root of time vs cumulative percentage release and shown in the Fig.16 From the graph $R^2$ value was found to be 0.9877as depicted in Fig.10.

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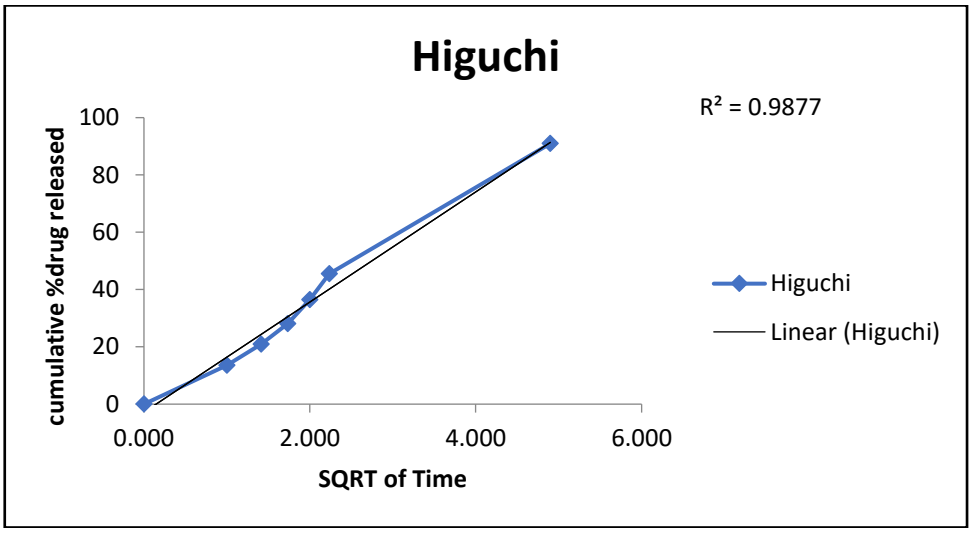


*Fig. 10 Higuchi plot*

**Korsmeyer peppas**

Log time vs log cumulative percentage and shown in the Fig. 17 From the graph was found to be 0.9692 and n value is 0.167as depicted in Fig.11.

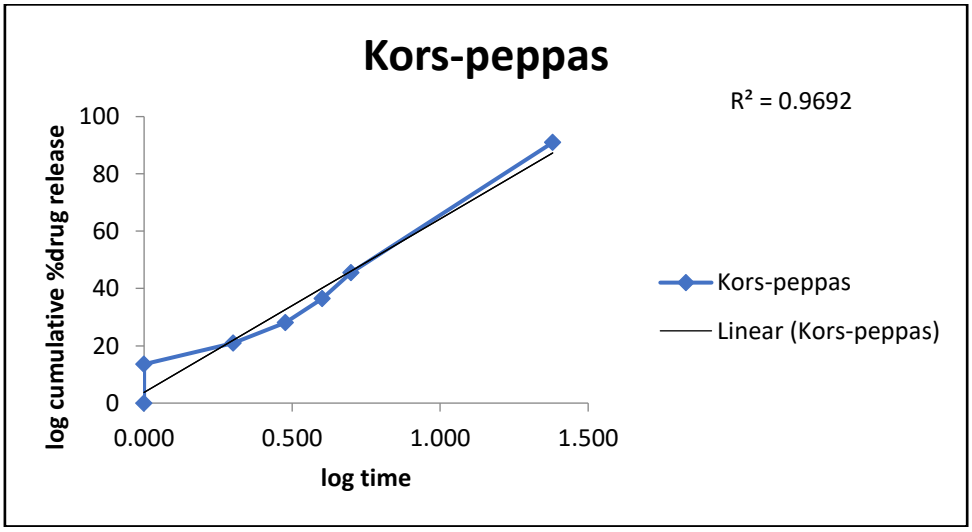


*Fig. 11 Korsmeyer peppas*

Based on the release kinetic analysis, the release data were best fitted with the Higuchi release with the $R^2$ value being 0.9877. To understand the mechanism of drug release, the data were fitted in the Korsmeyer- Peppas equation which states the type of diffusion. The corresponding plot for the Korsmeyer Peppas equation also shows good linearity. The release exponent "n" was found to be 0.167, confirming that the formulation followed Fickian diffusion kinetics.

From the release kinetics data, it can be concluded that the formulation *Pterocarpus marsupium* Roxb. Silver nanoparticle fit with the highest correlation ($R^2$) was obtained with Higuchi and followed by Korsmeyer Peppas zero-order, and first order.

**INVITRO ALPHA AMYLASE INHIBITION STUDY**

Alpha amylase is responsible for postprandial glucose levels therefore, *Pterocarpus marsupium* plant extracts with alpha-amylase inhibitory activity are being investigated that might decrease postprandial blood glucose levels, thus being an interesting and novel therapeutic target for diabetes mellitus treatment. A possible strategy to block dietary carbohydrate absorption is to use natural resources as carbohydrate digestive enzyme inhibitors as they have fewer side effects than synthetic drugs. Alpha-amylase inhibitory activity of *Pterocarpus marsupium* and their phytochemical compounds are explored that might be helpful within the treatment of diabetes mellitus. Acarbose is used as a standard here which is a good antidiabetic drug and works by slowing the action of certain chemicals that break down food to release glucose into our blood.The result suggests that *Pterocarpus marsupium* Roxb. exhibits good alpha amylase inhibition under in vitro condition.

**Alpha amylase inhibitory effect of positive control.**

The absorbance of control without sample is taken and it is 1.2442. Percentage inhibition of α amylase for the positive control Acarbose was found to be 33.61% at concentration 0.2mg/ml. When the concentration is increased to 0.4 mg/ml percentage inhibition is increased by 1.1fold of when the concentration was increased to 0.6mg/ml, so the percentage inhibition is increased by 1.2fold, further the concentration was increased to 0.8mg/ml and the percentage inhibition was found to increase by 1.1-fold, when the concentration was increased to 1 mg/ml which resulted in the increase of percentage inhibition was observed by 1-fold.

**α- Amylase inhibitory effects of *Pterocarpus marsupium* Roxb. silver nanoparticles**

Percentage inhibition of α amylase for the *Pterocarpus marsupium* Roxb. silver nanoparticles were found to be 29.14% for the concentration 0.2mg/ml. When the concentration was increased to 0.4mg/ml percentage inhibition was observed to increase by 1-fold when the concentration was increased to 0.6mg/ml, so the percentage inhibition was also found to increase by 1.1-fold. Further the concentration was increased to 0.8mg/ml then the percentage inhibition was increased by 1.1-fold, when the concentration was increased to 1mg/ml which resulted in the increase of percentage inhibition observed to 1.4-fold high.

**Comparison of α-amylase inhibition of *Pterocarpus marsupium* silver nanoparticles & acarbose on alpha amylase enzyme**

Comparison of alpha amylase inhibition of acarbose vs *Pterocarpus marsupium* Roxb. Silver nanoparticles were shown in Fig.12. From the figure we compare the percentage inhibition of both positive control and test drug.

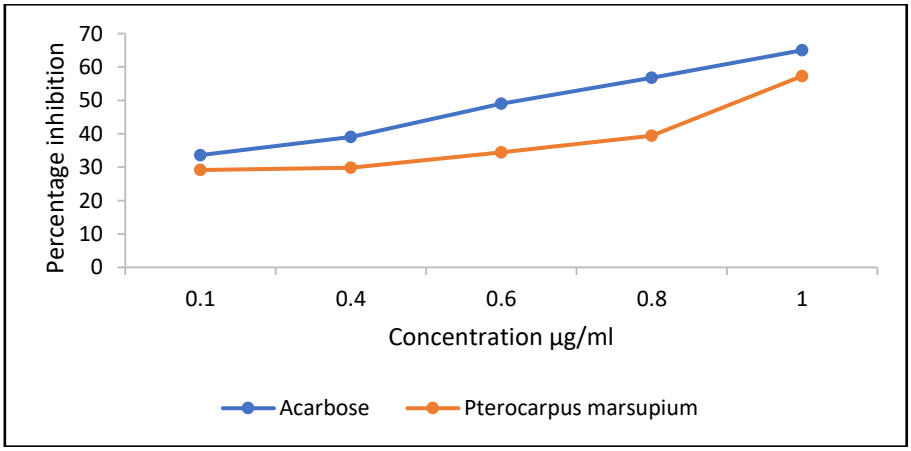

*Fig. 12 Comparison of alpha amylase inhibition of acarbose vs Pterocarpus marsupium Roxb. Silver nanoparticles*

The percentage α amylase inhibition of positive control Acarbose at lower(0.2mg/ml) and higher (1 mg/ml) concentration were found to be 33.61% and 65% respectively and for the test *Pterocarpus marsupium* Roxb. silver nanoparticles, the percentage α- amylase inhibition at lowest (0.2mg/ml) and highest (1 mg/ml) concentration were found to be 29.14% and 57.28% respectively.

**PHARMACOKINETIC INTERACTION STUDY OF GREEN SYNTHESIZED NANOPARTICLES AND ANTIDIABETIC DRUGS**

**ESTIMATION OF METFORMIN USING UV SPECTROPHOTOMETRY**

The calibration graph of metformin was revalidated. Metformin shows good linearity with the range of 2 to 10 µg/ml measured at 233 nm using uv spectrophotometry .

**DETERMINATION OF PROTEIN BINDING OF METFORMIN USING EQUILIBRIUM DIALYSIS METHOD**

The concentration of unbound metformin and percentage protein binding of metformin was determined using the equilibrium dialysis method. The study was carried out with concentration (3.8 x 10 $^{-8}$ M) of metformin.

In case of 3.8x10$^{-8}$ M concentration of metformin individually, the percentage protein binding of metformin at different time intervals like 10, 30, 60,120, 180, 240, 300 min was 41%, 33%, 31%, 30%, 24%, 21% respectively as depicted in Fig .13.

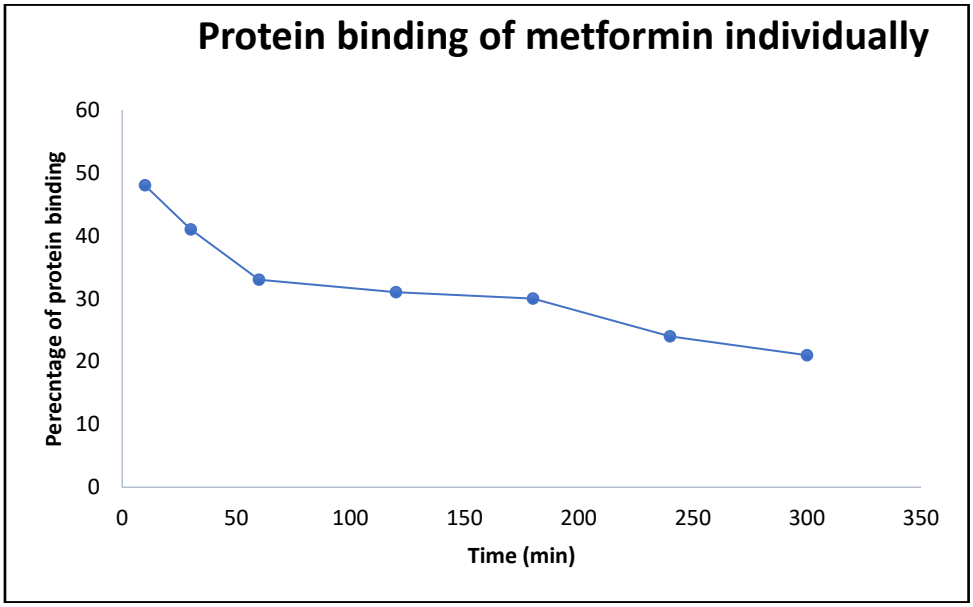


*Fig.13 Protein binding of metformin individually*

As the time of contact with drug and protein increases, there is a decrease in percentage of protein binding which may be due to the number of binding sites and affinity constant.

**INTERACTION STUDY OF METFORMIN WITH *Pterocarpus marsupium* SILVER NANOPARTICLES**

The concentration of unbound drug and percentage protein binding of metformin in presence

of *Pterocarpus marsupium* silver nanoparticles is determined using equilibrium dialysis method. The study was carried out with concentration (3.8 x 10 $^{-8}$ M) of metformin and 20 ml of *Pterocarpus marsupium* silver nanoparticles.
The percentage protein binding of metformin in presence of silver nanoparticles at different time intervals like 10, 30, 60, 120, 180, 240, 300 min was 45%, 44%, 41%, 38%, 31%, 30%, 28% respectively as represented in Fig.14. The concentration of metformin with silver nanoparticles was found to increase by 1.03, 1.02, 1.05, 1.10, 1.01, 1.03-fold respectively.

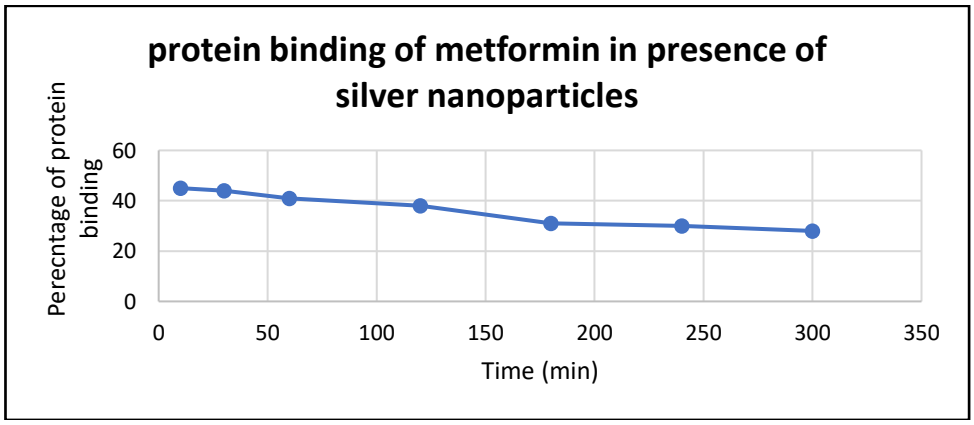


*Fig.14 Protein binding of metformin in presence of Pterocarpus marsupium silver nanoparticles (1:1) mixture*

**DETERMINATION OF PROTEIN BINDING PARAMETERS: SCATCHARD PLOT**

In case of scatchard plot, the x-axis is specific binding (usually labeled 'bound') and the y-axis is the ratio of specific binding to concentration of free radioligand (usually labeled 'unbound or free'). Numbers of binding sites were obtained by dividing the intercept (nk) by slope (k) of the straight lines. The values for affinity constants associated with the respective class of binding sites were obtained directly from the slope of the straight lines. **[30]**

**NUMBER OF BINDING SITES AND AFFINITY CONSTANT OF METFORMIN**

From scatchard plots, x – intercept was found to be 0.0025 and y – intercept was found to be 6.243. The number of binding sites for metformin individual in BSA was found to be 8.81 and affinity constant were found to be 0.70 respectively as represented in Fig.15.

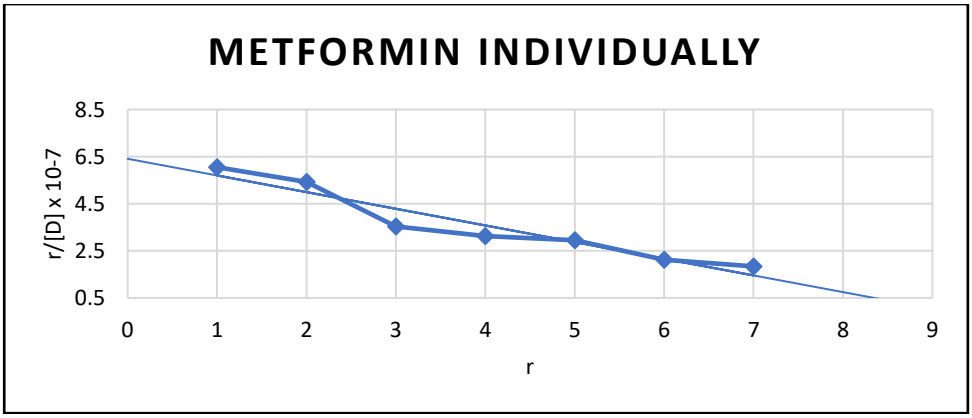


*Fig. 15 Scatchard plot for protein binding of metformin individually*

**NUMBER OF BINDING SITES AND AFFINITY CONSTANT OF METFORMIN IN PRESENCE OF *Pterocarpus marsupium* SILVER NANOPARTICLES**

From scatchard plots, x – intercept was found to be 0.00036 and y – intercept was found to be 2.490. The number of binding sites for metformin in presence of *Pterocarpus marsupium* silver nanoparticles in BSA was found to be 11.19 and affinity constants were found to be 0.22 respectively. When metformin is combined with silver nanoparticles the number of binding sites found to be increased when compared to metformin individually as represented in Fig.16

.

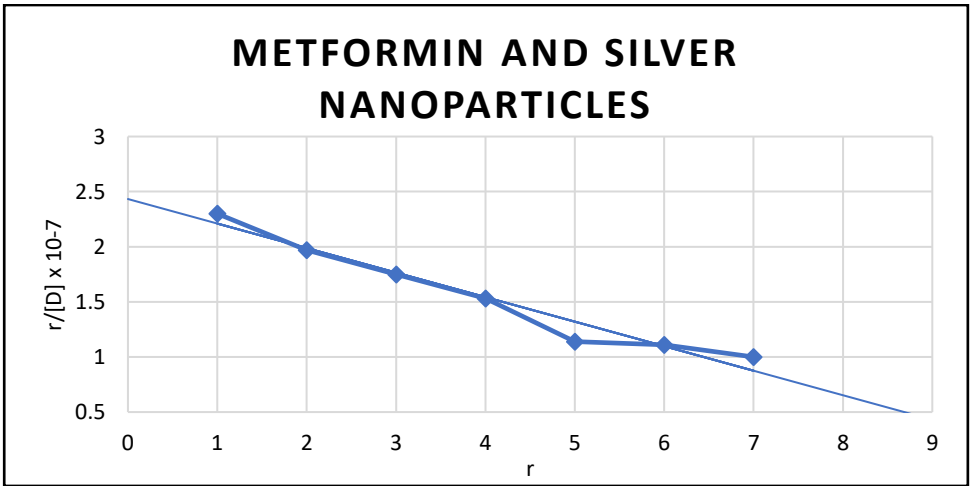


*Fig. 16 Scatchard plot for protein binding of metformin in presence of Pterocarpus marsupium silver nanoparticles (1 :1 mixture)*

**ESTIMATION OF GLIMEPIRIDE USING UV SPECTROPHOTOMETRY**

The calibration graph of glimepiride was revalidated. Glimepiride shows good linearity with the range of 9 to 36 µg/ml measured at 229 nm using Uv spectrophotometry.

**DETERMINATION OF PROTEIN BINDING OF GLIMEPIRIDE USING EQUILIBRIUM DIALYSIS METHOD**

The concentration of unbound glimepiride and percentage protein binding of glimepiride is determined using equilibrium dialysis method. The study was carried out with concentration ($1.8 \times 10^{-9}$ M) of glimepiride.

In case of $1.8 \times 10^{-9}$M concentration of glimepiride, the percentage protein binding of glimepiride at different time intervals like 10, 30, 60,120, 180, 240, 300 min was 88%, 85%, 83%, 81%, 75%, 70%, 67%. The concentration of glimepiride individually was found to increase by 1.11,1.2,1.08,1,1.2-fold respectively as represented in Fig.17

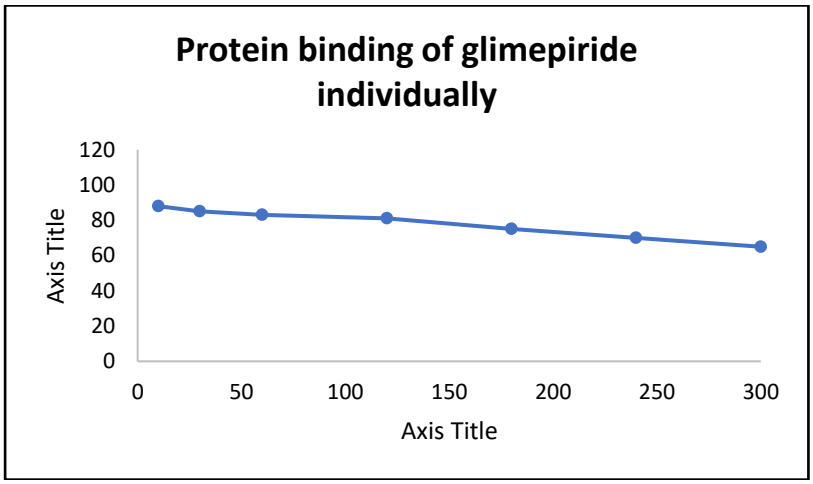


*Fig.17 Protein binding of glimepiride individually*

As the time of contact with drug and protein increases, there is a decrease in percentage of protein binding which may be due to the number of binding sites and affinity constant.

**INTERACTION STUDY OF GLIMEPIRIDE WITH *Pterocarpus marsupium* SILVER NANOPARTICLES**

The concentration of unbound drug and percentage protein binding of glimepiride in presence of *Pterocarpus marsupium* silver nanoparticles is determined using equilibrium dialysis method. The study was carried out with concentration (1.8 x $10^{-9}$ M) of glimepiride and 20ml *of Pterocarpus marsupium* silver nanoparticles.

The percentage protein binding of glimepiride in presence of silver nanoparticles at different time intervals like 10, 30, 60, 120, 180, 240, 300 min was 68%, 61%, 58%, 57%, 52%, 51%, 50% respectively. The concentration of glimepiride with silver nanoparticles was found to increase by 1.16,1.05,1.01,1.0,1.01,1.01-fold respectively as represented in Fig.18

.

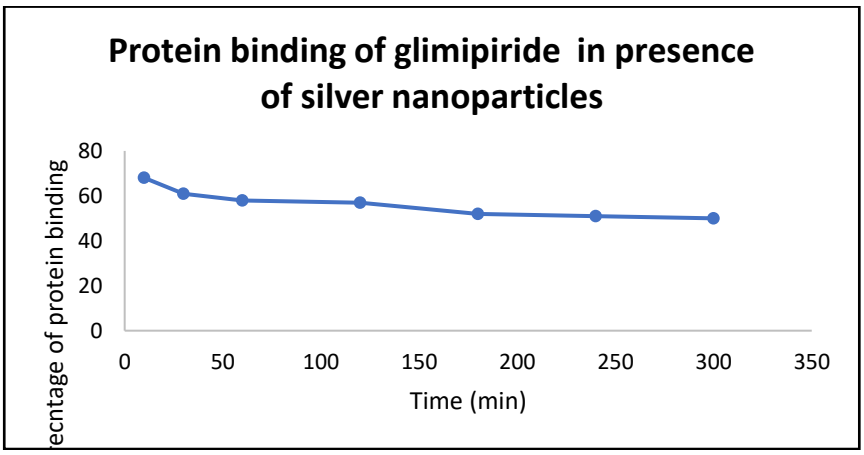


*Fig.18 Protein binding of glimepiride in presence of Pterocarpus marsupium silver nanoparticles (1:1) mixture*

**Determination Of Protein Binding Parameters**

**Number of Binding Sites and Affinity Constant of Glimepiride**

From scatchard plots, x – intercept was found to be 0.00031and y – intercept was found to be 7.310. The number of binding sites for glimepiride individual in BSA was found to be 8.50 and affinity constant were found to be 0.85 respectively are as represented in Figure 19.

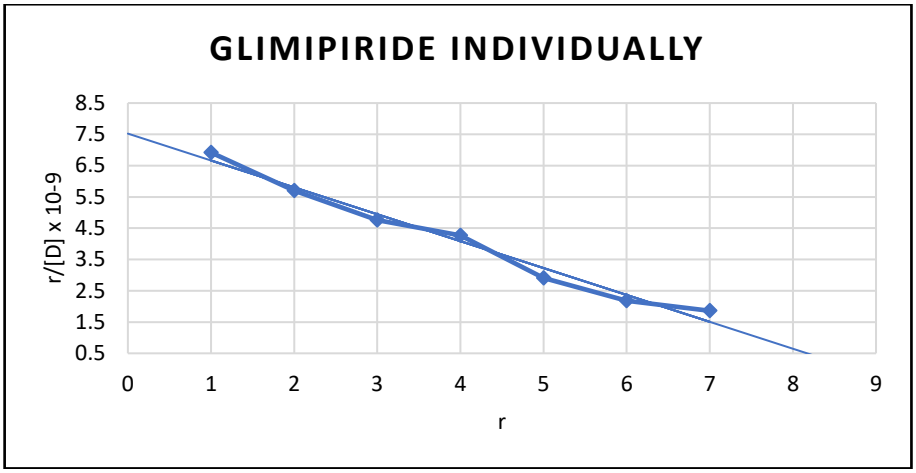


*Fig.19 Scatchard plot for protein binding of glimepiride individually*

**Number of Binding Sites and Affinity Constant of Glimepiride in Presence of *Pterocarpus Marsupium* Silver Nanoparticles**

From scatchard plots, x – intercept was found to be 0.00022and y – intercept was found to be 2.346. The number of binding sites for glimepiride in presence of *Pterocarpus marsupium* silver nanoparticles in BSA was found to be 19.32and affinity constants were found to be 0.12 respectively. When glimepiride is combined with silver nanoparticles the number of binding sites found to be increased when compared to glimepiride individually as represented in Fig.20

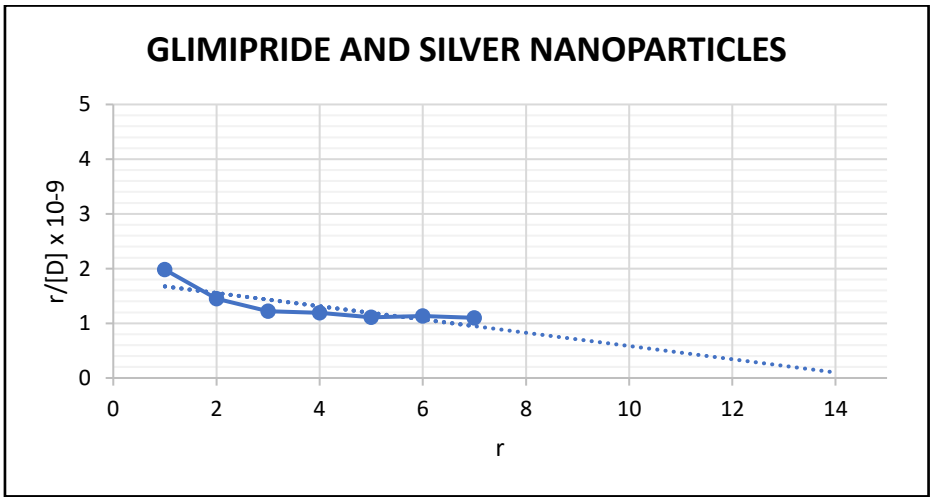


*Fig. 20 Scatchard plot for protein binding of glimepiride in presence of Pterocarpus marsupium silver nanoparticles (1 :1 mixture)*

## CONCLUSION

Green synthesis of silver nanoparticles was prepared using the *Pterocarpus marsupium* Roxb. Bark since this method helps bark extract (*Pterocarpus marsupium*) to serve as a reductant and a stabilizing agent for the synthesis of AgNPs. The UV–visible spectra peak at 429 nm confirmed the reduction of silver ions leading to AgNPs synthesis. FTIR observed that *Pterocarpus marsupium* extract acted as a stabilizer, reducing and capping agent for the synthesis of AgNPs. A drug entrapment efficacy test was carried out and the percentage of drug entrapment efficacy was found to be 95%. The stability of the nanoparticles was found to be stable with -22mV zeta potential estimated using Dynamic Light Scattering. Particle

size distribution was analyzed by using a zetasizer and the average particle size was found to be 36.44 nm with a polydispersity index. The size and morphology of particles were characterized using Scanning electron Microscopy. In Vitro drug release data have been observed in different time intervals, the release data were best fitted with Higuchi release with $R^2$ value being 0.9877. In vitro α amylase exhibited potential inhibitory activity for the aqueous extract of *Pterocarpus marsupium* silver nanoparticles and it was compared with standard acarbose. Literature surveys regarding diabetes mellitus have revealed that the patient administered with complementary or alternative medicine like herbal medicine along with synthetic drugs has led to the herb – drug interaction. Hence this research work has been carried out to find any pharmacokinetic interaction between green synthesized *Pterocarpus marsupium* silver nanoparticles with metformin and glimepiride using equilibrium dialysis method. The study was carried out by assessing percentage protein binding using metformin and glimepiride individually and in combination with green synthesized silver nanoparticles of *Pterocarpus marsupium*. The percentage protein binding of metformin and glimepiride individually and in combination with silver nanoparticles were found to decrease with the increase in time of contact. The concentration of metformin and glimepiride was found to be altered at different time intervals. From the scatchard plot the number of binding sites and affinity constant was found. The interaction study of green synthesized silver nanoparticles with metformin was observed to increase the free drug concentration of metformin in blood plasma. The interaction study of green synthesized silver nanoparticles with glimepiride was observed to increase the free drug concentration of glimepiride in blood plasma.

Hence, it can be inferred that, the concomitant administration of *Pterocarpus marsupium* silver nanoparticles with metformin and glimepiride should be avoided as the pharmacokinetic properties of the metformin and glimepiride was getting altered in presence of *Pterocarpus marsupium* silver nanoparticles.

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