EVALUATION OF ANTIDIABETIC ACTIVITY OF AQUEOUS EXTRACT OF BARK OF *Pterocarpus marsupium* SILVER NANOPARTICLES AGAINST STREPTOZOTOCIN AND NICOTINAMIDE INDUCED TYPE 2 DIABETES IN RATS

A Dissertation submitted to
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MASTER OF PHARMACY
IN
BRANCH- I - PHARMACEUTICS

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MARCH 2020

Certificate

This is to certify that the M. Pharm dissertation entitled **"Evaluation of Antidiabetic Activity of bark of *Pterocarpus marsupium* Silver nanoparticles against streptozotocin and nicotinamide induced type 2 Diabetes in rats."** being submitted to The Tamil Nadu Dr. M.G.R. Medical University, Chennai was carried out by **Ms. B Sai Krishna Priya (Registration No: 261810153)** in the Department of Pharmaceutics, College of Pharmacy, Sri Ramakrishna Institute of Paramedical Sciences, Coimbatore, under my direct supervision, guidance and to my fullest satisfaction.

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LIST OF ABBREVIATIONS

DDS	-	Drug delivery system
NDDS	-	Novel Drug Delivery System
NP	-	Nanoparticles
AgNPs	-	Silver nanoparticles
AEPM	-	Aqueous extract of *Pterocarpus marsupium*
PMAgNPs	-	*Pterocarpus marsupium* silver nanoparticles
FT-IR	-	Fourier Transform Infrared specrtroscopy
UV	-	Ultraviolet
FESEM	-	Field Emission Scanning Electron Microscope
EDX	-	Electron Dispersive X-ray spectroscopy analysis
rpm	-	Rotation per minute
cm	-	Centimeter
nm	-	Nanometer
mM	-	Milli Molar
et al.	-	and others
g	-	Gram(s)
hrs	-	Hour(s)
min(s)	-	Minutes
mg	-	Milligrams

ml	-	Millilitre
nm	-	Nanometer
µg	-	Micrograms
λ_{max}	-	Absorption maxima
KBr	-	Potassium Bromide
PARP	-	Poly (ADP ribose) Polymerase
JNK	-	C-Jun N-terminal kinase
NAD+	-	Nicotinamide Adenine Dinucleotide
ADP	-	Adenosine Di Phosphate
ATP	-	Adenosine Tri Phosphate
ROS	-	Reactive Oxygen Species
NO	-	Nitric oxide
DNA	-	Deoxy Ribo Nucleic Acid
SOD	-	Superoxide Dismutase
CAT	-	Catalase
GSSH	-	Glutathione reductase
GPx	-	Glutathione peroxidase
GSH	-	Reduced glutathione
MDA	-	Malondialdehyde
H_2O_2	-	Hydrogen peroxide

NADPH	-	Nicotinamide Adenine Dinucleotide Phosphate
SEM	-	Standard Error of Mean
STZ	-	Streptozotocin
TBA	-	Thiobarbituric Acid
TCA	-	Trichloroacetic Acid
WHO	-	World Health Organisation

INTRODUCTION

Delivering active compound to target site is one of the major problems in treatment of diseases. A conventional application of drug is characterized by limited effectiveness, poor biodistribution, lack of selectivity and these type of limitations can be overcome by controlling drug delivery. In controlled drug delivery, the drug is transported to the target place and its influence on vital diseases and unwanted side effects can be minimized. DDS protects the drug from rapid degradation or clearance and enhances drug concentration in target tissues, therefore lower doses are required. From this, new ideas on controlling the pharmacokinetics, pharmacodynamics, non-specific toxicity, immunogenicity, biorecognition and efficacy of drugs were generated. These new developments are called as drug delivery systems. Currently drug delivery technology has become refined and it takes into consideration, several factors such as bioavailability, drug absorption processes, pharmacokinetic processes, timing for optimal drug delivery etc. **(Krishna A *et al.,* 2011)**

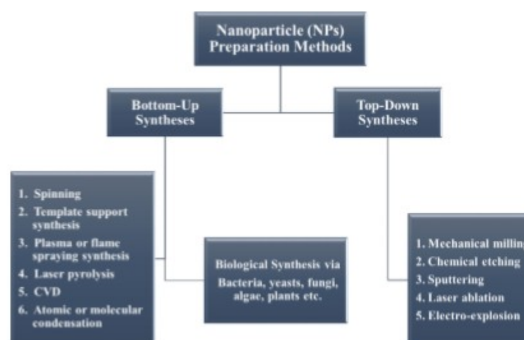
Targeted drug delivery, also known as smart drug delivery, is a method of treatment that involves the increase in medicament in one or few body parts in comparison to others. Therefore, it delivers the medication only to areas of interest within the body. This offers an improved efficacy of treatment and also reduces side effects. It differs from the conventional drug delivery system in that, it gets release in a dosage form while the former functions by the absorption of drug across biological membrane. Greogoriadis, in 1981, described the use of novel drug delivery for drug targeting as “old drug in new clothes”. There are four principle requirements for a successful targeted drug delivery system: retain, evade, target and release. Drug targeting to an area of interest within the body increases the therapeutic effectiveness as well as it reduces the toxicity that may arise otherwise. Two strategies are widely used for drug targeting to the desired organ/tissue i.e. active and passive targeting. **(Nidhi Mishra *et al.,* 2016)**

NANOPARTICLES:

Nanomaterials can be well-defined as a material with sizes ranged between 1 and 1000 nm, which influences the frontiers of nanomedicine starting from biosensors, microfluidics, drug delivery, and microarray tests to tissue engineering. Hence, they can move more freely in the human body as compared to bigger materials. Nanoscale sized particles exhibit unique structural, chemical, mechanical, magnetic, electrical, and biological properties. Nanomedicines have become well appreciated in recent times due to the fact that nanostructures could be utilized as delivery agents by encapsulating drugs or attaching therapeutic drugs and deliver them to target tissues more precisely with a controlled release.

Nanostructures stay in the blood circulatory system for a prolonged period and enable the release of amalgamated drugs as per the specified dose. Thus, they cause fewer plasma fluctuations with reduced adverse effects. Being nanosized, these structures penetrate in the tissue system, facilitate easy uptake of the drug by cells, permit an efficient drug delivery, and ensure action at the targeted location. The uptake of nanostructures by cells is much higher than that of large particles with size ranging between 1 and 10 µm. Hence, they directly interact to treat the diseased cells with improved efficiency and reduced or negligible side effects. **(Jayanta Kumar Patra *et al.,* 2018)** .

Synthesis of Nanoparticles



Figs. 1 preparation methods of nanoparticles

Types of Nanoparticles

NPs are broadly divided into various categories depending on their morphology, size and chemical properties. Based on physical and chemical characteristics, some of the well known classes of NPs are given as below.

Carbon-based NPs

- Fullerenes and carbon nanotubes (CNTs) represent two major classes of carbon-based NPs. Fullerenes contain nanomaterial that are made of globular hollow cage such as allotropic forms of carbon.
- They have created noteworthy commercial interest also in nanocomposites for many commercial applications such as fillers, efficient gas adsorbents for environmental remediation, and as support medium for different inorganic and organic catalysts.

Ceramics NP

- Ceramics NPs are inorganic nonmetallic solids, synthesized via heat and successive cooling. They can be found in amorphous, polycrystalline, dense, porous or hollow forms
- Therefore, these NPs are getting great attention of researchers due to their use in applications such as catalysis, photocatalysis, photodegradation of dyes, and imaging applications.

Semiconductor NP

- Semiconductor materials possess properties between metals and nonmetals and therefore they found various applications in the literature due to this property.
- Semiconductor NPs possess wide bandgaps and therefore showed significant alteration in their properties with bandgap tuning. Therefore, they are very important materials in photocatalysis, photo optics and electronic devices. As an example, variety of semiconductor NPs are found exceptionally efficient in water splitting applications, due to their suitable

bandgap and band edge positions.

Polymeric NPs

- These are normally organic based NPs and in the literature a special term polymer nanoparticle (PNP) collective used for it. They are mostly nanospheres or nanocapsular shaped.
- The former are matrix particles whose overall mass is generally solid and the other molecules are adsorbed at the outer boundary of the spherical surface.
- In the latter case the solid mass is encapsulated within the particle completely. The PNPs are readily functionalize and thus find bundles of applications in the literature.

Lipid-based NPs

- These NPs contain lipid moieties and effectively using in many biomedical applications. Generally, a lipid NP is characteristically spherical with diameter ranging from 10 to 1000 nm. Like polymeric NPs, lipid NPs possess a solid core made of lipid and a matrix contains soluble lipophilic molecules.
- Surfactants or emulsifiers stabilized the external core of these NPs. Lipid nanotechnology is a special field, which focus the designing and synthesis of lipid NPs for various applications such as drug carriers and delivery and RNA release in cancer therapy.

Metal NPs

- Metal NPs are purely made of the metals precursors. Due to well-known localized surface plasmon resonance (LSPR) characteristics, these NPs possess unique optoelectrical properties. NPs of the alkali and noble metals i.e. Cu, Ag and Au have a broad absorption band in the visible zone of the electromagnetic solar spectrum.
- The facet, size and shape controlled synthesis of metal NPs is important in

present day cutting-edge materials. Due to their advanced optical properties, metal NPs find applications in many research areas. **(Ibrahim Khan et al., 2019)**

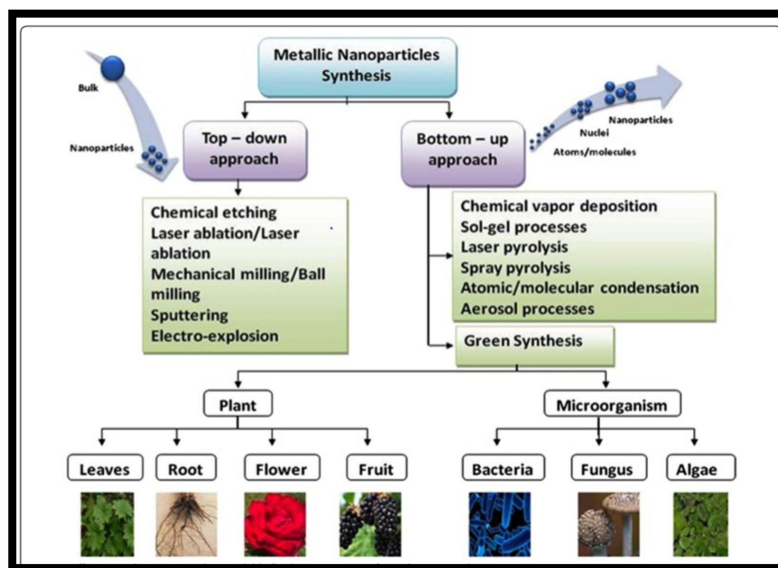


Fig 2 Synthesis of metallic nanoparticles

SILVER NANOPARTICLES

- Metal nanoparticles (MNPs) exhibit novel and size-related physico-chemical properties significantly different from their bulk counter part.
- The unique properties of MNPs have been ambassador of their potential uses in medicine, catalysis, optics, cosmetics, renewable energies, inks, microelectronics, medical imaging, environmental remediation, and biomedical devices. Besides, Ag-NPs exhibit a broad spectrum of bactericidal and fungicidal activity.
- Therefore, the use of MNPs became exceptionally trendy for the wide range of consumer goods, including plastics, soaps, pastes, food, and textiles, to enhance their market value.
- Among the wide range of metal nanoparticles, silver nanoparticles (Ag-NPs or nano silver) were the most popular, due to their unique physical, chemical, and biological properties when compared to their macro scaled

counterparts.

Advantages

The advantage of the nano silver over the other noble metals with respect to their physico-chemical properties are:

- Small loss of the optical frequency during the surface-plasmon propagation
- Non-toxic,
- High electrical and thermal conductivity,
- Stability at ambient conditions,
- Low cost than the other noble metals such as gold and platinum,
- High-primitive character,
- Wide absorption of visible and far IR region of the light,
- Surface-enhanced Raman scattering, chemical stability, catalytic activity, and nonlinear optical behavior. **.(Neelu chouhan 2017)**

Synthesis of silver nanoparticles

Nanosized metallic particles are unique and can considerably change physical, chemical, and biological properties due to their surface-to-volume ratio therefore, these nanoparticles have been exploited for various purposes.

In order to fulfill the requirement of AgNPs, various methods have been adopted for synthesis. Generally, conventional physical and chemical methods seem to be very expensive and hazardous. Interestingly, biologically-prepared AgNPs show high yield, solubility, and high stability.

Among several synthetic methods for AgNPs, biological methods seem to be simple, rapid, non-toxic, dependable, and green approaches that can produce well-defined size and morphology under optimized conditions for translational research. In the end, a green chemistry approach for the synthesis of AgNPs

shows much promise. **(Xi-Feng Zhang *et al*., 2016)**

Chemical method of synthesis can be subdivided into chemical reduction, electrochemical, irradiation assisted chemical and pyrolysis methods. Ag NPs synthesis in solution requires metal precursor, reducing agents and stabilizing or capping agent. Commonly used reducing agents are ascorbic acid, alcohol, borohydride, sodium citrate and hydrazine compounds.

However, physical methods do not require lethal and highly reactive chemicals and generally have a fast processing time.

Physical methods have another advantage over chemical methods in that the Ag NPs have a narrow size distribution, while the main demerits are consumption of high energy.

Thus, biological synthesis of Ag NPs from herbal extract and/or microorganisms has appeared as an alternative approach as these routes have several advantages over the chemical and physical methods of synthesis. It is also a well-established fact that these routes are simple, cost-effective, eco-friendly and easily scaled up for high yields and or production.

Biosynthesis of metal and metal oxide nanoparticles using biological agents such as bacteria, fungi, yeast, plant and algal extracts has gained popularity in the area of nanotechnology. **(Khwaja Salahuddin Siddiqi *et al.,* 2018)**

Physical and chemical methods

Generally, the synthesis of nanoparticles has been carried out using three different approaches, including physical, chemical and biological methods.

Physical method

The physical synthesis of AgNP includes the evaporation–condensation

approach and the laser ablation technique. Both approaches are able to synthesize large quantities of AgNPs with high purity without the use of chemicals that release toxic substances and jeopardize human health and environment. However, agglomeration is often a great challenge because capping agents are not used. In addition, both approaches consume greater power and require relatively longer duration of synthesis and complex equipment, all of which increase their operating cost. The evaporation–condensation technique typically uses a gas phase route that utilizes a tube furnace to synthesize nanospheres at atmospheric pressure. Various nanospheres, using numerous materials, such as Au & Ag have been synthesized by this technique. The center of the tube furnace contains a vessel carrying a base metal source which is evaporated into the carrier gas, allowing the final synthesis of NPs. The size, shape, and yield of the NPs can be controlled by changing the design of reaction facilities.

Advantages

- Speed
- Radiation used as reducing agents
- No hazardous chemicals involved

Disadvantages

- The tube furnace occupies a large space.
- Consumes high energy elevating the surrounding temperature of the metal source,
- Requires a longer duration to maintain its thermal stability
- Low yield
- Solvent contamination (**Sang Hun Lee, Bong-Hyun Jun 2017)**

Chemical methods

- Chemical methods use water or organic solvents to prepare the silver nanoparticles. This process usually employs three main components, such

as metal precursors, reducing agents, and stabilizing/capping agents.

- Basically, the reduction of silver salts involves two stages.

(1) nucleation

(2) subsequent growth.

- In general, silver nanomaterials can be obtained by two methods, classified as “top-down” and “bottom-up”.

Advantages

- Ease of production,
- Low cost
- High yield.

Disadvantages

- Extremely expensive.
- Toxic and hazardous.
- The manufactured particles are not of expected purity, as their surfaces were found to be sedimented with chemicals.
- Very difficult to prepare AgNPs with a well-defined size, requiring a further step for the prevention of particle aggregation.
- In addition, during the synthesis process, too many toxic and hazardous by products are excised out.

Chemical methods make use of techniques such as

- Cryochemical synthesis,
- Laser ablation ,
- Lithography,
- Electrochemical reduction ,
- Laser irradiation

- Sono-decomposition ,
- Thermal decomposition,
- Chemical reduction. **(Xi-Feng Zhang *et al.*, 2016)**

GREEN CHEMISTRY APPROACH FOR THE SYNTHESIS OF SILVER NANOPARTICLES

To overcome the shortcomings of chemical methods, biological methods have emerged as viable options.

- Recently, biologically-mediated synthesis of nanoparticles have been shown to be simple, cost effective, dependable, and environmentally friendly approaches and much attention has been given to the high yield production of Ag NPs of defined size using various biological systems including bacteria, fungi, plant extracts, and small biomolecules like vitamins and amino acids as an alternative method to chemical methods—not only for AgNPs, but also for the synthesis of several other nanoparticles, such as gold and graphene.
- Several studies reported the synthesis of AgNPs using green, cost effective and biocompatible methods without the use of toxic chemicals in biological methods.

The biological synthesis of nanoparticles depends on three factors,

(a) the solvent;
(b) the reducing agent
(c) the non-toxic material.

Advantages

- The major advantage of biological methods is the availability of amino acids, proteins, or secondary metabolites present in the synthesis process.
- The elimination of the extra step required for the prevention of particle

aggregation.

- The use of biological molecules for the synthesis of AgNPs is eco-friendly and pollution-free.
- Biological methods seem to provide controlled particle size and shape, which is an important factor for various biomedical applications.
- Using bacterial protein or plant extracts as reducing agents, we can control the shape, size, and mono dispersity of the nanoparticles.
- The availability of a vast array of biological resources,
- A decreased time requirement,
- High density & stability,
- The ready solubility of prepared nanoparticles in water. **(Xi-Feng Zhang *et al.*, 2016)**

Applications of silver nanoparticles

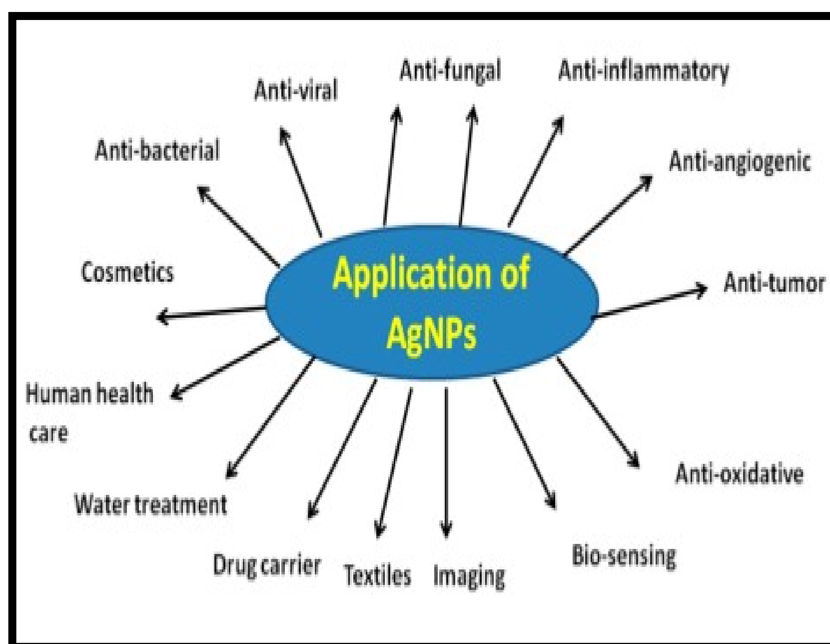


Figure no: 3 Applications of silver nanoparticles

DIABETES MELLITUS

Diabetes mellitus refers to the group of diseases that lead to high blood

glucose levels due to defects in either insulin secretion or insulin action. Diabetes develops due to a diminished production of insulin (type 1) or resistance to its effects (in type 2) both of which leads to hyperglycemia. Apart from insulin deficiency excess of other hormones like growth hormones, glucocorticoids and glucagon may also be involved. When the renal threshold for glucose reabsorption exceeds, glucose spill over into urine(glucosuria) and causes an osmotic diuresis (polyuria), which in turn results in dehydration, thirst and increased drinking (polydipsia). All forms of diabetes are treatable since insulin became medically available in 1921, but there is no cure. The injections by a syringe, insulin pump or insulin pen which is the basic treatment of type 1 diabetes. Type 2 is managed with combination of dietary treatment, exercise, medications and insulin. **(Rang HP *et al.,* 2008)**

Symptoms

The classic symptoms of untreated diabetes are weight loss, polyuria, polydipsia & polyphagia. Symptoms may develop rapidly in type 1 diabetes while they usually develop much more slowly and may be subtle or absent in type 2 diabetes. **(Nathaniel G *et al.,* 2007).** Several other signs and symptoms include blurry vision, headache, fatigue, slow healing of cuts, itchy skin. Prolonged high blood glucose can cause glucose absorption in the lens of the eye, which leads to change in its shape resulting in vision changes. A number of skin rashes that can occur in diabetes are collectively known as diabetic dermadromes. People may also experience episodes of diabetic ketoacidosis, a type of metabolic problem characterized by nausea, vomiting and abdominal pain, the smell of acetone in the breath, deep breathing known as kussmaul breathing and in severe cases a decreased level of consciousness. A rare but equally severe possibility is hyper osmolar non ketotic state which is more common in type2 diabetes and is mainly the result of dehydration. **(Suresh B 2016)**

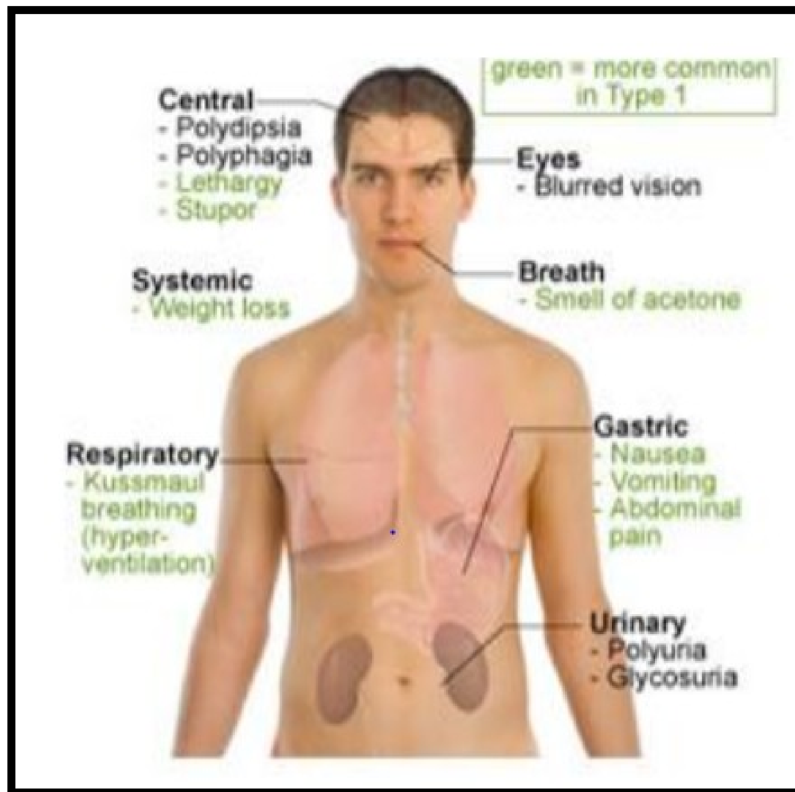


Fig: 4 Symptoms of diabetes mellitus

Classification of diabetes mellitus

Several international bodies like American Diabetic Association (ADA), World Health Organization (WHO), European Association for Study Diabetes (EASD), International Diabetic Federation (IDF) has attempted to classify diabetes and by now there is an universal consensus on the common classification. **(Baynes HW 2015)**

- Type 1 diabetes – Immune mediated & Idiopathic
- Type 2 diabetes
- Other specific types
- Gestational diabetes mellitus

Type 1 diabetes

Insulin dependent diabetes mellitus is a chronic disease characterized by hyperglycemia, impaired metabolism and storage of important nutrients, evidence of autoimmunity and long term vascular and neurologic complications. Also characterized by the loss of insulin producing beta cells of islets of Langerhans I the pancreas leading to deficiency. In type 1 diabetes, the body does not produce insulin and daily insulin injections are required. This is usually diagnosed during childhood or early adolescence and it affects about 1 in every 600 children. **(Baynes HW 2015)**

A) Immune mediated diabetes

Type 1 or juvenile onset diabetes results from a cellular mediated autoimmune destruction of the beta cells of the pancreas. In this form of diabetes, the rate of beta cell destruction is quite variable being rapid in some individuals and slow in others. **(Diabetes Care, 1997)**

B) Idiopathic diabetes

Some of these patients have permanent insulinopenia and are prone to ketoacidosis, but have no evidence of autoimmunity. Only a minority of patients with type 1 diabetes fall into this category. Individuals with this form of diabetes suffer from episodic ketoacidosis and exhibit varying degrees of insulin deficiency between episodes. **(Diabetes Care, 1997)**

Type 2 diabetes

Once known as adult – onset or noninsulin dependent diabetes is a chronic condition that affects the way the body metabolizes glucose which is the main source of fuel. In type 2 diabetes, the body either resists the effects of insulin or does not produce enough insulin to maintain a normal glucose level and if untreated, this can be life threatening. It is the result of failure to produce sufficient insulin and insulin resistance. Elevated blood glucose levels are managed with reduced food intake, increased physical activity and eventually oral

medications or insulin. **(Aguilar – Bryan L, *et al.,* 1995)**

Gestational diabetes mellitus

This type affects females during pregnancy. Some women have very high levels of glucose in their blood and their bodies are unable to produce enough insulin to transport all of the glucose into their cells, resulting in progressively rising levels of glucose. Undiagnosed or uncontrolled gestational diabetes can raise the risk of complications during childbirth. The baby may be bigger than he\ she should be. The majority of gestational diabetes patients can control their diabetes with exercise and diet. Between 10% to 20% of them will need to take some kind of blood-glucose- controlling medications. **(Abdulfatai B *et al.*, 2012)**

Diagnosis

Diabetes mellitus is diagnosed by demonstrating any one of the following:

- Fasting plasma glucose level ≥7.0 mmol/l (126 mg/dl)
- Plasma glucose ≥ 11.1 mmol (200 mg/dl) two hours after a 75g oral glucose load as in a glucose tolerance test
- Symptoms of hyperglycemia and casual plasma glucose ≥ 11.1 mmol/l (200 mg/dl)
- Glycated hemoglobin (Hb A 1 C) ≥6.5 %.

A positive result, in the absence of unequivocal hyperglycemia should be confirmed by a repeat of any of the above methods on a different day. It is preferable to measure a fasting glucose level because of the ease of measurement and the considerable time commitment of formal glucose tolerance testing, which takes 2 hours to complete and offers no prognostic advantage over the fasting test. According to the current definition, two fasting glucose measurements above 126 mg/dl (7.0 mmol/l) is considered diagnostic for diabetes mellitus. As per the WHO, people with fasting glucose levels from 6.1 to 6.9 mmol/l (110 to 125 mg/dl) are considered to have impaired fasting glucose. People with plasma glucose at or above 7.8 mmol/l (140 mg/dl), but not over 11.1 mmol/l (200 mg/dl),

2 hours after a 75g oral glucose load are considered to have impaired glucose tolerance. Of these two prediabetic states, the latter in particular is a major risk factor for progression to full – blown diabetes mellitus, as well as cardiovascular disease. The American diabetes association since 2003 uses a slightly different range for impaired fasting glucose of 5.6 to 6.9 mmol/l (100 to 125 mg/dl). Glycated hemoglobin is better than fasting glucose for determining risks of cardiovascular disease and death from any cause.

The rare disease diabetes insipidus has similar symptoms to diabetes mellitus, but without disturbances in the sugar metabolism and does not involve the same disease mechanisms. **(Diabetes care 2019).**

WHO diagnosis criteria (Mayfield J 1998)

Table : 1 WHO diagnosis criteria

CONDITION	2 HOUR GLUCOSE mmol/l (mg/dl)	FASTING GLUCOSE mmol/l (mg/dl)	HbA_{1c} (%)
Normal	< 7.8 (140)	<6.1 (<110)	<6.0
Impaired fasting glycaemia	< 7.8 (140)	<6.1 (<110) & <7.0(<126)	6.0 – 6.4
Impaired glucose tolerance	≥7.8 (≥140)	<7.0(<126)	6.0 – 6.4
Diabetes mellitus	≥11.1 (≥200)	≥7.0(≥126)	≥6.5

Prevalence of diabetes

The prevalence of diabetes for all age groups worldwide was estimated to be 2.8% in 2000 and 4.4% in 2030. The total number of people with diabetes is projected to rise from 171 million in 2000 to 366 million in 2030. The prevalence of diabetes is higher in men than women, but there are more women with diabetes than men. The urban population in developing countries are projected to double between 2000 and 2030. The most important demographic change to diabetes prevalence across the world appears to be the increase in the proportion of people

> 65 years of age. **(Sarah W, Gojka R, Anders G. 2004)**

List of countries with the highest numbers of estimated cases of diabetes for 2000 and 2030

The top three countries are the same as those identified for 1995 (India, China, U.S.). Bangladesh, Brazil, Indonesia, Japan and Pakistan also appear in the lists for both 2000 and 2030. The Russian federation and Italy appear in the list 2000 but are replaced by the Philippines and Egypt for 2030, reflecting anticipated changes in the population size and structure in these countries between the two time period. **(Wild S *et al.*, 2004)**

Table 2: List of countries with the highest numbers of estimated cases of diabetes for 2000 and 2030

Ranking	**2000**		**2030**	
	Country	**People with diabetes (millions)**	**Country**	**People with diabetes (millions)**
1	India	31.7	India	79.4
2	China	20.8	China	42.3
3	U.S.	17.7	U.S.	30.3
4	Indonesia	8.4	Indonesia	21.3
5	Japan	6.8	Japan	8.9
6	Pakistan	5.2	Pakistan	13.9
7	Russian	4.6	Brazil	11.3
8	Brazil	4.6	Bangladesh	11.1
9	Italy	4.3	Philippines	7.8
10	Bangladesh	3.2	Egypt	6.7

Drugs used in the induction of diabetes

Alloxan

Alloxan is the most prominent chemical compound used in diabetogenic research. In research, it is used for the induction of Type 1 diabetes. Alloxan is a urea derivative which causes selective necrosis of the beta cells of pancreatic islets. It has been widely used to induce experimental diabetes in animals such as rabbits, rats, mice and dogs with different grades of disease severity by varying the dose of alloxan use. **(Dsouza D, Lakshmidevi N. 2015)**

Streptozotocin (STZ,2-deoxy-2-(3-(methyl-3-nitrosoureido)-glucopyranose)

Streptozotocin is the permanent diabetes inducing drug. It is synthesized by a strain of the soil microbe *streptomyces achromogenes* (gram positive bacterium) with a broad spectrum of antibacterial properties. This is an unusual aminoglycoside containing a nitrosoamino group. The nitrosoamino group enables the metabolite to act as a nitric oxide (NO) donor. NO is an important messenger molecule involved in many physiological and pathological processes in the body. Streptozotocin is widely used to induce diabetes in rodent models. The frequently used single intravenous dose in adult rats to induce IDDM is between 40 and 60 mg/kg b.w. but higher doses are also used. STZ is also efficacious after intraperitoneal administration of a similar or higher dose, but single dose below 40mg/kg b.w. may be ineffective. NIDDM can easily be induced in rats by intravenous or intraperitoneal treatment with 100mg/kg b.w. STZ on the day of birth. Streptozotocin action in B cells is accompanied by characteristic alterations in blood with insulin and glucose concentrations. Two hours after injection, the hyperglycemia is observed with a concomitant drop in blood insulin. About 6 hours later, hypoglycemia occurs with high levels of blood insulin. Finally, hyperglycemia develops and blood insulin level decrease. These changes in blood glucose and insulin concentrations reflect abnormalities in B cell function. STZ

impairs glucose oxidation and decreases insulin biosynthesis and secretion. It was observed that STZ at first abolished the B cell response to glucose. Temporary return of responsiveness then appears which is followed by its permanent loss and cells are damaged. STZ is taken up by pancreatic B cells via glucose transporter GLUT2. A reduced expression of GLUT2 has been found to prevent the diabetogenic action of STZ. Intracellular action of STZ results in changes of DNA in pancreatic B cells comprising its fragmentation. The main reason for the STZ-induced B cell death is alkylation of DNA.

STZ is a nitric oxide (NO) donor and NO was found to bring about the destruction of pancreatic islet cells, it was proposed that this molecule contributes to STZ-induced DNA damage. STZ was found to generate reactive oxygen species, which also contribute to DNA fragmentation and evoke other deleterious changes in the cells. The formation of superoxide anions results from STZ action on mitochondria. These effects strongly limit mitochondrial ATP production and cause depletion of this nucleotide in beta cells. STZ induced DNA damage activates poly ADP-ribosylation. This process leads to depletion of cellular NAD^+ further reduction of the ATP content and subsequent inhibition of insulin synthesis. **(Szkdelski T.2001)**

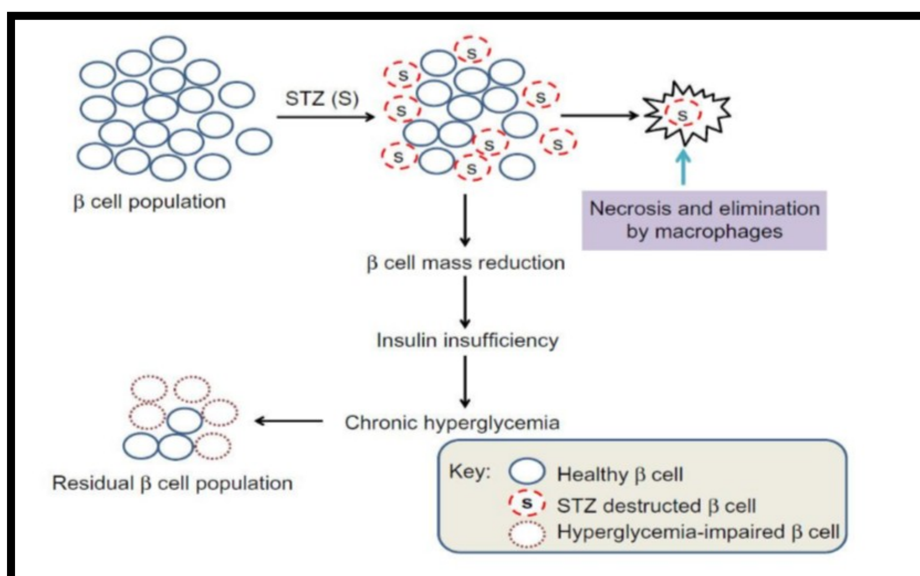


Fig:5 Mechanism of action of streptozotocin

Mechanism of action of streptozotocin and nicotinamide

Exposure of STZ to pancreatic β-cells results in damage via different pathways:

a. STZ destructs β-cells by damaging the major macromolecule i.e. DNA by alkylating. Alkylation of DNA results in fragmentation of DNA in β-cells. DNA injury by STZ leads to overstimulation of PARP-1 [(poly (ADP ribose) polymerase-1] in the insulin-secreting cells and is harmful to the cell as a result of a substantial depletion of the intracellular PARP-1 substrate, NAD+. NAD+ is an important molecule implicated

b. STZ also decrease the activity of islet of mitochondrial aconitase, reduces oxygen consumption by mitochondria and decreases the mitochondrial membrane potential

c. Generation of nitric oxide may play a role in the cytotoxic action of STZ on insulin-secreting cells. This assumption is supported by results demonstrating that scavengers of nitric oxide (NO) attenuate early DNA-strand breaks induced by STZ.

d. STZ generates low amounts of ROS in pancreatic β-cells. These effects may partially contribute to β-cells damage induced by STZ because of a weak antioxidant defense in these cells.

e. It has also been revealed that c-Jun N-terminal kinase (JNK) is also involved in the cytotoxicity of STZ. Increased activity of this enzyme is observed in the case of cellular stress leading to cell death. Studies on insulin-secreting cells exposed to STZ demonstrated increased activity of JNK, whereas inhibitors of this enzyme attenuated the cytotoxic action of STZ. Activation of JNK by STZ is supposed to be preceded by increased activity of PARP-1 since PARP-1 inhibitors are able to decrease the activity of both PARP 1 and JNK.

Nicotinamide

- NIC (pyridine-3-carboxamide) also known as niacinamide, is an active and water soluble form of vitamin B3 (niacin). Niacin converted into NIC in the body and is a food additive.
- NIC is essential to the coenzymes NADH and NADPH and consequently for numerous enzymatic reactions in the body including formation of ATP.
- NIC has neuro-protective and antioxidant functions and is given to animals to partially protect pancreatic beta cells against STZ.
- Numerous in vitro experimental studies conducted on isolated rat pancreatic islets have revealed that effects of STZ like reduction of pro-insulin biosynthesis, inhibition of glucose stimulated insulin secretion etc. were modulated by NIC.
- NIC shows its partial protection against STZ induced destruction of β-cells by two major mechanisms i.e. either by inhibiting PARP-1 or by increasing the concentration of NAD+ **(Kishore L Kajal 2017)**

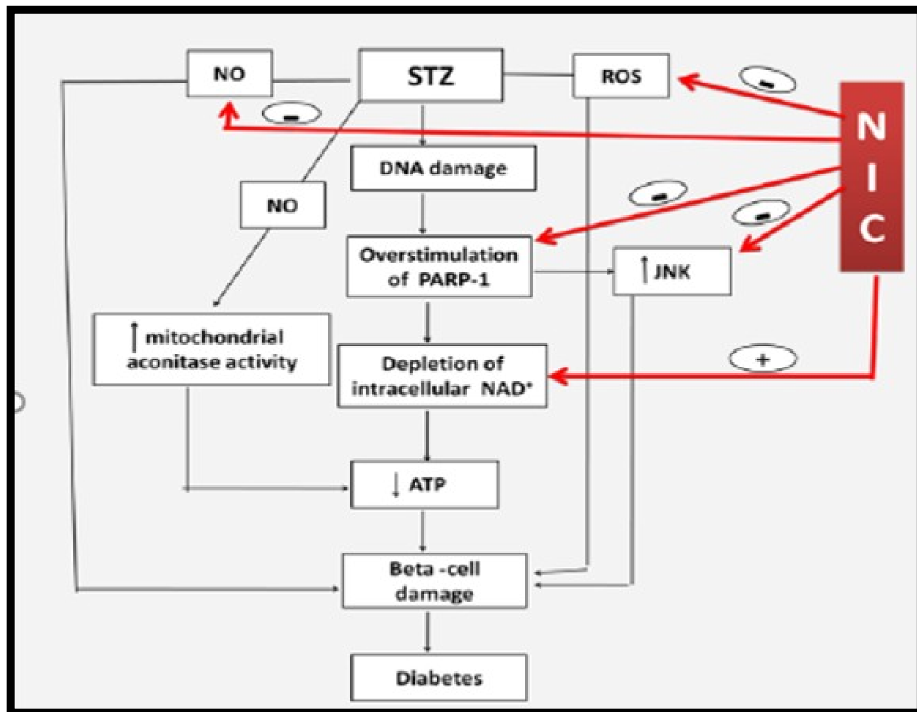


Fig :6 Role of nicotinamide on the action of streptozotocin

Effect of free radicals in diabetes mellitus

Diabetes mellitus results in severe metabolic imbalances and pathological changes in many tissues. Oxidative stress is believed to play a role in the development of complications in these tissues. There is an increasing evidence that in certain pathogenic states, especially chronic diseases, the increased production or ineffective scavenging of reactive oxygen species (ROs) may play a critical role. **(Johnas S *et al.*,2018)** Our body possess defense mechanisms, which in a healthy individual adequately control plasma ROS concentration under most conditions. However, in persons with diabetes, increased plasma ROS generation and a marked reduction in antioxidant defenses result in oxidative stress, which in turn can lead to many of the deleterious effects of diabetes. It is critical therefore that any therapy for diabetes mellitus include the direct and/or indirect reduction of oxidative stress. **(Bhor VM *et al* ., 2004)** Hyperglycemia causes the auto-oxidation of glucose, glycation of proteins and the activation of polyol metabolism. These changes accelerate generation of ROS and increase the oxidative chemical modification of lipids, DNA and proteins in various tissues. Oxidative stress may play an important role in the development of complications in diabetes such as lens cataracts, nephropathy and neuropathy. Glycation reactions occur in vivo as well as in vitro and are associated with chronic complications of diabetes such as aging and age- related diseases by an increase in oxidative chemical modifications of lipids, DNA and proteins. In particular, long-lived proteins such as lens crystallines, collagens and hemoglobin may react with reducing sugars to form advanced glycation end products (AGEs). It has been found out that hexanoyl modification formed by the reaction of oxidized lipids and proteins may be an important factor in oxidative stress. Macrophages and neutrophils play an important role in oxidative stress during hyperglycemia. Glutathione is thought to be an important factor in cellular function and defense against oxidative stress. **(Rahimi R *et al.*, 2005)**

Biogenesis of free radicals

Free radicals are the natural by products of many biochemical process within the cells and are essential part of aerobic life and metabolic processes. They are continuously produced by the body by normal use of oxygen such as respiration and also by some cell medicated immune functions. They are also found or generated through environmental pollutants, cigarette smoke, automobile exhaust fumes, radiation, air pollutants, pesticides, everyday stress such as inflammation, exercise, alcohol, UV light, fatty diet, toxins and drugs. Free radicals are also produced when phagocytes the cells that fight infection, destroy cells infected with bacteria or viruses with bursts of nitric oxides, super oxides and hydrochlorides. Hydroxyl radical is the most dangerous among all reactive oxygen intermediate species. **(Bruce BL *et al.*, 2000)**

Table: 3 Types of free radicals

S.NO.	FREE RADICALS	STRUCTURE
1	Superoxides	O_2
2	Nitric oxide	NO
3	Peroxy radical	RCOO
4	Hydrogen	H
5	Alkoxy	RO
7	Hydroxyl	OH
8	Singlet oxygen	O
9	Hydrogen peroxide	H_2O_2
10	Lipid peroxy radical	LO_2
11	Hypochlorous acid	HOCL
12	Peroxy nitrite	ONOO

13	Carbon centered free radicals	CCL_3

Diseases caused by free radicals

Diabetes mellitus- diabetic retinopathy, nephropathy, Cardiovascular diseases- atherosclerosis, alcohol induced cardiomyopathy, heart attack, hypertension, coronary artery disease, Gastrointestinal tract- pancreatitis, liver injury, ulcers, Eyes – cataract, glaucoma, degenerative retinal disease, ocular degeneration, Kidneys- kidney failure, nephrotic syndrome, Nephrotoxicity, Glomerulonephritis, Nervous system- Alzheimers disease, Parkinson's, Senile dementia, Memory loss, Multiple sclerosis, Lungs- Lungs cancer, Emphysema. **(Osawa T. Kato Y. 2005)**

ANTIOXIDANTS AND ITS ROLE IN TREATMENT OF DIABETES

Antioxidants are scavengers of free radicals, unstable and potentially damaging molecules generated by normal chemical reactions in the body. They reduce the effect of dangerous oxidants by binding together with these harmful molecules and decrease their destructive power. It can also help to repair damage already sustained by cells. Any therapy for diabetes, especially type 2 include the direct or indirect reduction of oxidative stress. Modification of certain environmental factors, for example, exercise can effectively prevent and even reverse the effects of diabetes, in part by reducing oxidative stress. Various hypoglycemic agents reduce oxidative stress indirectly by lowering blood glucose levels and directly acts as free radical scavengers. For example, gliclazide, a sulphonyl urea normally used to augment insulin release is an effective scavenger of superoxide and hydroxyl radicals. Recent studies have demonstrated that gliclazide can decrease oxidation of low-density lipoproteins and monocyte adhesion to the endothelium, the events that contribute to the development of atherosclerosis in diabetes mellitus. The insulin sensitizing agent troglitazone also appears to possess some antioxidant activity. **(Neeti S. 2014)** Certain antioxidant enzymes are produced within the body. The most commonly recognized of these

naturally occurring antioxidants are superoxide dismutase, catalase and glutathione. Superoxide dismutase changes the structure of oxidants and breaks them down into hydrogen peroxide. Catalase in turn breakdown hydrogen peroxide into water and tiny oxygen particles or gases. Glutathione is a detoxifying agent which binds with different toxins to change their form so that they are able to leave the body as waste. People with diabetes have elevated levels of free radicals and lower levels of antioxidants. Therefore, it is reasonable that antioxidants can play an important role in the improvement of diabetes. Use of antioxidants reduces oxidative stress and alleviates diabetic complications. Oxidative stress may play an important role in the pathogenesis of diabetic neuropathy, a condition characterized by pain and numbness of the extremities. Antioxidant treatment has demonstrated to prevent nerve dysfunction in experimental diabetes. Among their benefits, antioxidants make cholesterol less likely to stick to artery walls. **(Jacob V, Michel A. 1999)**

There are 3 types of antioxidants and it includes,

Primary antioxidants

This group prevents the formation of new radical species, that is either by converting existing free radical units to harmless molecule or by preventing formation of free radicals from other molecules. **(Rojita M. 2011)**

- Superoxide dismutase (SOD) which convert O_2 to H_2O_2
- Glutathione peroxidase (GPX) which converts H_2O_2 less harmful molecules
- Metal binding proteins, eg. Ferritin and ceruloplasmin which limits the availability of Fe^{2+} necessary for the formation of OH radicals. **(Birangane R, Chole D, Sathya P. 2011)**

Secondary antioxidants

- This type can retard lipid oxidation through a variety of mechanisms, including chelating of transition metal ions, oxygen scavenging,

replenishing hydrogen to primary antioxidants, absorbing UV radiation and deactivation of reactive species eg. Vitamin E, C, beta carotene, uric acid and albumin. **(Birangane R, Chole D, Sathya P. 2011)**

Tertiary antioxidants

- They repair biomolecules damaged by free radicals eg DNA repair enzymes, methionine sulphoxide reductase. **(Salem Z, Yousry MN, Camacho LH 2013)**

Treatment for type 1 diabetes

- Exercising regularly and maintaining a healthy weight
- Eating healthy foods
- Monitoring blood sugar regularly
- Injecting insulin

The goal is to keep the blood sugar level as close to normal as possible to delay or prevent complications. Although there are exceptions, generally, the goal is to keep the daytime blood glucose levels before meals between 80 and 120 mg/dL (4.4 to 6.7 mol/L) and your bedtime level between 100 and 140 mg/dL (5.6 to 7.8 mol/L) **(Singh PS *et al.*, 2003)**

Treatment for type 2 diabetes

- **Oral hypoglycaemic drugs**
- ✓ **Sulphonyl ureas –**

First generation	**Second generation**
Tolbutamide	Glibenclamide
Chlorpropamide	Glipizide
	Gliclazide & Glimiperide

- ✓ **Biguanides**

  Metformin

- ✓ **Meglitinides**

Repaglinide and Nateglinide

- ✓ **Thiazolidine diones**

  Rosiglitazon and Pioglitazone

- ✓ **Alpha glucosidase inhibitors**

  Acarbose and Miglitol.

The actions of antidiabetic drugs are given in the table 4

Table : 4 **Actions of antidiabetic drugs and side effect profile (Manisha M *et al.*,2007)**

Medication	Action	Advantages	Side effects
Meglitinides Repaglinide (prandin) Nateglinide (starlix)	Stimulate the release of insulin	Works quickly	Hypoglycaemia, weight gain, nausea, back pain, headache.
Biguanides Metformin (fortamet, Glucophage)	Inhibit the release of glucose from the liver, improve sensitivity to insulin	May promote modest weight loss and modest decline in LDL cholesterol	Well tolerated weight loss, nausea, diarrhea rarely lactic acidosis
Thiazolidinediones Rosiglitazone (avandia) Pioglitazone (actos)	Improve sensitivity to insulin; inhibit the release of glucose from the liver	May slightly increase the HDL level	Heart failure, stroke, heart attack, liver disease.
Alpha glucosidase inhibitors Acarbose (precose) Miglitol (glyset)	Slow the breakdown of some starch and some sugars	Doesn’t cause weight gain	Stomach pain, gas, diarrhoea

MEDICINAL PLANTS:

Nature always stands as a golden ark to exemplify the outstanding phenomenon of symbiosis. The biotic and abiotic elements of the nature are all independent. The plants were indispensable to man for his life. A nest of other useful products are supplied to him by the plant kingdom. Nature has provided a complete range of remedies to came an ailments of mankind. The knowledge of drugs has accumulated thought of years of a result of meaning inquisitive nature so that today we possess many health care. Archaeological evidence indicates that the use of medicinal plants data of least the paleotic, approximately 60000 years age. In india, medicinal plants are widely used in traditional systems of medicine like ayurvedic, unani, siddha and homeopathy. India with its valuable resources of natural flora has always been one of the richest sources of medicinal plants in the world. **(Bonaventura C *et al.*, 2015)**

Importance of herbal drugs

Antidiabetic allopathic drugs have their own side effect & adverse events like hypoglycemia, nausea, vomiting, hyponatremia, flatulence, diarrhea or constipation, alcohol flush, headache, weight gain, lactic acidosis, pernicious anemia, dyspepsia, dizziness, joint pain. So instead of allopathic drugs, herbal drugs are a great choice which is having more or less no side effect & adverse effects. Around 800 Indian herbs possess antidiabetic activity. Though complementary & alternative medicine (CAM) treatments are popular, scientific evidence support their application to diabetes care is scare. Instead of focusing on single modalities CAM practitioners prescribe complex, multi dietary intervention. Ayurvedic interventions may benefit patients with higher base line HbA1c value, warranting future research. **(Bonaventura C *et al.*, 2015)**

Natural origin and fewer side effects promote the use of herbal drugs in both developing and developed countries. In the last few years there has been an

exponential growth in the use of herbal drugs. Many traditional medicines in use are derived from medicinal plants, minerals and organic matter. According to WHO there are about 21000 plants which are used for medicinal purposes around the world. Among these 2500 species are found in India. India is called as the botanical garden of the world because of the rich herbal medicine resources. Very recently, two exhaustive reviews have been published based on global literature survey on 150 plants from india and 343 plants from different parts of the world. Some plats like *allium cepa, Allium sativum, Syzygium cumini, Momordica charantia, Gymnema sylvestre, Moringa pterygosperma* etc are well noticed by scientists as well as laymen in recent years. Biological activities of the plant products used against diabetes are related to their phytochemistry. Herbal products or plant products are rich in phenolic compounds, flavonoids, terpenoids, coumarins and other constituents which reduces the blood glucose levels. **(Sindhu M. Tanu S. 2013)**

HERBAL DRUG AS METALLIC NANOPARTICLE:

In biologically created nanoparticles, metals are combined with herbs which help in assimilation and delivery of the ingredients into the human body.

Counterparts are stable over longer period of time, require lower dosages, are easy to store and have sustainable availability. Synthesis of nanoparticles using biological entities has great interest due to their unusual optical, chemical, photo electro-chemical and electronic properties.

The synthesis and assembly of nanoparticles would benefit from the development of clean, nontoxic and environmentally acceptable 'green chemistry' procedure.

Due to distinct properties of silver nanoparticles such as good conductivity, chemically stable, catalytic activity, surface enhanced Raman scattering and antimicrobial activity have attracted and demandable research of interest in the field of nanotechnology.

In this era silver is used as antimicrobial agent. Recent focuses towards silver nanoparticle synthesis for increasing the treat of antibiotic resistance, caused by the misuse of antibiotic.

Herbal substances act as chelating agent for biosynthesized nanoparticles and because of such property the drug gets easily absorbed in the body and they target drug delivery and easily eliminated out of body.

Herb reduces the toxicity of metal, converting it to herbo -metallic form, enhancing its therapeutic quality so that it is effectively used by the body. Acharya Charaka says ' by this the quality of herbal drugs is attributed to mineral drugs. (**Meena Shamrao Deogade *et al.*, 2016)**

REVIEW OF LITERATURE

CorneliuTanase *et al*., (2019) Green synthesis is one of the rapid and best ways for silver nanoparticles (AgNP) synthesis. In the present study, synthesis and bioactivity of AgNPs has been demonstrated using water beech (Fagus sylvatica L.) bark extract. The physical and chemical factors such as time, metal ion solution, and pH, which play a vital role in the AgNPs synthesis, were assessed. The AgNPs were characterized by ultraviolet-visible (UV-Vis) spectrometry, Fourier transform infrared spectroscopy (FT-IR), and transmission electron microscopy (TEM). Antioxidant and antimicrobial activity of the obtained AgNPs was evaluated. AgNPs were characterized by color change pattern, and the broad peak obtained at 420–475nm with UV-Vis confirmed the synthesis of AgNPs. FT-IR results confirmed that phenol sand proteins of beech bark extract are mainly responsible for capping and stabilization of synthesized AgNPs. TEM micrographs showed spherical or rarely polygonal and triangular particles with an average size of 32 nm at pH = 9, and 62 nm at pH = 4. Furthermore, synthesized AgNPs were found to exhibit antioxidant activity and have antibacterial effect against Staphylococcus aureus, methicillin-resistant Staphylococcus aureus (MRSA), Escherichia coli, and Pseudomonas aeruginosa. These results indicate that bark extract of F. sylvatica L. is suitable for synthesizing stable AgNPs, which act as an excellent antimicrobial agent.

Sayan *et al*., (2019) Background: Diabetes mellitus (DM) can be defined as chronic hyperglycemia due to lack in insulin secretion and/or action. This study was designed to compare the antidiabetic activity of Coriandrum sativum L. with the standard antidiabetic drug, Metformin in Streptozotocin induced diabetic rats. Methods: Streptozotocin (STZ) was used to induce diabetes in the rats. Standard drug was metformin and test drug were Coriandrum sativum seed extract. 4 groups of 8 rats each were taken (normal control, diabetic control, streptozotocin

+ Coriandrum sativum and streptozotocin + metformin). Blood Sugar Levels (BSL) and HbA1C levels were estimated on day 0, 14 and 28 and day 28 respectively. Results: Streptozotocin administration resulted in significant rise in BSL. This rise was reduced with the administration of CS seed extract, but the reduction was more with chronic administration. It also reduced the HbA1C levels but couldn't attain total normoglycemia. However, the reduction of BSL was superior with Metformin compared to the test drug. Conclusions: Oral administration of Coriandrum sativum seed extract at a dose of 40 mg/kg has shown antihyperglycemic activity in streptozotocin induced diabetic rats. Thus, Coriandrum sativum may have considerable therapeutic benefit as an antidiabetic agent and can be suggested as a potential dietary add on.

Chen yu *et al.*, (2019): This article reports on silver nanoparticles (AgNPs) that were green-synthesized by using Eriobotrya japonica (Thunb.) leaf extract and their use for the catalytic degradation of reactive dyes. The properties of biogenic AgNPs were characterized using UV-vis absorption spectroscopy, field emission scanning electron microscope (FESEM), X-ray powder diffraction (XRD), transmission electron microscope (TEM), Fourier transforming infrared spectroscopy (FTIR), energy dispersive X-ray spectroscopy (EDS), and selected area electron diffraction (SAED) analysis. The UV-vis spectroscopy and X-ray analyses confirmed the formation of AgNPs and showed the strong absorbance around 467 nm with surface plasmon resonance (SPR). The mean diameter of biogenic AgNPs at room (20 ◦C), moderate (50 ◦C), and high temperatures (80 ◦C) were 9.26 ± 2.72, 13.09 ± 3.66, and 17.28 ± 5.78 nm, respectively. The reaction temperature had significant impacts on the sizes of synthesized AgNPs. The higher the synthesis temperature, the larger size and the lower catalysis activity for reductive decomposition of reactive dyes via $NaBH_4$. The results supported a bio-green approach for developing AgNPs with a small size and stable

degradation activity of reactive dyes over 92% in 30 min by using Eriobotrya japonica (Thunb.) leaf extract at pH 7, 20 ◦C, and 1:10 ratio of silver nitrate added to the leaf extract.

Jerushka S Moodley *et al.*, (2018) In this study we report on the synthesis of silver nanoparticles (AgNPs) from the leaf extracts of Moringa oleifera using sunlight irradiation as primary source of energy, and its antimicrobial potential. Silver nanoparticle formation was confirmed by surface plasmon resonance at 450 nm and 440 nm, respectively for both fresh and freeze-dried leaf samples. Crystallinity of AgNPs was confirmed by transmission electron microscopy, scanning electron microscopy with energy dispersive x-ray spectroscopy and Fourier transform infrared (FTIR) spectroscopy analysis. FTIR spectroscopic analysis suggested that flavones, terpenoids and polysaccharides predominate and are primarily responsible for the reduction and subsequent capping of AgNPs. X-ray diffraction analysis also demonstrated that the size range of AgNPs from both samples exhibited average diameters of 9 and 11 nm, respectively. Silver nanoparticles showed antimicrobial activity on both bacterial and fungal strains. The biosynthesised nanoparticle preparations from M. oleifera leaf extracts exhibit potential for application as broad-spectrum antimicrobial agents.

Mudassir khan *et al.*, (2018) Metallic nanoparticles are mostly used in medical fields as it has small size and can easily be used in different applications. In this review, silver nanoparticles and its antimicrobial activities are elaborated for ease of study from different research papers. It has been proven by researchers that nanoparticles have antimicrobial properties. In all metallic nanoparticles, silver nanoparticles have much attention towards antimicrobial properties. Chemicals are used as reducing agents to synthesize nanoparticles, hence it can be used in various biological risks and activities because of its toxic nature also having environmental friendly nature. Biological molecules screened out from

plant extracts are used in green synthesis as they are prominent over chemical methods. Plants have vital role in synthesis of metal nanoparticles because plants have biological molecules. This review describes plant diversity which mostly meets with silver nanoparticles having antimicrobial activities.

Manjula *et al.*, (2018) investigated the In-vitro anti-diabetic activity of root and aerial parts of Barleria noctiflora L.f. (Acanthaceae). The present investigation deals with morphological and in-vitro anti diabetic study of ethanolic extracts of root and aerial parts of selected plants. The plant material was extracted using soxlet apparatus and ethanol as a solvent. In-vitro anti-diabetic activity was determined by inhibition of α-glucosidase and inhibition of α-amylase studies. The extract showed a significant level of anti-diabetic activity when compared with standards. The results of ethanolic extracts of Barleria noctiflora are in support of traditional uses of the species to reduce blood glucose levels. It is highly likely that long term treatment may achieve the desired results with diabetes mellitus patients. The results obtained indicated that the extracts possessed significant level of activity in the highest concentration of extract was high effective as an anti-diabetic agent.

Graeme *et al.,* (2018) evaluated the in vitro antidiabetic activity and mechanism of action of Brachylaena elliptica (Thunb.) The aim of this study was to investigate the antidiabetic activity and mechanism of action of aqueous leaf extract prepared from Brachylaena elliptica. The inhibitory effects of the extract on the activities of different enzymes including alpha-amylase, alpha-glucosidase, pancreatic lipase, dipeptidyl peptidase IV (DPP-IV), collagenase, and CYP3A4 enzymes were evaluated. The extract was also tested against protein glycation using standard published procedure. The plant extract displayed low level of toxicity, where both concentrations tested did not induce 50% cell death. The extract caused a significant increase in glucose uptake in HepG2 liver cells, with efficacy significantly higher than the positive control, berberine. The crude extract also displayed no significant effect on muscle glucose uptake, triglyceride

accumulation in 3T3-L1, glucose metabolism in INS-1 cells, alpha-amylase, alpha-glucosidase, DPP-IV, lipase, protein glycation, and collagenase compared to the respective positive controls. The extract displayed a proliferative effect on INS-1 cells at 25 μg/ml when compared to the negative control. The findings provide evidence that B. elliptica possess antidiabetic activity and appear to exert its hypoglycemic effect independent of insulin.

Neelu Chauhan (2017) Day by day augmenting importance of metal nanoparticles in the versatile fields like, catalyst, electronic, magnetic, mechanic, optical optoelectronic, materials for solar cell and fuel cell, medical, bioimaging, cosmetic, ultrafast data communication and optical data storage, etc, is increasing their value. Nanoparticles of alkali metals and noble metals (copper, silver, platinum, palladium, and gold, etc.) have a broad absorption band in the visible region of the electromagnetic spectrum of light, because the solutions of these metal nanoparticles show the intense color, which is absent in their bulk counterparts as well as their atomic level. The main cause behind this phenomenon is attributed to the collective oscillations of the free conductive electrons that are induced by an interaction with electromagnetic field. The whole incidence is known as localized surface plasmonic resonance. Out of these, we have selected the silver nanoparticles for the studies. In this article, we will discuss the synthesis, characterization, and application of the silver nanoparticles. Future prospective and challenges in the field commercialization of the nanosilver is also discussed.

Nafeesa Khatoon *et al.,* (2017), Silver and its compound have been widely used since from ancient time for the treatment of bacteria and wound infections especially in patients of serious burns. The use of silver compounds has been deteriorated due to emergence of new therapeutic agents. In the past decade nanotechnology has acquired pace due to its ability of modifying metals ions into their nano range, which dramatically changes their chemical, physical and optical

properties. Silver nanoparticles have been proved a potential antimicrobial agent. Recently, the use of silver nanoparticles (AgNPs) has been greatly enhanced, due to the development of antibiotic resistance against several pathogenic bacteria. The silver nanoparticles have been widely employed in biomedical industry as coatings in dressings, in medicinal devices and in the form of nanogels in cosmetics and lotions, etc. There are well established protocols for the preparation of silver nanoparticles can be broadly classified into physical, chemical and biological protocols. The physical and chemical processes often involve high temperatures/pressure for the reaction and the use of hazardous chemicals. Therefore the research in synthesis of nanoparticles by biological methods is gaining importance. Plant extracts are considered cost-effective, environment friendly and efficient alternative for the large-scale synthesis of nanoparticles. In the present review, we critically assess the role of plants in synthesis of silver nanoparticles and their biomedical applications.

Chayarop *et al.,* (2017) reported the hypoglycaemic activity of Mathurameha, a Thai traditional herbal formula aqueous extract, and its effect on biochemical profiles of streptozotocin-nicotinamide induced diabetic rats. Extract of the herbal formula was the most potent extract for improving glucose tolerance of streptozotocin-nicotinamide-induced diabetic rats after single oral administration. After 2 weeks of daily oral administration and showed a dose-dependent glucose lowering effect. Most of the biochemical profiles of diabetic rats were improved, including the total cholesterol (TC), alkaline phosphatase (ALP), total protein, albumin, globulin, creatinine, and uric acid levels. The significantly increased triglyceride (TG) level observed in treated diabetic rats indicated a lack of a beneficial effect of the extract on lipid homeostasis. Nevertheless, there were no signs or symptoms of acute toxicity observed after oral administration of aqueous extract (5g/kg) to both male and female rats.

Taheri ***et al.,*** **(2017)** investigated the effect of pomegranate fresh juice versus pomegranate seed powder on metabolic indices, lipid profile, inflammatory biomarkers, and the histopathology of pancreatic islets of langerhans in streptozotocin-nicotinamide induced type 2 diabetic Sprague–Dawley rats. Type 2 diabetes mellitus (T2DM) is associated with hyperglycemia, inflammatory disorders and abnormal lipid profiles. Several functional foods have therapeutic potential to treat chronic diseases including diabetes. The present study aimed to evaluate the effects of pomegranate juice and seed powder on the levels of plasma glucose and insulin, inflammatory biomarkers, lipid profiles, and health of the pancreatic islets of langerhans in streptozotocin (STZ)-nicotinamide (NAD) induced T2DM in SpragueDawley (SD) rats. Forty healthy male SD rats were induced to diabetes with a single intraperitoneal injection of STZ (60 mg/kg b.w.)-NAD (120 mg/kg b.w.). Diabetic rats were orally administered with 1 mL of pomegranate fresh juice (PJ) or 100 mg pomegranate seed powder in 1 mL distilled water (PS), or 5 mg/kg b.w. of glibenclamide every day for 21 days. Rats in all groups were sacrificed on day 22. The obtained data was analyzed by SPSS software using One-way analysis of variance.

Rajgovind ***et al.,*** **(2015)**, In present study, copper oxide nanoparticles (CuONPs) synthesized by quick and eco-friendly phytogenicreduction of copper salt (copper sulphate CuSO4 .H2 O) solution with Pterocarpus marsupium extract. UV-VIS spectrometry indicated formation of nanoparticles via absorption spectra of copper colloidal solution at 442 nm. Phytosynthesis of CuONPs were further characterized by Transmission electron microscopy; scanning electron microscopy and Fourier transform infrared spectroscopy. The experimental results showed that diameter of CuONPs in colloidal solution were < 40 nm. Further, antibacterial activities of CuONPs were determined against Gram negative Escherichia coli-MTCC-9721, Proteus vulgaris- MTCC-7299, Klebsiella pneumonia- MTCC-9751 and Gram positive i.e. Staphylococcus aureus- MTCC-9442, Staphylococcus.

epidermidis- MTCC- 2639, Bacillus cereus- MTCC-9017 bacteria by well agar diffusion and microdilution method. Notably, The CuONPs showed an effective antibacterial activity against all test microorganisms where *K. pneumonia* and E.*coli* showed maximum ZOI and MIC respectively i.e. 24 mm and 6 µg/m

Busineni *et al.,* (2015) examined that streptozotocin-a diabetogenic agent in animal models. Streptozotocin is a permanent diabetogenic compound, it induces diabetes mellitus in laboratory animals by killing insulin-producing pancreatic β-cells. Streptozotocin is a toxic glucose analogue that preferentially accumulate in pancreatic beta cells via the low affinity glucose transporter GLUT2. The toxic effector mechanism of STZ starts with its decomposed products and the free radicals generated, which destroy the pancreatic β-cells by alkylating DNA, impairing mitochondrial system and inhibiting O-GlcNAcase. Its β-cell toxicity is reasoned through carbamoylation of proteins, alkylation of DNA, release of free radicals (ROS and RNS) and inhibition of O-GlcNAcse. β-cell insulin production is impaired by methylation of DNA through formation of carbonium ion (CH3+), resulting in the provocation of nuclear enzyme poly ADP ribose synthetase (PARP) and therefore, depletion of NAD+ and ATP. Free radicals generated during decomposition and metabolism of STZ diminish the activities of mitochondrial enzymes and inhibit O-GlcNAcse a (glycoside hydrolase) causing to tarnish energy levels of cell and suppressing biological function of proteins of islet cells. The above-mentioned harmful events induced by STZ are responsible for necrosis of pancreatic β-cells and induction of experimental diabetes mellitus in laboratory animal models.

Babak Sadeghi, F. Gholamhoseinpoor (2014), Biomolecules present in plant extracts can be used to reduce metal ions to nanoparticles in a single-step green synthesis process. This biogenic reduction of metal ion to base metal is

quite rapid, readily conducted at room temperature and pressure, and easily scaled up. Mediated Synthesis by plant extracts is environmentally benign. The involved reducing agents include the various water-soluble plant metabolites (e.g. alkaloids, phenolic compounds, terpenoids) and co-enzymes. Silver (Ag) nanoparticles have the particular focus of plant-based syntheses. Extracts of a diverse range of Ziziphora tenuior (Zt) have been successfully used in making nanoparticles. The aim of this study was to investigate the antioxidant properties of this plant and its ability to synthesize silver nanoparticles. Z. tenuior leaves were used to prepare the aqueous extract for this study. Silver nanoparticles were characterized with different techniques such as UV–vis spectroscopy, Fourier transform infrared (FT-IR) spectroscopy, X-ray diffraction (XRD), Scanning electron microscopy (SEM), and transmission electron microscopy (TEM). Transmission electron microscopy experiments showed that these nanoparticles are spherical and uniformly distributed and its size is from 8 to 40 nm. FT-IR spectroscopy revealed that silver nanoparticles were functionalized with biomolecules that have primary amine group (ANH2), carbonyl group, AOH groups and other stabilizing functional groups. X-ray diffraction pattern showed high purity and face centered cubic structure of silver nanoparticles with size of 38 nm.

U. K. Patil and M. K. Tripathy (2014) The antidiabetic potential of ethanolic extract of bijasar (Pterocarpus marsupium Roxb.) heartwood was evaluated in the streptozotocin-nicotinamide induced type 2 diabetic model. Graded doses of the ethanolic heartwood extract were administered to normal and experimental diabetic rats for 12 days. Significant ($p < 0.05$) reduction in fasting blood glucose levels were observed in the normal as well as in the treated diabetic animals. In addition, changes in body weight, serum lipid profiles, thiobarbituric acid reactive substance levels, glycosylated hemoglobin and liver glycogen levels assessed in the extract treated diabetic rats were compared with diabetic control

and normal animals. Significant results were observed in the estimated parameters, thereby justifying the use of the bijasar heartwood by Indian herbal practitioners for treatment of diabetics in different part of the Indian subcontinent.

Vishnu Kiran M. and Murugesan S. (2013) Diabetes mellitus is a multifunctional disorder characterized by hyperglycemia resulting from increased hepatic glucose production, diminished insulin secretion resulting in impaired insulin action. The intestinal digestive enzymes α-glucosidase and α-amylase plays a key role in carbohydrate digestion, one main antidiabetic approach is to reduce the post prandial glucose level in blood by inhibition of alpha glucosidase and alpha amylase enzymes. Silver nanoparticles were prepared by green synthesis, where silver nitrate was taken as a metal precursor and marine red alga Halymenia poryphyroides as a reducing and capping agent. The formation of silver nanoparticles was characterized by UV–Nano photometer, FT-IR, SEM and XRD. In the present study *invitro* antidiabetic activity was studied from the biosynthesis of silver nanoparticles from the marine red alga Halymenia poryphyroides as a pre-requisite for the in vivo studies further. The assay results of silver nanoparticles showed dose dependent significantly ($P<0.005$) increase in percentage inhibitory activity against α–amylase enzyme, at a concentration of 0.2 mg/ml 26.20 ± 0.02% inhibition was seen and at 1.0 mg/ml 91.30 ± 0.02% inhibition was observed, similarly dose dependent significantly ($P<0.005$) increase in percentage inhibitory activity against α-glucosidase enzyme was also observed where in at lower concentration of 0.2 mg/ml 33.20 ± 0.01% of inhibition and at higher concentration of 1.0 mg/ml 89.10 ± 0.01% inhibition were recorded.

Naquvi *et al.,* (2012) studied the antidiabetic activity of aqueous extract of Coriandrum sativum L. (Apiaceae) on streptozotocin induced diabetic rats. In doses of 250mg/kg and 500 mg/kg the aqueous extract showed significant decrease in blood glucose level. It also decreased total cholesterol level and

increased high density lipid cholesterol significantly. The perusal of data revealed that the aqueous extract of fruits of C. sativum decreased the blood glucose level statistically significant when compared with diabetic control.The 500 mg/kg bw dose was found better than 250 mg/kg b.w however, the standard glimepiride was better in comparison to both doses. Treatment with aqueous extract decreased total cholesterol level and increased high density lipid cholesterol level, which was statistically significant when compared with normal control. The above findings justified the antidiabetic activity of fruits of C. sativum which proved the traditional claim of antidiabetic activity of the aqueous extract.

V. Vats *et.al.,* (2012) developed a research on the hypoglycemic effect of the aqueous (Aq) extract of the bark of Pterocarpus marsupium (PM) and alcoholic (Alc) extract of seeds of Trigonella foenum-graecum (FG) and leaves of Ocimum sanctum (OS) was investigated in both normal and alloxan-induced diabetic rats. The aqueous extract of PM (1 g/kg PO) significantly ($P< 0.001$) reduced the blood sugar levels from 72.32± 5.6 to 61.35± 1.2 mg% 2 h after oral administration of the extract and also significantly lowered the blood glucose in alloxan diabetic rats from 202.91± 5.44 to 85.22± 11.28 mg% 21 days after daily oral administration of the extract ($P< 0.001$). Similarly, reduction was seen with alcoholic extract of FG (74.33± 4.77 to 60.56± 1.9 in normal rats and 201.25± 7.69 to 121.25± 6.25 in diabetic rats) ($P< 0.001$) and OS (204.48± 11.0 to 131.43± 7.86 in normal rats and 73.54± 3.7 to 61.44± 2.3 in diabetic rats ($P< 0.001$. Although the study was concluded that the anti-hyperglycemic effect with OS and FG plateaued at the end of the second week and was less pronounced than PM.

Szekudelski *et al., (*2001) reported that alloxan and streptozotocin are widely used to induce experimental diabetes in animals. The mechanism of their action in beta cells of the pancreas has been intensively investigated and now is quite well understood. The cytotoxic action of both these diabetogenic agents is

mediated by reactive oxygen species, however, the source of their generation is different in the case of alloxan and streptozotocin. Alloxan and the product of its reduction, dialuric acid, establish a redox cycle with the formation of superoxide radicals. These radicals undergo dismutation to hydrogen peroxide. There after highly reactive hydroxyl radicals are formed by the fenton reaction. The action of reactive oxygen species with a simultaneous massive increase in cytosolic calcium concentration causes rapid destruction of beta cells. Streptozotocin enters the beta cell via a glucose transporter (GLUT2) and causes alkylation of DNA. DNA damage induces activation of poly ADP-ribosylation, a process that is more important for the diabetogenicity of streptozotocin than DNA damage itself. Poly ADP-ribosylation leads to depletion of cellular NAD+ and ATP. Enhanced ATP dephosphorylation after streptozotocin treatment supplies a substrate for xanthine oxidase resulting in the formation of superoxide radicals. Consequently, hydrogen peroxide and hydroxyl radicals are also generated. Furthermore, streptozotocin liberates toxic amounts of nitric oxide that inhibits aconitase activity and participates in DNA damage. As a result of the streptozotocin action, B cells undergo the destruction by necrosis.

Bandana *et al.*, (2003) studied the analgesic, anti-inflammatory and local anaesthetic activity of *Moringa pterygosperma* in laboratory animals. *Moringa pterygopserma* Gaertn (Moringaceae) (Drum stick tree) is known to possess various medicinal properties. It is grown in the sub-Himalayan ranges and is commonly cultivated in India and Burma. Because of its medicinal properties, it was used in the treatment of rheumatism, venomous bite, gout and also as rubefacient and vesicant. There is no proper scientific evaluation of the plant regarding its pharmacological and toxicological aspects, hence, a detailed study of its analgesic, anti-inflammatory and local anaesthetic properties was undertaken in order to establish its traditional claim. Analgesic activity was tested in mice using methanolic extract of plant.acetic acid induced writhing episodes were

significantly and dose-dependently reduced. Carrageenin induced paw oedema in mice was significantly reduced after oral administration. Furthermore, its local anaesthetic activity were tested in frog in guinea pig models, and it was seen that in both animals, the plant produces significant local anaesthetic activity.

Asgari *et al.*, (1999) reported the anti-oxidant effect of flavonoids on hemoglobin glycosylation. A high glucose concentration has been found to lead to the glycosylation of amino groups of lysine residue in proteins. The addition of reducing agent not only prevents this reaction but also reverses it. On the other hand, flavonoids which found in plant sources have antioxidant properties. Since the glycosylation of protein is an oxidation reaction, therefore, antioxidants should be able to prevent this reaction. In this study, the best concentration and time to incubate glucose with hemoglobin was investigated. Then the glycosylation degree of hemoglobin in the presence of flavonoids and their absence was measured by means of a colorimetric method.

AIM AND OBJECTIVE

AIM

The aim of the present work was to formulate & evaluate the antidiabetic activity of the bark of *Pterocarpus marsupium* silver nanoparticles against streptozotocin and nicotinamide induced type 2 diabetes in rats.

OBJECTIVE

Diabetes mellitus is a major health problem. The disease is found in all parts of the world and is rapidly increasing in most parts of the world. Diabetes mellitus is characterized by increased concentration of blood glucose due to derangement in carbohydrates metabolism and defective insulin production. These metabolic disturbances result in acute and long term diabetic complications. Free radicals and oxidative stress may act as common pathway to diabetes itself as well for later complications. The increased oxidative stress in diabetes includes the autoxidation of glucose and non-enzymatic glycation and also changes in antioxidant defense system.

Even though there are many allopathic drugs like biguanides and sulfonyl are available along with insulin for the treatment of diabetes mellitus but have side effects associated with their uses. Most of the plants contain substances like glycosides, alkaloids, terpenoids, flavonoids etc and they are considered to be safe and effective for the treatment of diabetes.

An attempt has been made to formulate silver nanoparticles with the herbal drug and evaluated against streptozotocin and nicotinamide induced type 2 diabetes in *Wistar* rats. Also this research work focuses on green chemistry which is an eco-friendly & environmentally accepted procedure. Herbal substances act as chelating agent for biosynthesized nanoparticles and because of such property the drug gets easily absorbed in the body and they target drug delivery and easily eliminated out of body.

Pterocarpus marsupium is one of the traditional herbal medicine reported to possess anti-inflammatory, cardiovascular effect.

PLAN OF WORK

STEP 1

Review of literature.

STEP 2

Collection & authentication of the bark of *Pterocarpus marsupium.*

STEP 3

Processing of the sample - Drying & Pulverizing.

STEP 4

Aqueous extraction of the bark of *Pterocarpus marsupium* .

STEP 5

Processing of the material into silver nanoparticles.

STEP 6

Separation of silver nanoparticles.

STEP 7

Characterization of *Pterocarpus marsupium* silver nanoparticles.

STEP 8

Invitro antidiabetic activity.

STEP 9

In vivo screening of the *Pterocarpus marsupium* silver nanoparticles against streptozotocin and nicotinamide induced antidiabetic activity in rats.

STEP 10

Tabulation, compilation of results and statistical analysis of data obtained.

PLANT PROFILE

Scientific classification

Kingdom: Plantae

Subkingdom: Tracheobionta

Super division: Spermatophyta

Division: Magnoliophyta

Class: Magnoliopsida

Sub class: Rosidae

Order: Fabales

Family: Fabaceae

Genus: Pterocarpus Jacq

Species: Pterocarpus marsupium Roxb.

Different species in the genus of *Pterocarpus* family

Pterocarpus acapulcensis, Pterocarpus claessensil, Pterocarpus lucens, petrocarpus officinalis, Pterocarpus osun, petrocarpus rohrii, Pterocarpus mildbraedii, Pterocarpus orbiculatus, Pterocarpus mutondo, petrocarpus amazonum, Pterocarpus angolensis.**(kritikar rao and Basu 1981)**

Vernacular names of *Pterocarpus marsupium* family

English: Indian kino

Tamil: vengai

Malayalam: Malabar kino

Hindi: vijayasar

Description: It is a deciduous tree about 90 feet or more high.

Bark: stem bark is grey brown. Heart wood is yellow and bark yields a reddish gum.

Leaves: 3-5 inches long have 5-7 leaflets long, margin wavy and obtuse. The petioles are round, smooth and waved from leaftlet, 5 or 6 inches long and no stipules.

Flowers: 1.5 c.m. long, very numerous, white, with a small tinge of yellow, stamens are 10. United near the base but soon dividing into two parcels of 5 each; anthers are globose and 2 lobed.

Origin, Distribution & Habitat

P. marsupium is growing in defoliate and evergreen jungles of Southern, Western and Central regions of India. It is present generally in Gujarat, Bihar, West Bengal, Orissa, Uttar Pradesh, Western Ghats, Kerala, Karnataka, and Madhya Pradesh, of prominent India, Srilanka and Nepal. It develops generally on hills or undulating lands or rocky grounds up to a height of 150 to 1100 meter. The usual rainfall of its habitat ranges starting from 750 to 2000 mm and even

more in Southern India. The maximum temperature was ranged from 35°C to 48°C and minimum temperature was ranged from 0°C to 18°C. It can increase in huge variety of soils and geographical situations like quartzite, shale, conglomerates, lateritic, gneiss and sandstone. It favors well-drained sandy and sedimentary soil to loamy soil. The species is adequate light loving and the young seedlings are frost-tender.

Traditional uses: *Pterocarpus marsupium* has been traditionally used in the treatment of leucoderma, elephantiasis, diarrhea, cough, discoloration of hair and rectalgia.14 It is nontoxic and useful in jaundice, fever, wounds, diabetes, stomach ache and ulcer. **(Mohd SaidurRahman *et al.,* 2018)**

Parts used: Bark, **(Trease and Evans 2002)** wood, Flower, Stem, Heartwood, Leaves, Gum **(Kritikar rao and Basu 1981)**

Ethnobotanical Uses

The phloem of stem contains red astringent fluid present in secretory cell, which exudes after given incision. Kino is odourless but has astringent taste and sticks in the teeth, colouring the saliva red in colour. As astringent it is used in diarrhoea, dysentery etc. Bruised leaves are applied on fractures, leprosy, leucoderma, skin diseases, sores and boils, Constipation, depurative, rectalgia, ophthamology, hemorrhages and rheumatoid arthritis. Bark is used as diuretic in Gabon and fresh leaves are used as food in Nizeria. Leaves are used in GIT disorders. Stem in the treatment of neurological problems.

Bark and wood have been used to treat diabetics marsupin and Pterostilbene significantly lower the blood glucose levels useful in NIDDM. Traditionally the plant has been used for Angina, Cancer, and used as Cardiotonic and brain tonic **(Mohd Saidur Rahman et al., 2018)**

Reported activities

Antidiabetic, Antioxidant, Antihyperlipidemic, Antibacterial, Hepatoprotective activity, Anti-inflammatory, Antidiarrheal, Antiulcer, Cardiotonic activity, Nootropic activity, Antiglycation, Anticataract, Aphrodisiac activity, Anthelmintic activity, Analgesic activity. **(Mohd Saidur Rahman et al., 2018)**

Chemical constituents

Pterostilbene 4-5%, alkaloids 0.4%, tannins, protein pentosan, pterosupin, pseudobaptigenin, liquiritigenin, isoliquiritigenin, garbanzol, 5-de-oxykaempferol, epicatechin, marsupol, carsupin, marsupinol. Aqueous extract of the heartwood of PM contains five new flavanoids c-glucosides namely 6- hydroxyl-2-(4-hydroxybenzyl)-benzo-furan-7-c-a-D- glucopyranoside, 3-(a-methoxy-4-hydroxybenzylidene)-6-hydroxybenzo-2(3H)furanonE-7-C-a-D glucopyranoside, lucopyranoside, 8-(C-a-Dglucopyranosyl)-7,3,4-trihydroxyflavone and 1,2-bis (2,4-dihydroxy, 3-Cglucopyranosy) – ethanedione and two known compounds C-a-D-glucopyranosyl 2, 6-dihydroxyl benzene and sesquiterpene. **(J.Bagyalakshmi and Haritha 2017)**

Fig : 7 *Pterocarpus marsupium* roxb.

EXCIPIENT PROFILE

SILVER NITRATE

IUPAC name : Silver nitrate

Synonyms : Nitric acid silver (1+) salt

Molecular Formula : Ag No3

Molecular Weight : 169.872

Colour : White

Odour : Odour less solid

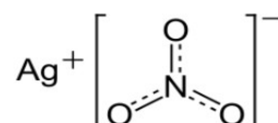
Density : 4.35 g/cm3

Melting point : 209.7 °C

Boiling point : 440 °C pH : 5.4-6.4 (100g/l, H2O, 20°C)

Storage temperature : 2- 8ºC

Structure of silver nitrate



Solubility : Soluble in Water, Acetone, Ammonia, Ether, Glycerol,

Uses : Anti-Infective Agents, Disinfection, destruction of cutaneous warts, Precursor to other silver compounds, Halide abstraction, Organic synthesis

Safety

As an oxidant, silver nitrate should be properly stored away from organic compounds. Despite its common usage in extremely low concentrations to prevent gonorrhea and control nose bleeds, silver nitrate is still very much toxic and corrosive. Brief exposure will not produce any immediate side effects other than the purple, brown or black stains on the skin, but upon constant exposure to high concentrations, side effects will be noticeable, which include burns. Long term exposure may cause eye damage. Silver nitrate is known to be a skin and eye irritant **(https:/wiki/Silver_nitrate).**

MATERIALS AND EQUIPMENT'S

SI NO	MATERIALS USED	SOURCE
1	Silver nitrate	Qualigens fine chemicals, Mumbai
2	Pterocarpus marsupium Roxb Bark	Natural Source., Thalassery
3	Sodium potassium tartrate	Merck
4	Starch	SD chemical limited
5	α amylase enzyme	Himedia, Mumbai
6	3,5- Dinitrosalicylic acid	Himedia, Mumbai
7	Sodium hydroxide	Sigma
8	Sodium chloride	SD Chemical Limited
9	Sodium phosphate monobasic	Sigma
EQUIPMENTS USED		
1	Magnetic Stirrer	REMI – 2MLH
2	UV Spectrophotometer	JASCO V-530
3	FT-IR Spectrometer	FTIR JASCO – 4100
4	pH meter	pH TESTER 1,2 (EUTECH)
5	Zeta Sizer	MALVERN
6	SEM	Hitachi X650, Tokyo, Japan
7	Dialysis membrane 50	Himedia, Mumbai

REAGENTS & INSTRUMENTS USED FOR INVIVO STUDIES

Chemicals used

Ethylacetate, Glucose, streptozotocin, nicotinamide, glibenclamide, potassium carbonate, ethanol, bovine serum albumin, sodium carbonate, sodium bicarbonate, potassium dihydrogen phosphate, sodium hydroxide, thiobarbituric acid, trichloro acetic acid, potassium dichromate, NADPH, glutathione, tris-HCl buffer, sodium azide, ellman's reagent, hydrogen peroxide and glacial acetic acid were procured from sigma Aldrich.

Instruments used

Centrifuge (Remi instruments Ltd., Kolkata), digital balance (Sartorius Ltd.,USA), Shimadzu-Jasco V-630 UV/Vis spectrophotometer, ELECO 1/27 pH meter.

EXPERIMENTAL METHODOLOGY

SAMPLE COLLECTION

Pterocarpus marsupium bark were collected from Thalassery, Kerala.

Authentication

The plant specimen was identified and authenticated by Botanical survey of India, Southern regional centre, Coimbatore.

Drying and Pulverizing

The bark and wood were collected and shade dried. It was grounded into fine coarse powder with electronic blender and passed and kept in a well closed container in a dry place.

Preparation of aqueous extract of *Pterocarpus marsupium* Roxb

Fifty grams of the bark powder was stirred with 500 mL of deionised 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. **(Chen yu *et al.,* 2018)**

Phytochemical screening:

Preparation of Test Solution

The filtered aqueous extract of bark of *Pterocarpus marsupium* Roxb was used as a test solution for preliminary screening of phytochemical constituent.

PRELIMINARY QUALITATIVE PHYTOCHEMICAL ANALYSIS

Phytochemical screening

Chemical tests were carried out using the extract of *Pterocarpus*

marsupium for the presence of phytochemical constituents.

Tests for tannins and phenolics

- To the solution of the extract, a few drops of 0.1% ferric chloride was added and observed for brownish green or a blue-black coloration.

Tests for saponins

- About 10ml of the extract was mixed with 5ml of distilled water and shaken vigorously for a stable persistant-froth. The frothing was mixed with 3 drops of olive oil and shaken vigorously and then observed for the formation of emulsion.

Test for flavonoids

- To a portion of the extract concentrated H2SO4 was added. A yellow colouration indicates the presence of flavonoids. The yellow coloration disappeared on standing.
- Few drops of 1% AlCl3 solution was added to a portion of extract. A yellow coloration indicates the presence of flavonoids .
- A portion of the extract was heated with 10ml of ethyl acetate over a steam bath for 3min. The mixture was filtered and 4ml of the filtrate was shaken with 1ml of dilute ammonia solution. A yellow coloration indicates a positive test for flavonoids.

Tests for terpenoids

- About 5ml of the extract was treated with 2ml of chloroform and about 3ml concentrated H_2SO_4 was carefully added to form a layer. A reddish brown coloration of the interface indicates the presence of terpenoids.

Tests for alkaloids

- A small portion of the extract was stirred with few drops of dil.HCl and filtered.
- To the filtrate, dragendroff's reagent (potassium bismuth iodide solution) was added and an orange brown precipitate indicates the presence of alkaloids.
- To the filtrate, Mayer's reagent was added and a cream precipitate indicates the presence of alkaloids.

Test for Starch

- To the aqueous extract add weak aqueous Iodine solution.

Test for Proteins

- **Warming Test**: Heat the test solution in a boiling water bath.

Test for Steroids

- **Salkowski Test**: Treat the extract with few drops of concentrated sulphuric acid**. (C. K.Kokate *et.al*)**

PREFORMULATION STUDY:

Solubility Test

About 1 mg of *Pterocarpus marsupium* Roxb. bark extract powder was taken in a test tube and solubility in ethanol, water, chloroform and diethyl ether, dimethyl sulphoxide were checked.

UV- VIS spectral analysis of *Pterocarpus marsupium* Roxb bark

Preparation Of Calibration Curve Of Pterocarpus marsupium Roxb Bark Extract

Twenty-five milligrams of crude extract was dissolved in phosphate buffer with a pH 7.4 and further diluted to 50mL of solvent, in volumetric flask to get a concentration of 500 µg/mL. This was treated as stock solution. Various aliquots of stock solution were diluted further to get different concentrations. The resultant

solutions were scanned for λ max in the range of 200-400nm using UV-spectrometer. **(Xi-Feng Zhang *et al*., 2016)**

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 disc under hydraulic pressure. Then FTIR spectra were recorded in the 4000- 400cm-1 range **(Holler, Skoog, Crouch).**

FTIR spectroscopy of silver nitrate

100mg of Silver nitrate was mixed with 100 mg of spectral grade KBr and pressed into disc under hydraulic pressure. Then FTIR spectra were recorded in the 4000- 400cm-1 range **(Holler, Skoog, Crouch).**

GREEN SYNTHESIS OF SILVER NANOPARTICLES

Preparation of Stock Solution

1 mg of aqueous extract was weighed and diluted to 10 ml with distilled water.

Preparation of 1mM silver nitrate aqueous solution

0.017g of silver nitrate was dissolved in 100 ml of distilled water to prepare 1mM solution of silver nitrate and stored in amber coloured bottle until further use

Synthesis of silver nanoparticles

An aliquot (1ml, 2ml,3ml, 4ml, 5ml) of aqueous plant extract sample was separately added to 10ml of 1mM aqueous $AgNO_3$. To drive nanoparticle formation the reaction mixtures were kept in magnetic strirrer with constant

stirring at 120 rpm. Colour change of the reaction mixtures were monitored to determine silver nanoparticle formation which is indicated by a colloidal brown colour. **(Shakeel Ahmed *et al.,* 2016)**

Purification of Agnp:

The purification of AgNPs from the final reaction mixture was adopted from [33]. The reaction mixture (10ml AgNO3 + 5ml leaf extract sample) was split into two equal parts and transferred to pre-weighed sterile 15ml centrifuge tubes (United Scientific, South Africa). The preparations were then centrifuged at 4000rpm for 20 mins. (Eppendorf centrifuge 5810 R, Germany). Supernatants were discarded and the pellets were collected & stored. The pellets were washed in 10ml of distilled water to remove any contaminating plant material before centrifugation for 1hr. This wash step was repeated twice to remove water soluble biomolecules such as proteins and cellular metabolites & then dried in an oven at 37 °C for 1 hr. **(Jerushka S Moodley *et al.,* 2018)**

Characterization of silver nanoparticles:

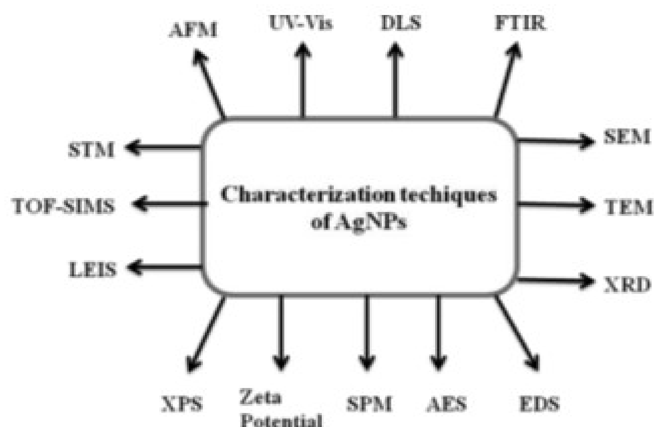


Fig : 8 characterization of silver nanoparticles

The present study includes time dependent formation of silver nanoparticles

employing UV–Vis spectrophotometer, shape by employing FESEM and understanding of *Pterocarpus marsupium* silver nanoparticles interaction from Fourier transform infrared (FT-IR) spectroscopy, particle size measurement, stability from zeta potential and drug entrapment & Energy dispersive x-ray spectroscopy (EDS) for elemental analysis.

Visual examination

UV- vis spectroscopy

UV-vis spectroscopy is a very useful and reliable technique for the primary characterization of synthesized nanoparticles which is also used to monitor the synthesis and stability of AgNPs . AgNPs have unique optical properties which make them strongly interact with specific wavelengths of light. In AgNPs, the conduction band and valence band lie very close to each other in which electrons move freely. These free electrons give rise to a surface plasmon resonance (SPR) absorption band, occurring due to the collective oscillation of electrons of silver nano particles in resonance with the light wave.

Fourier transform infrared spectroscopy

FTIR is able to provide accuracy, reproducibility, and also a favorable signal-to-noise ratio. FTIR is a suitable, valuable, non-invasive, cost effective, and simple technique to identify the role of biological molecules in the reduction of silver nitrate to silver.

Field emission scanning electron microscopy

Among various electron microscopy techniques, SEM is a surface imaging method, fully capable of resolving different particle sizes, size distributions, nanomaterial shapes, and the surface morphology of the synthesized particles at the micro and nanoscales. Using SEM, we can probe the morphology of particles

and derive a histogram from the images by either by measuring and counting the particles manually, or by using specific software.

Energy dispersive x-ray spectroscopy

The combination of SEM with energy-dispersive X-ray spectroscopy (EDX) can be used to examine silver powder morphology and also to conduct chemical composition analysis.

Determination of Zeta potential

Zeta potential is a measure of surface charge. The surface charge (electrophoretic mobility) of nanoparticles can be determined by using Zeta sizer (Malvern Instrument) having zeta cells, polycarbonate cell with gold plated electrodes and using water as medium for sample Preparation. It is essential for the characterisation of stability of the silver nanoparticles .[36]

Particle Size Determination

The average mean diameter and size distribution of silver nanoparticles is found by Dynamic Light Scattering method using Malvern zeta sizer at 25°C.The dried nanoparticles were dispersed in water to obtain proper light scattering intensity for silver nanoparticles.

Determination of Entrapment Efficiency

The entrapment efficiency of nanoparticles was determined by adding 10 ml of phosphate buffer of pH 7.4 and sonicated in a bath sonicator and filtered. 1 ml of filtrate is made up to 10 ml with phosphate buffer and was assayed spectrophotometrically at 337 nm (UV visible spectrophotometer, JASCO V-530. The amount of entrapped drug was calculated from the equation. **(www.scieconf.com)**

In-vitro **methods**

α- Amylase inhibitory effect

Pancreatic α-amylase, an important enzyme of digestive system hydrolyzes starch into mixture of smaller oligosaccharides comprising of maltose, maltotriose and oligoglucans which are further degraded by glucosidase into glucose that enters the blood stream upon absorption. This leads to elevated post-prandial hypergylcemia (PPHG). Hence, it is important to control these two aspects in the treatment of type 2 diabetes.

Procedure

From 1mg/ml stock solution different concentrations of plant extracts were prepared in phosphate buffer. About 500µl of test/standard was added to 500 µl of α-amylase (0.5mg/ml) is incubated for 10min at room temperature. Then added 500µl of 1% starch solution and incubated for another 10minutes. After that 1ml of coloring reagent was added to reaction mixture it is prepared by mixing sodium potassium tartrate solution (12g dissolved in 8ml of 2M NaOH) and 96Mm 3,5-Dinitrosalicylic acid and heated in boiling water bath for 15minutes after cooling, 10ml of distilled water is added. To measure the absorbance of colored extracts blank is prepared for each set of concentration of test sample by replacing the enzyme with buffer. Control incubations representing 100% enzyme activity was prepared by replacing test drug with buffer. Absorbance measured at 540 nm .

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

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 to after meals. **(Vishnu Kiran M. Murugesan S. 2013)**

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 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 drug with respect to different time intervals were obtained from curves of the absorbance A versus concentration C of *Pterocarpus marsupium* silver nanoparticles in PBS based on Lambert-Beer law. **(Guo-Ping Yan *et al.,* 2010).**

Drug release mechanism

In order to understand the mechanism of drug release, in vitro drug release data were treated to kinetic models such as zero order, first order and Higuchi model and Korsmeyer-Peppas model.

Zero order:

Zero-order release kinetics describe systems where the drug release rate is constant over a period of time, where Q_t is the cumulative amount of drug released at time t, $Q_0$is the initial amount of drug, K is the release kinetic constant, and t is the time at which the drug release is calculated or measured**, (Z Saqib *et al.*, 2005)**

$$Q_t = Q_0 + K_t$$

First order:

The pharmaceutical dosage forms following this dissolution profile, release the drug in a way that is proportional to the amount of drug remaining in its interior, in such way, that the amount of drug released by unit of time diminish.

The following relation can be used to express this model. **(Z Saqib *et al.*, 2005)**

$$\log Q_t = \log Q_0 + K_t / 2.303$$

Q_t =Amount of drug dissolved in time ″t″

Q_0 =Initial amount of drug in the solution

K_t =First order release constant

Higuchi Model:

This model helps to study the release mechanism of water-soluble and less water soluble drugs incorporated in semi-solid and solid matrixes. **(VK Mourva TR Saini 1997)** The mathematical expression for drug release is,

$$Q = [D (2C-Cs) Cs.t]^{1/2}$$

Whereas,

Q= Cumulative % of drug released in time ″t″ per unit area.

C =Initial drug concentration

Cs= Drug solubility in the matrix media

D= Diffusion co efficient

Assuming that diffusion co efficient and other parameters remain constant during release, the above equation reduces to

$$Q = k.t^{1/2}$$

Thus, for diffusion controlled release mechanism, a plot of cumulative % of drug released vs. square root of time should be linear. The linearity of the plots can be checked by carrying out linear regression analysis and determination of regression coefficient of the plot.

Korsmeyer-Peppa’s model

To verify the fact that whether the diffusion follows Ficks law or not, the drug release data can also be plotted against Log time according to Peppas equation. **(NS Murthy 1997)**

The drug release can be expressed as,

Q= K t n

Taking log on both sides of equation,

Log Q= Log K + n Log t

Where Q is the cumulative % drug release

t is the time

n is the slope of linear plot of Log Q Vs Log t

Table 6 DIFFUSION MECHANISM & DIFFUSION EXPONENT (n)

DIFFUSION EXPONENT (n)	DIFFUSION MECHANISM
<0.5 or 0. 5	Fickian diffusiom
0.5< n < 1.0	Non fickian diffusion
1.0	Case 2 transport
>1.0	Supercase 2 transport

RESULTS & DISCUSSION

SAMPLE COLLECTION

The bark of *Pterocarpus marsupium* collected from Thalassery, Kannur district in Kerala and it is cleaned with distilled water to remove any substance on the surface of the bark.

AUTHENTICATION

The bark of *Pterocarpus marsupium* had been collected from Coimbatore, Tamilnadu. The plant was identified and authenticated by Dr.C Murugan, Scientist 'D', Botanical Survey of India, Tamilnadu Agricultural University (TNAU), Coimbatore, India and voucher specimen has been given the code BSI/SRC/5/23/2020Tech/505.

DRYING AND PULVERIZING

The collected bark were shade dried & it was grounded into fine powder with the help of electronic blender. Then the powder obtained was stored in well closed container and kept in dry place.

PREPARATION OF AQUEOUS EXTRACT OF *Pterocarpus marsupium*

Fifty grams of the bark powder was stirred with 500 mL of deionised 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 kept in air tight container & stored at 4 °C for future use

PREFORMULATION STUDIES

1.1 Physical Characteristics

Pterocarpus marsupium was checked for its colour, odour and texture.

It is light yellow coloured powder in appearance and has a pleasant odour

The results are shown in table no. 6.

Table 7 Phytochemical analysis

Chemical Constituent	Tests	PM Ag NPs
Carbohydrate	Molisch's Test	+
	Benedict's Test	-
	Fehling's Test	-
	Barford's Test	-
Proteins	Million's Test	-
	Biuret's Test	-
	Ninhydrin's Test	-
Alkaloids	Mayer's Test	+
	Wagner's Test	+
	Dragendorf's Test	+
	Hager's Test	+
Glycosides	Modified Borntrager's	-
	Legal's Test	+
	Balget's Test	+
Tannins	Ferric Chloride Test	+
	Lead Acetate Test	+
	Gelatin Test	+
Flavonoids	Shinoda Test	+
	Ferric Chloride Test	+
	Mineral Acid Test	+
	Lead-Acetate Test	+
Steroids and Triterpenes	Liberman-Burchard's	+
	Salkowski's Test	+
Saponins	Foam Test	+

(+) Presence of Phytoconstituents and (-) Absence of Phytoconstituents

Preliminary phytochemical screening of plant provided information regarding chemical nature of plant such as presence and absence of various phytoconstituents. The phytoconstituents screening was done for extracts of *Pterocarpus marsupium.*

Pterocarpus marsupium was an excellent bio-source of alkaloids, glycosides, carbohydrates and flavonoids, whereas tannins and phenols, saponins test were found to be negative. These are the results for the screening of phytoconstituents present in *Pterocarpus marsupium. Pterostilbene* is the flavonoid present in the bark of *Pterocarpus marsupium* which is responsible for the antidiabetic activity. **(Treas and Evans 2002)**

Solubility studies

Solubility test for *Pterocarpus marsupium* was carried out in different solvents such as ethanol, water, chloroform and results are given in Table 7.

Table 8 solubility studies of *Pterocarpus marsupium*

S.No	Solvent	Sparingly soluble	Insoluble	soluble
1	Ethanol	-	-	✓
2	Water	-	-	✓
3	Chloroform	✓	-	-
4	Phosphate buffer 7.4	-	-	✓
5	Dimethyl sulphoxide	✓	-	-

From the solubility studies, it has cleared that the *Pterocarpus marsupium* bark extract is soluble in water, ethanol & phosphate buffer 7.4 and sparingly

soluble in chloroform & dimethyl sulphoxide. Hence *Pterocarpus marsupium* aqueous extract of bark is more soluble in polar solvents than non polar solvents.

Selection of wavelength

The *Pterocarpus marsupium* stock solution of concentration 500µg/mL was scanned in the range of 200 – 800 nm for $\lambda_{max.}$ using double beam UV Spectrophotometer.

The UV visible spectra is shown in the figure no:10

Uv visible spectra of Pterocarpus marsupium bark

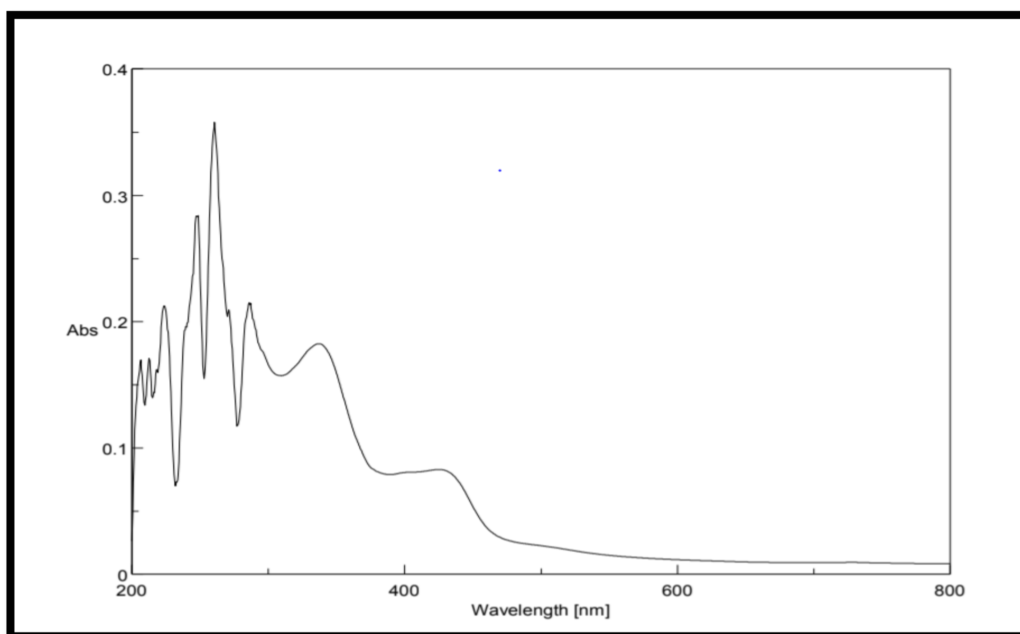


Fig. : 9 UVvisible spectra of *Pterocarpus marsupium* bark

The maximum absorption of Pterocarpus marsupium was found to be at 337nm and hence it is selected as the wavelength for further studies.

Construction of calibration cure of *Pterocarpus marsupium* bark

To construct a calibration curve, 40 – 200 µg/ml of *Pterocarpus marsupium* was taken & checked the linearity at 337 nm.The calibration data is shown in the table no. 7

Calibration data

Table 9 Calibration data of *Pterocarpus marsupium* bark

S.NO	Concentration(µg/ml)	Absorbance at 337 nm
1	40	0.18243
2	80	0.3644
3	120	0.5369
4	160	0.75138
5	200	0.8907

Construction of calibration curve of *Pterocarpus marsupium* bark

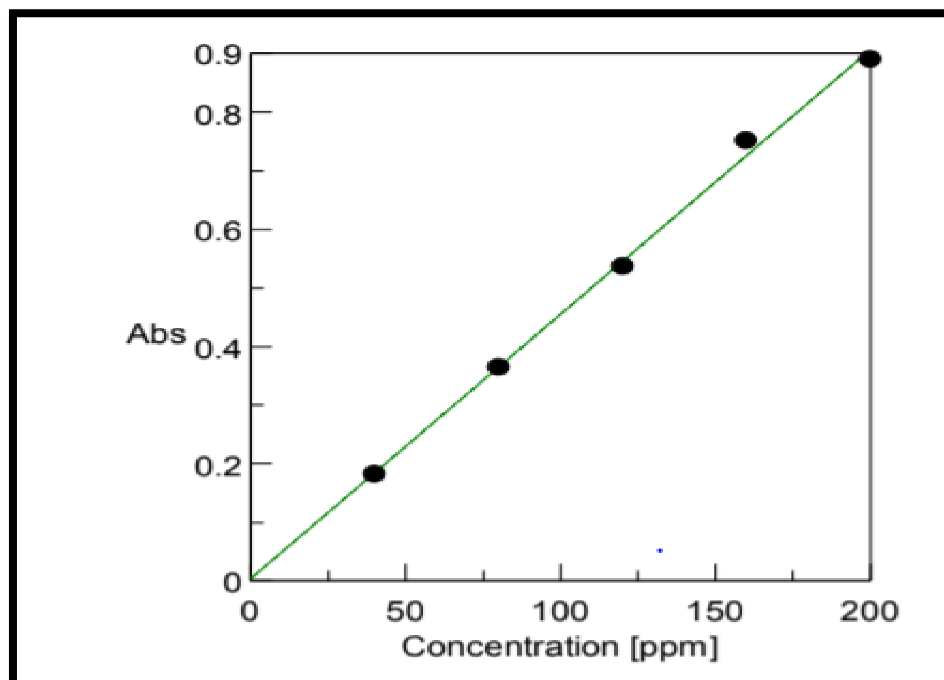


Fig. no: 10 calibration curve of *Pterocarpus marsupium* bark

In the calibration curve, linearity was obtained between 40-200µg/ml concentration of *Pterocarpus marsupium* bark and the regression value was found to be r^2 = 0.99837. Hence we can conclude that *Pterocarpus marsupium* bark obeys Beer Lambert's Law at the concentration between 40-200 µg/ml.

FTIR SPECTROSCOPY OF *Pterocarpus marsupium*

Fourier Transform Infrared (FT-IR) spectra of the samples were obtained using a FTIR Jasco 4100 Spectrometer by KBr disc method. The spectrums were recorded for the pure drug and physical mixture of drug and excipient and are shown in Figure

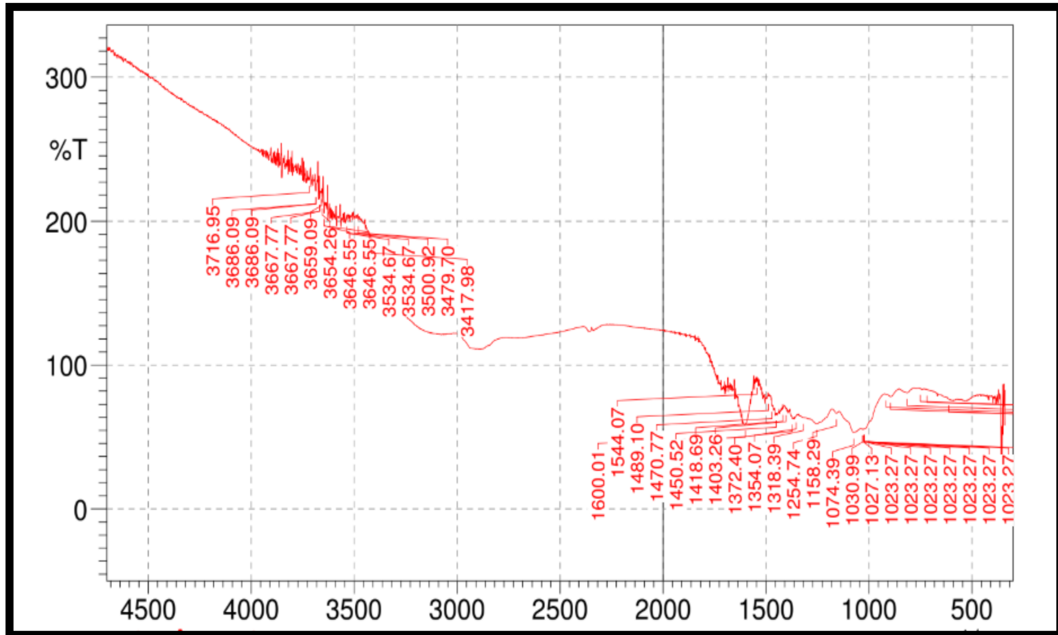


Fig. : 11 FTIR spectra of *Pterocarpus marsupium bark*

Materials	Standard wave number cm $^{-1}$	Test wave number cm $^{-1}$	Functional group assessment
Pterocarpus marsupium bark		3417.98	O-H stretching
		3479.70	O-H stretching
		3500.92	O-H stretching
		3534.67	O-H stretching
		1254.74	CH_3 bending
		1074.39	C-O stretching
		1158.29	C-O stretching

FTIR SPECTROSCOPY OF SILVER NITRATE

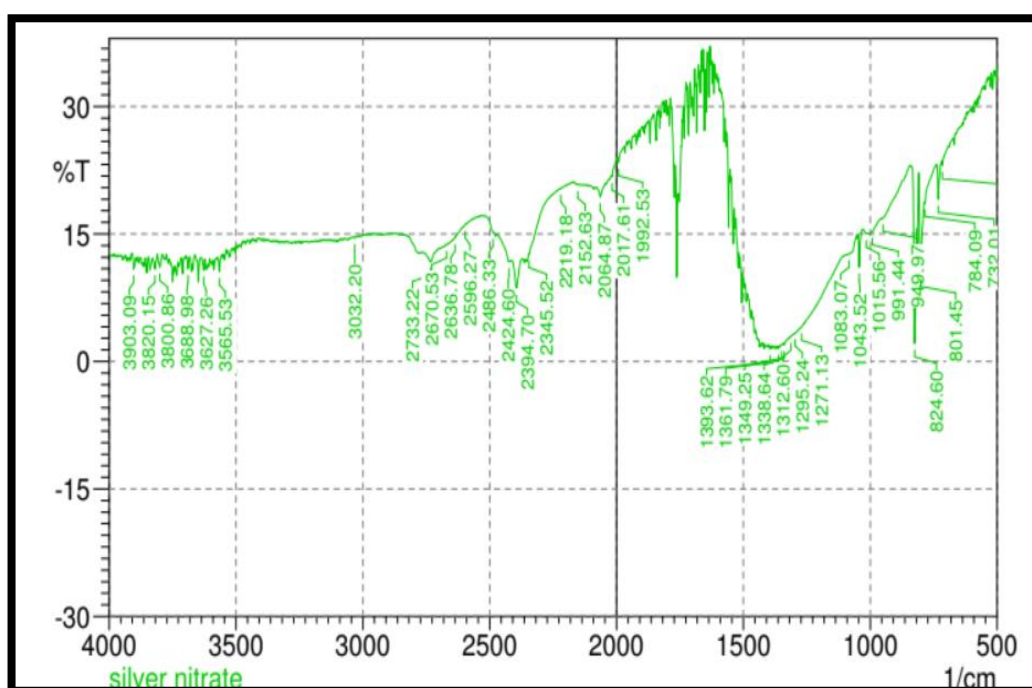


Fig. 12 : FTIR spectroscopy of silver nitrate

Table : FTIR interpretation of silver nitrate

Material	Standard wave number (cm-1)	Test wave number (cm-1)	Functional group assessment
Silver nitrate		1312.60	N-O stretching
		1338.64	N-O stretching
		1349.25	N-O stretching
		1361.79	N-O stretching
		1393.62	N-O stretching

GREEN SYNTHESIS OF SILVER NANOPARTICLES

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

Fig. 13: Formation of Silver Nanoparticles Indicated by Color Change

SEPARATION OF SILVER NANOPARTICLES

The colloidal solution was centrifuged at 4000 rpm for 20 minutes and after centrifugation, the pellets were collected and the supernatant fluid was discarded then the pellets were thoroughly washed with deionized water to separate the silver nanoparticles and they were kept for drying in desiccator to avoid contact with moisture or dust from air. After drying,, the formulated *Pterocarpus marsupium* silver nanoparticles were stored in air tight container and used for further evaluation studies.

Fig. 14 : After centrifugation of colloidal *Pterocarpus marsupium* loaded silver solution

CHARACTERIZATION OF *Pterocarpus marsupium* BARK SILVER NANOPARTICLES

FORMULATION OF *Pterocarpus marsupium* LOADED SILVER NANOPARTICLES

F1, F2, F3, F4, F5 formulations of *Pterocarpus marsupium* silver nanoparticles were prepared by green synthesis method.

Formulation	Drug (*Pterocarpus marsupium)* (ml)	AGNO3 Solution (millimolar)	Observation
F1	1	10ml of 1mM AgNo3 solution	Faint yellow color was formed
F2	2	10ml of 1mMAgNO3 solution	Faint light yellow color was formed
F3	3	10 ml of 1mM AgNO3 solution	Light yellow to yellowish brown color was formed
F4	4	10 ml of 1mM solution	Yellowish brown color was formed
F5	5	10ml of 1mM solution	Colloidal brown color was formed and it does not change after different time intervals

In all experiments, addition of plant extract of *Pterocarpus marsupium* into the beakers containing aqueous solution of silver nitrate led to the change in the colour of the solution from yellowish to reddish brown within reaction duration due to excitation of surface plasmon vibrations in silver nanoparticles which was further confirmed by UV Vis spectral analysis**.** On addition of different concentration (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 faint 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. (**Shakeel Ahmed *et al.,* 2015**)

UV Vis spectral analysis of *Pterocarpus marsupium* silver nanoparticles

Periodic sampling of 30 mins, 60 mins, 90 mins, 210 mins & 24 hrs were taken by using distilled water as blank from the wavelength of 300 – 800 m which is depicted in the figure 15.   .

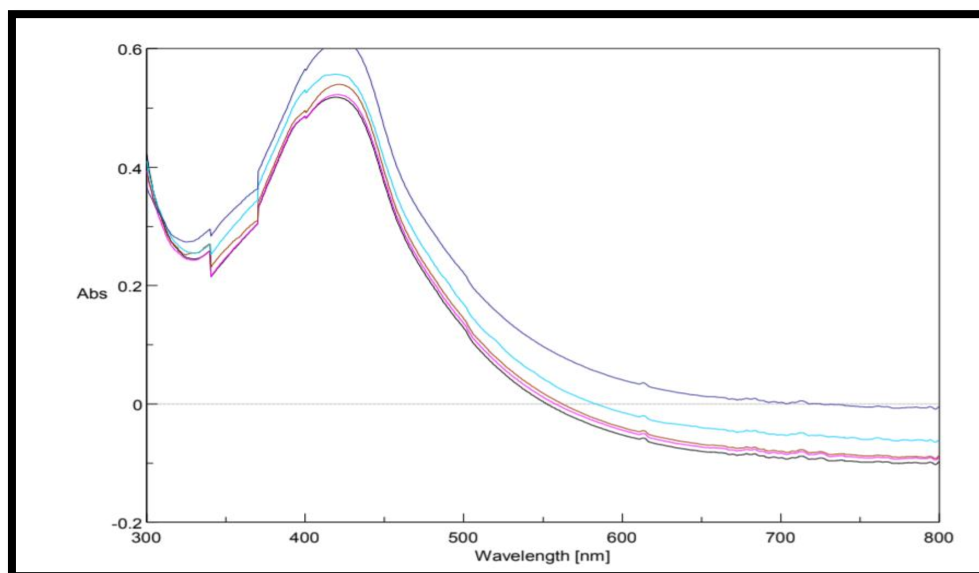


Fig. : 15 Uv- visible absorption spectra of pterocarpus marsupium Roxb. silver nanoparticles at different time intervals

In AgNPs, the conduction band and valence band lie very close to each other in which electrons move freely. These electrons give rise to a surface plasmon resonance absorption band in the visible region occurring due to the collective oscillation of electrons of silver nanoparticles in resonance with the light wave.

The silver surface plasmon resonance was observed at 427nm which steadily increases in intensity as a function of time of reaction (ranging from 30 min to 5 h) without showing any shift of the wavelength maximum. **(Xi-Feng Zhang *et al.,* 2016)**

FTIR SPECTROSCOPY OF *pterocarpus marsupium* SILVER NANOPARTICLES

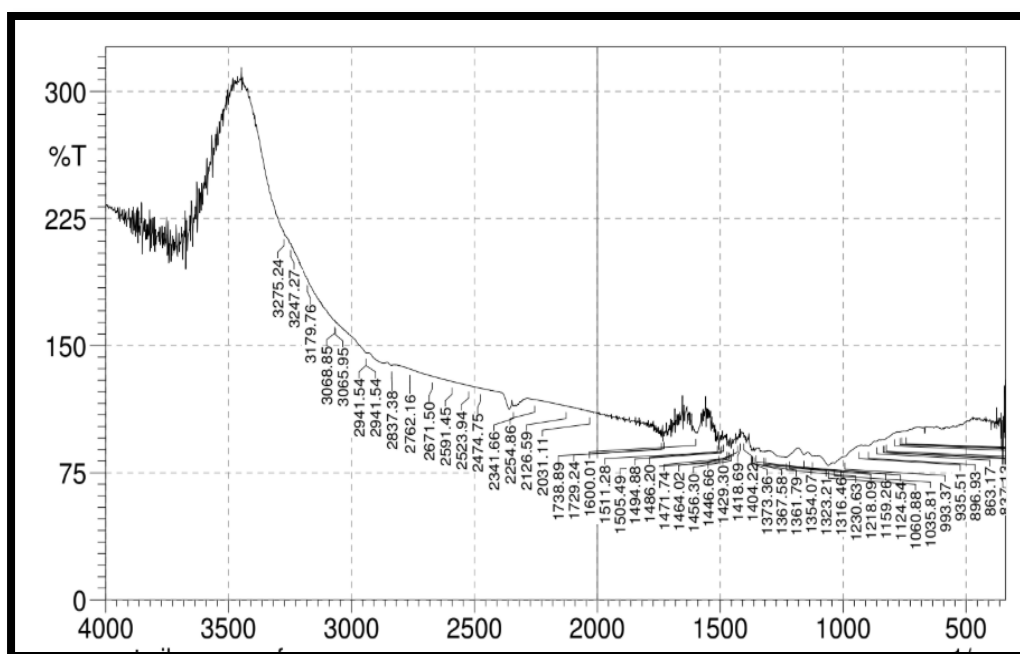


Fig. 16 : FTIR spectra of *Pterocarpus marsupium* silver nanoparticles

Table FTIR Interpretation of *Pterocarpus marsupium* silver nanoparticles

Material	Standard wave number (cm-1)	Test wave number (cm-1)	Functional group assessment
Pterocarpus marsupium silver nanoparticles		3275.24	O-H stretching
		3247.27	
		3179.76	C-H asymmetric stretching
		2941.54	C-H symmetric stretching
		1060.88	C-O stretching
		1124.54	
		1159.26	
		1464.02	
		1354.07	N-O stretching
		1361.79	
		1367.58	
		1373.36	
		1404.22	

DRUG ENTRAPPMENT EFFICIENCY

The drug entrapment can be calculated after centrifugation by UV spectrophotometry at 427 nm.

The amount of drug present in the supernatant liquid was calculated by using the following formula.

% drug entrapment = W-w / W * 100

W – total amount used in the preparation of silver nanoparticles

w- drug present in the supernatant obtained from calibration curve

(W-w) – amount of drug entrapped. **(Peng-Fei Yue 2009)**

S. No	Formulation code	% drug entrapment
1	F1	81%
2	F2	83%
3	F3	85%
4	F4	89%
5	F5	93%

The % entrapment of drug or drug content of *Pterocarpus marsupium Roxb.* silver nanoparticles were found to be 81% for F1, 83% for F2, 85% for F3, 89% for F4 and 93% for F5. Hence the highest amount of drug was entrapped in the formulation F5 which is 93% also it was in colloidal brown color indicated the formation of *Pterocarpus marsupium* silver nanoparticles. From the drug

entrapment results, the formulation F5 was chosen for further evaluation studies because it possessed high drug entrapment than the other formulations.

ZETA POTENTIAL.

For *Pterocarpus marsupium* silver nanoparticles zeta potential was found to be -24.3 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 Figure 17

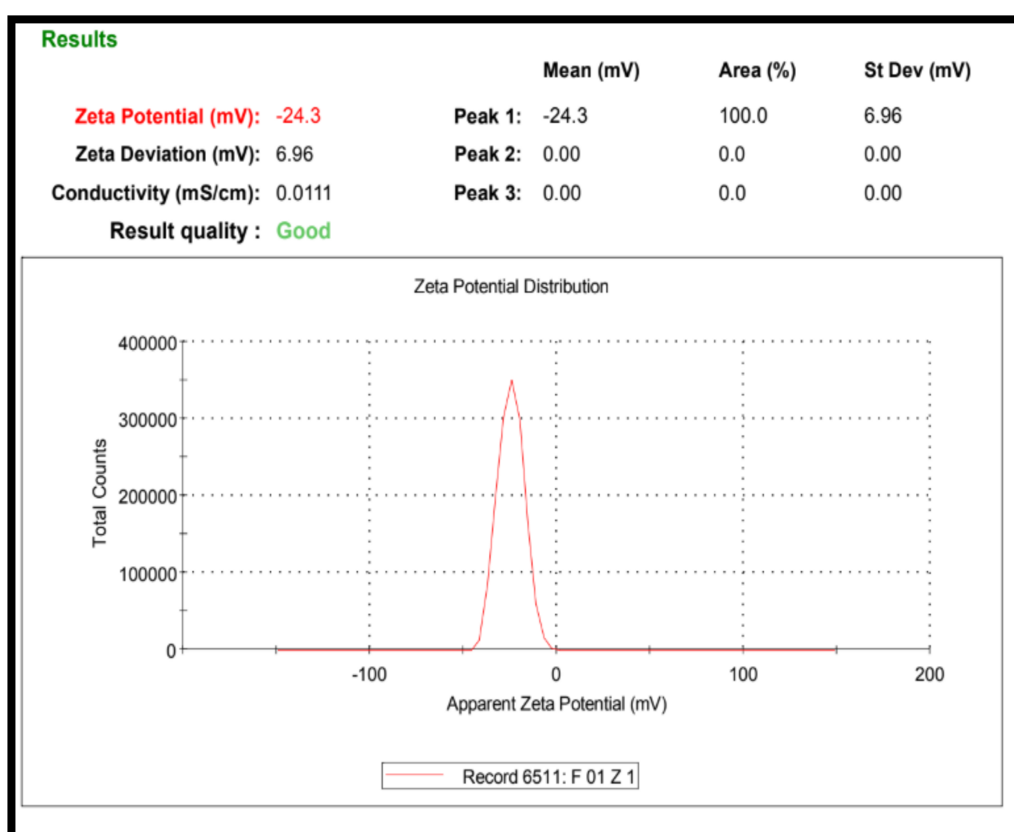


Fig.: 17 Determination of zeta potential of *Pterocarpus marsupium* silver nanoparticles

Zeta Potential was determined using Malvern zeta-sizer instrument. Zeta potential analysis is carried out to find the surface charge of the particles to know its stability during storage. The magnitude of zeta potential is predictive of the colloidal stability.

Nanoparticles with zeta potential value greater than +25 mV or less than -25 mV typically have high degrees of stability. If all the particles in suspension have a large negative or positive zeta potential then they will tend to repel each other and there will be no tendency for the particles to come together.

However, if the particles have low zeta potential values then there will be no force to prevent the particles coming together and flocculating.

PARTICLE SIZE MEASUREMENT

The average particle size (z-average) is found to be 132.6 nm. Particle size analysis showed the presence of nanoparticles with polydispersity indices PDI value 0.248 with intercept 0.643. It is presented in the Table & Fig. 18.

Parameter	value	Peak no.	Peak intensity	Peak size	Peak width
ZAverage	132.6	1	100.0	141.8	102.5
PDI	0.248	2	0.000	0.000	0.000
Intercept	0.643	3	0.000	0.000	0.000

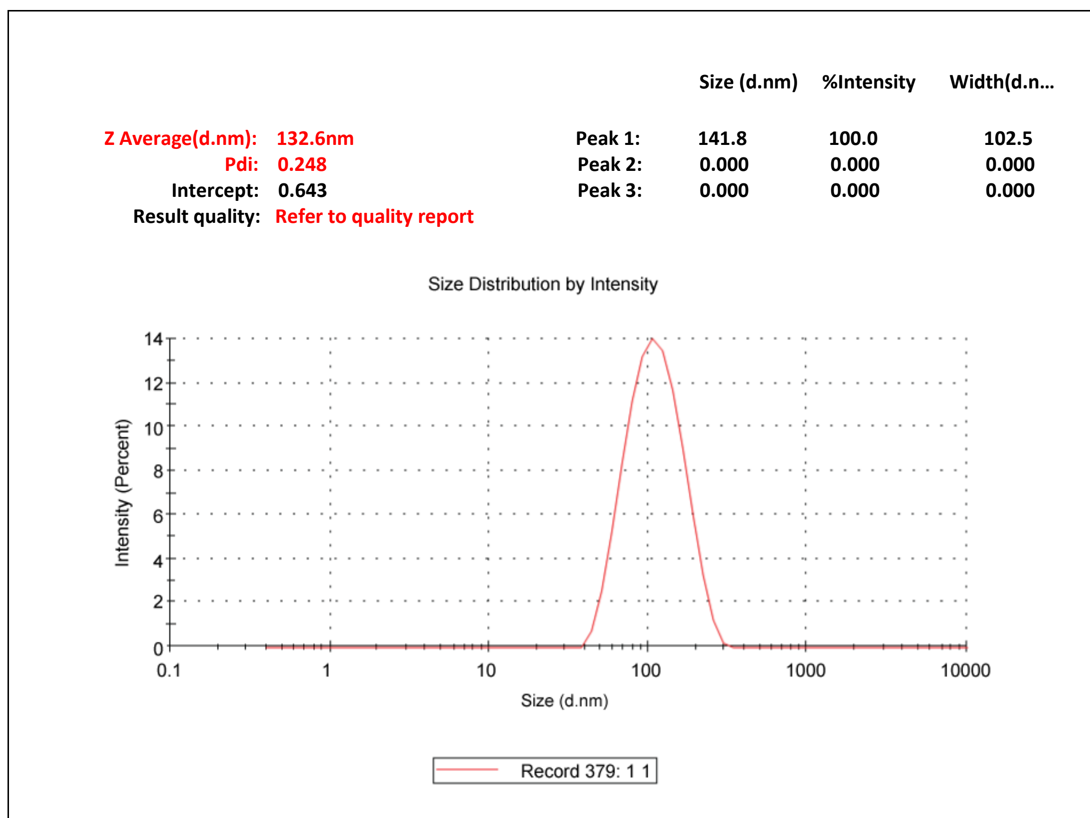


Fig.: 18 Particle size measurement of *Pterocarpus marsupium* silver nanoparticles

The particle size is one of the most important parameters for the characterisation of nanoparticles. The average particle sizes of the prepared nanoparticles were measured using Malvern zeta sizer. The colloidal solution having a Poly Dispersity Index less than 0.50 are considered as good quality. The results were in agreement with the statement reported before.

Physical properties vary greatly with size change in the nanoscale, melting points drop dramatically with smaller nanosize; optical absorption is also sensitive to size, where Ag exhibit plasmon absorptions in the 400–600 nm range wavelengths depending on size.

FIELD EMISSION SCANNING ELECTRON MICROSCOPY

FE SEM analyses of the formulated *Pterocarpus marsupium* silver nanoparticles were performed to evaluate the surface morphology of nanoparticles. The FESEM image of *Pterocarpus marsupium* silver nanoparticles displayed below, reveals that the particles were:

- Uniform in size
- Spherical in shape
- Segregated

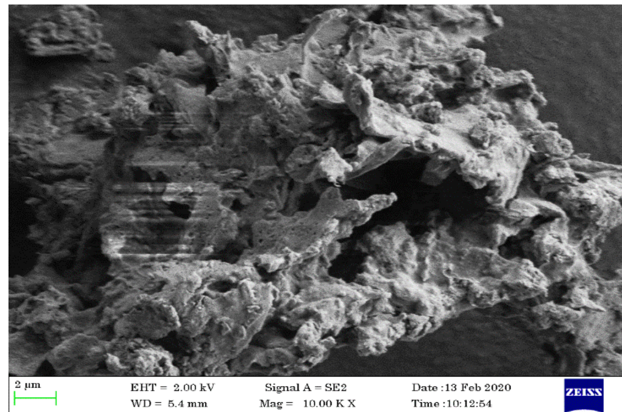


Fig.: 19 FESEM analysis of *Pterocarpus marsupium* silver nanoparticles with 1000 Maginification

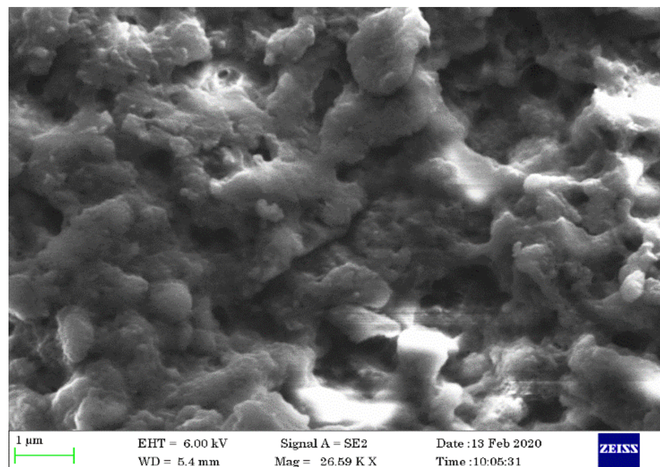


Fig.: 20 FE SEM analysis of Pterocarpus marsupium silver nanoparticles with 26500X maginification

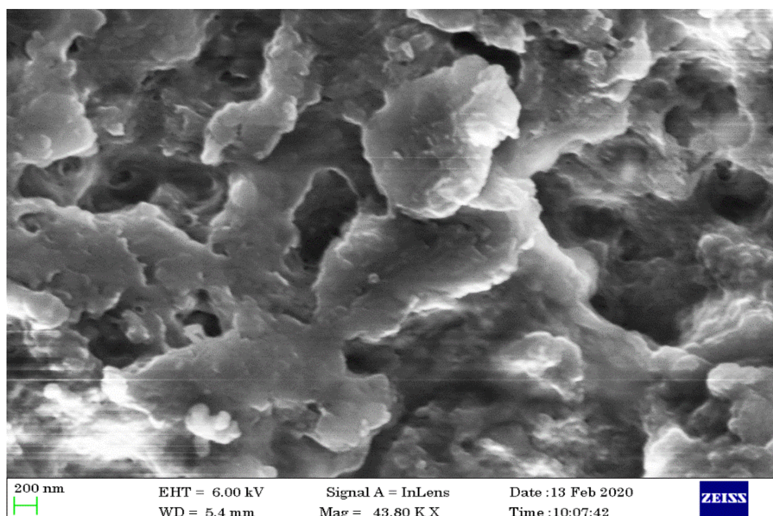


Fig. : 21 FESEM analysis of *Pterocarpus marsupium* silver nanoparticles with 43,800 X maginification

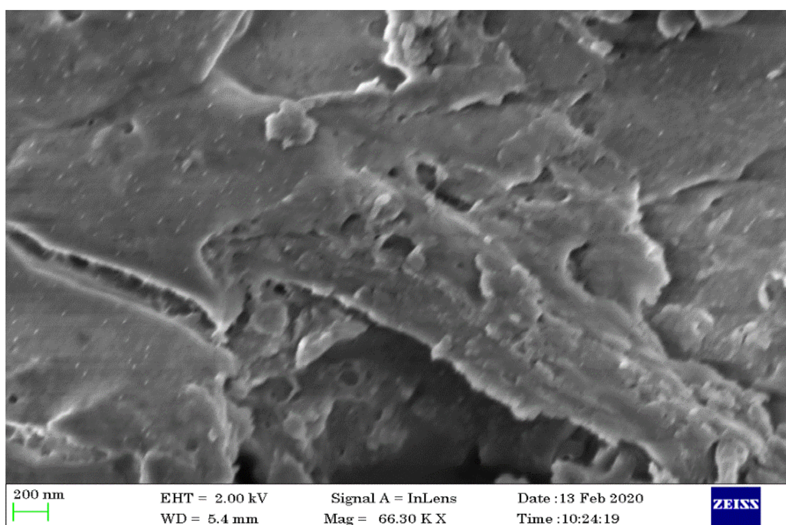


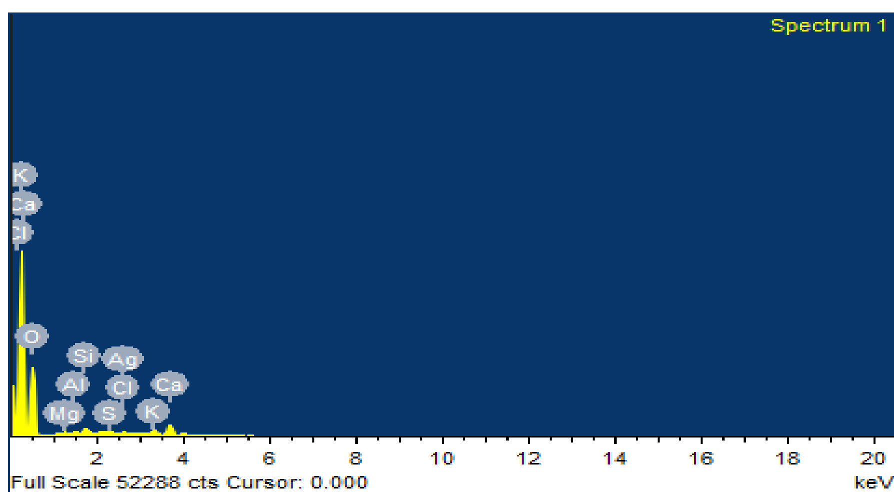
Fig.: 22 FESEM analysis of *Pterocarpus marsupium* silver nanoparticles with 66,300X maginification

The FESEM image of Silver nanoparticles synthesized by green synthesis process by using 5 % bark extract and 1mM AgNO3 concentration gave a clear

image of highly dense silver nanoparticles. The FESEM image showing silver nanoparticles synthesized using *Pterocarpus marsupium* aqueous extract of bark confirmed the growth of silver nanostructures.

ENERGY DISPERSIVE X-RAY SPECTROSCOPY

EDX analysis of AgNPs demonstrated a well defined silver signal at 3 keV along with



carbon, oxygen and nitrogen peaks, with the latter weaker signals probably representing surface biomolecule capping structures originating from the bark extracts.

Fig. 23: EDX spectrum of mineral crust

ELEMENT	WEIGHT %	ATOMIC %
O K	81.32	91.80
Mg K	1.03	0.75
Si K	2.2	1.40
S k	0.74	0.41
Cl K	0.85	0.43
K K	2.5	1.16
Ca K	7	3.34
Ag L	4.36	0.22

In addition to FESEM, along with EDX the signals coming from the sample can be used to get information about the composition of the materials**.** The EDX reading proved that the compulsory phase of silver (Ag) because of the silver nitrate solution used for the formulation of nanoparticles. Magnesium (Mg), calcium (Ca), potassium (K), and oxygen (O) are usually present in plants. Aqueous extract of *Pterocarpus marsupium* bark was used which is the reason for the presence of chlorine in the sample. The graph also shows the presence of sulfur (S) and Silicon (S) are present in the EDX picture of silver nanoparticles. Due to the carbon film on the Cu-grid and the silicon substrate on the mounting base small, intense peaks corresponding to C-Kα1 and Si-Kα are also seen in the EDX spectra.

***IN VITRO* DRUG RELEASE STUDY**

In vitro drug release study of the prepared *Pterocarpus marsupium* silver nanoparticles was carried out using dialysis bag diffusion method. Amount of drug released in different time intervals were observed.

Fig. : 24 *In vitro* drug release

In vitro drug release profile data obtained from *Pterocarpus marsupium* silver nanoparticles are given in Table 10.

Table: 10 *In vitro* drug release study of *Pterocarpus marsupium* Roxb silver Nanoparticles

Time (in hrs)	Log time	Square root of time	Cumulative amount of drug released	Cumulative % of drug released	Log cumulative amount of drug released
1	0	1	0.1853	18.53%	1.2678
2	0.3010	1.4142	0.2685	26.85%	1.4289
3	0.4771	1.7320	0.4198	41.98%	1.6230
4	0.6020	2	0.4736	47.36%	1.6754
5	0.6989	2.2360	0.7356	73.56%	1.8666
6	0.778	2.4494	0.8841	88.41%	1.9465

24	1.3802	4.8989	0.9280	92.8%	1.9675

At various time intervals, *in vitro* drug release profile for *Pterocarpus marsupium* silver nanoparticles were checked using dialysis bag diffusion method to find the cumulative percentage of drug release. From the *in vitro* drug release profile, it was concluded that the formulated *Pterocarpus marsupium* silver nanoparticles follows controlled drug delivery at different time intervals. The drug release of formulation for 1 hr was 18.53% and for 2 hrs it was increased to 26.85% which was 1.4 fold increased drug release compared to 1 hr. For 3 hrs, the drug release was 41.98 % which was 1.56 fold increase from 2 hrs of drug release. For 4 hrs, the drug release was 47.36% which was 1.12 fold increase from 3 hrs of drug release. Then for 5 hrs, the drug release was 73.56% which was 1.5 fold increase from 4 hrs of drug release and for 6 hrs, the drug release was 88.41% which was 1.2 fold increase from 5 hrs of drug release. Later in 24 hrs, the drug release was 92.8 % which was 1 fold increase from 6 hrs of drug release and 5 folds increased from 1 hr of drug release. Thus the formulated *Pterocarpus marsupium* silver nanoparticles released drug from 1 to 1.5 fold increase during every one hr interval of time. The *in vitro* drug release study was fitted into various kinetic models and the figures are depicted below

Drug release data fitted into different kinetic models

Zero order

From the *in vitro* drug release data, time & % cumulative amount of drug release is plotted to find the R^2 value which could give a clear data that the formulation follows zero order kinetics. The graph is shown in the fig. and the R^2 value was found to be 0.9633.

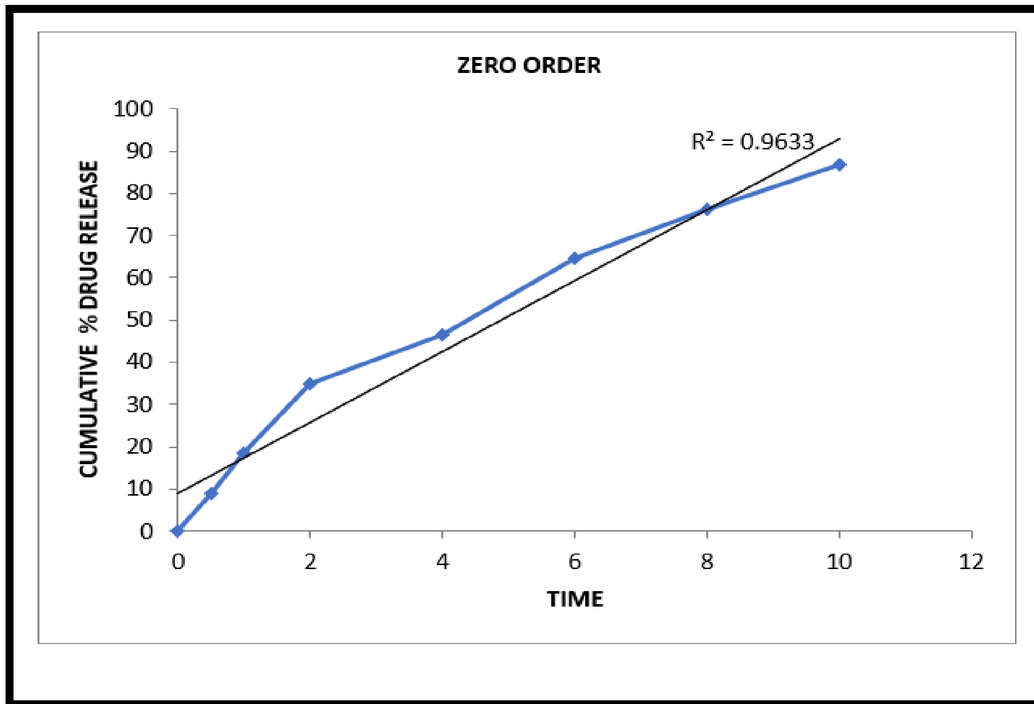


Fig. 25 Zero order plot

First order

From the *in vitro* drug release data, time & log cumulative amount of drug release is plotted to find the R^2 value which could give a clear data that the ormulation follows first order kinetics. The graph is shown in the fig. and the R^2 value was found to be 0.6109.

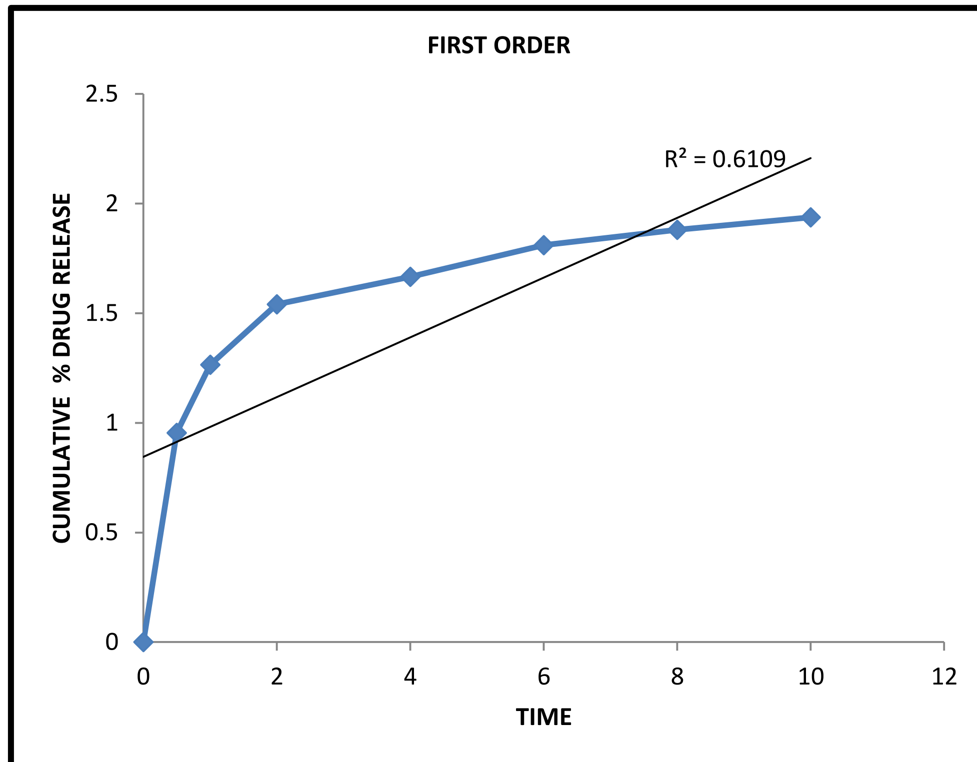


Fig. 26 First order plot

Higuchi 's plot

From the *in vitro* drug release data, square root of time & % cumulative amount of drug release is plotted to find the R^2 value which could give a clear data that the formulation follows first order kinetics. The graph is shown in the fig. and the value was found to be 0.9633.

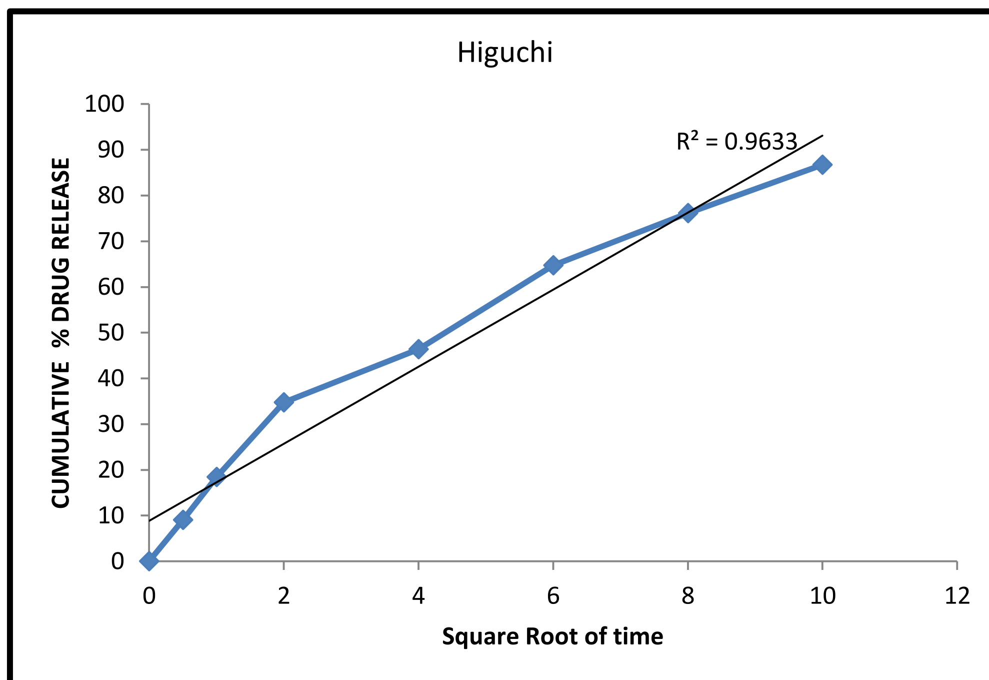


Fig. 27 Higuchi plot

Korsmeyer peppas

From the *in vitro* drug release data, log time & log cumulative amount of drug release is plotted to find the R^2 value which could give a clear data that the formulation follows first order kinetics. The graph is shown in the fig. and the R^2 value was found to be 0.5851.

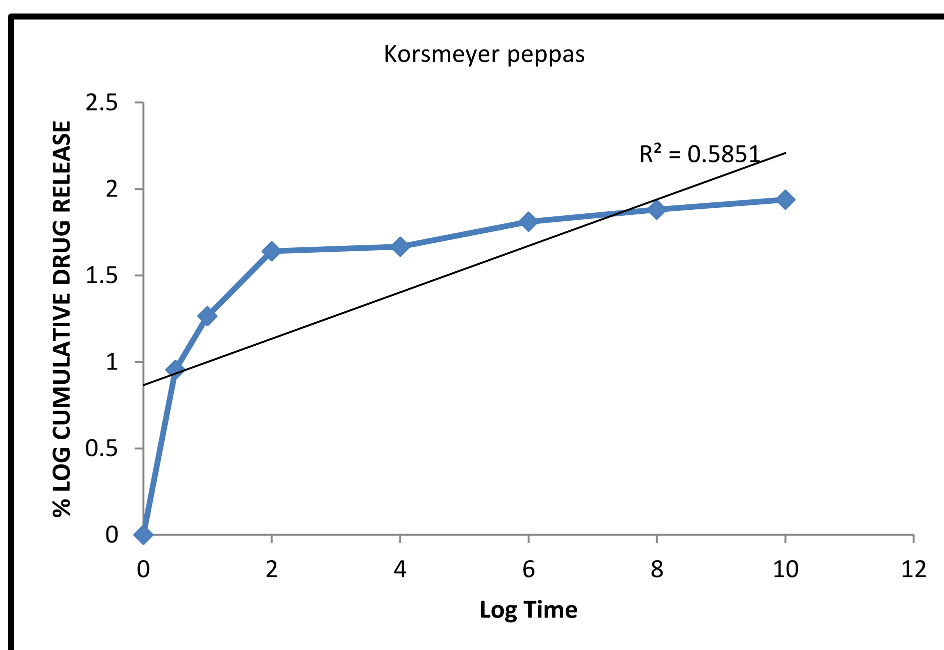


Fig. 28 Korsmeyer peppas

Table 18 R^2 value obtained for various kinetic models

FORMULATION	CORRELATION COEFFEICIENT (R^2)				
	Zero order R^2	**First order R^2**	**Higuchi R^2**	**Korsmeyer peppas**	
				R^2	**n**
Pterocarpus marsupium silver nanoparticles	0.9613	0.6109	0.9633	0.5851	0.1875

The mechanism of drug permeation through the dialysis membrane from the silver nanoparticles were determined by subjecting the *in vitro* drug permeation data to various kinetic models such as zero order, first order and Higuchi model and Korsmeyer-Peppas model. Then the linearity and correlation coefficient of all obtained from the plots were observed. The *in vitro* drug release plot has shown that the drug release of *Pterocarpus marsupium* silver nanoparticles follow higuchi plot and fickian model kinetics. Further more the korsmeyer peppas explains the drug release kinetics where the values of diffusion exponent n=0.1875 which confirms the fickian diffusion model.

***IN VITRO* ALPHA AMYLASE INHIBITION**

α-amylase is a key enzyme in carbohydrate metabolism. Inhibition of α-amylase is one of the strategy of treating diabetes. Inhibiting α-amylase will lower post prandial blood sugar. The result suggest that Pterocarpus marsupium silver nanoparticles exhibit good α amylase activity under *in vitro* condition. Dose dependent % inhibitory activity against α-amylase was noted. Our study indicates that Pterocarpus marsupium could be useful in the treatment of post prandial hyperglycaemia. The anti-diabetic activity may be attributed to the presence of flavonoids, tannins & anti α-amylase activity. Acarbose is used as a standard here which is a good antidiabetic drug and works by slowing the action of certain chemicals that breakdown food to release glucose into our blood.

The absorbance of control without sample is taken and it is 0.832

Table 19 : Alpha amylase inhibitory effects of positive control Acarbose

Concentration (mg/ml)	Absorbance	% Inhibiton
0.2	0.5671	31.83%
0.4	0.4103	50.68%
0.6	0.2280	72.59%
0.8	0.1086	86.94%
1	0.0679	91.83%

Table 20 : α- Amylase inhibitory effects of *Pterocarpus marsupium* Roxb. silver nanoparticles

Concentration (µg/ml)	Absorbance	Percentage inhibition
0.2	0.6183	25.68%
0.4	0.5347	35.73%
0.6	0.3641	56.23%
0.8	0.2576	69.03%
1	0.1152	86.15%

From the figure 29, we can compare the percentage inhibition of both positive control and test drug.

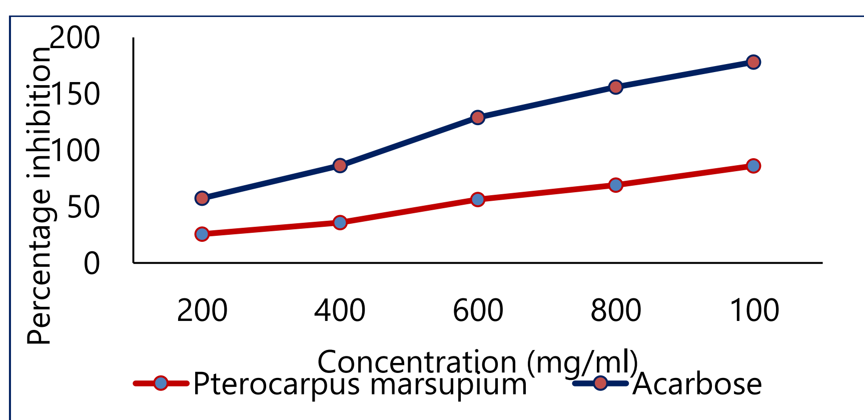


Fig. 29 α amylase inhibition of Pterocarpus marsupium silver nanoparticles & acarbose on alpha amylase enzyme

Percentage inhibition of α amylase for the positive control Acarbose was found to be 31.83% at concentration 0.2mg/ml. When the concentration is increased to 0.4 mg/ml percentage inhibition is increased by 1.5 fold then the concentration was increased to 0.6mg/ml so the percentage inhibition is increased by 1.1 fold, further the concentration was increased to 0.8mg/ml then the percentage inhibition is increased by 1.1 fold again the concentration was increased to 1mg/ml which resulted in the increase of percentage inhibition by 1 fold.

Percentage inhibition of α amylase for the *pterocarpus marsupium* Roxb. silver nanoparticles was found to be 25.68% for the concentration 0.2mg/ml. When the concentration is increased to 0.4mg/ml percentage inhibition is increased by 1.4 fold then the concentration was increased to 0.6mg/ml so the percentage inhibition was also increased by 1.5 fold, further the concentration was increased to 0.8mg/ml then the percentage inhibition is increased by 1.2 fold again the concentration was increased to 1mg/ml which resulted in the increase of percentage inhibition by 1.2 fold.

The percentage α amylase inhibition of positive control Acarbose at lower(0.2mg/ml) and higher (1mg/ml) concentration were found to be 31.83% and 91.83% and for test *pterocarpus marsupium* Roxb. silver nanoparticles percentage α amylase inhibition at lowest (0.2mg/ml) and highest (1mg/ml) concentration were found to be 25.68% and 86.15% respectively.

IN VIVO RESULTS

Acute toxicity studies and selection of dose for in-vivo studies

The aqueous extract of bark of *Pterocarpus marsupium* silver nanoparticles were selected and used for further in vivo evaluation. Acute toxicity was carried out as per OECD guidelines 420 employing fixed dose procedure for selecting the dose for biological activity.

For acute toxicity studies female *wistar* rats weighing 180-195gms were taken and they were fasted overnight before the experimental day. Overnight fasted rats were weighed and body weight determined for dose calculation and test compound were administered orally.

2000 mg/kg dose of Pterocarpus marsupium silver nanoparticles and the rats were observed for signs of acute toxicity. No toxic effect was observed after sufficient interval of time (2-3days).

Signs and symptoms of toxicity and death if any were observed individually for each rat at 0, 0.5, 1, 2, 3 and 4h for first 24h and thereafter daily for 14 days. Diet was given to the animals after 4th hour of dosing. The animals were observed twice daily for 14 days and body weight changes, food and water consumption were noted.

In acute toxicity studies, it was found that the animals were safe up to a maximum dose of 2000mg/kg of body weight. There were no changes in normal behavioural pattern and no signs and symptoms of toxicity and mortality in rats. As per the OECD 420 guidelines *Pterocarpus marsupium* silver nanoparticles can be included in the category 5 or unclassified category of globally harmonized classification system (GHS).

Hence based on these results the *Pterocarpus marsupium* silver nanoparticles were considered non-toxic and 1/20th dose were used for the biological evaluation (antidiabetic activity) and the studies were conducted at dose

levels of 200 mg/kg body weight.

Table 21 : OBSERVATIONS DONE FOR THE ACUTE ORAL TOXICITY STUDY OF TEST COMPOUNDS

Parameters observed		0 h	0.5h	1 h	2 h	4 h	Day 2&3	Day 4&5	Day 6&7	Day 8&9	Day 10&11	Day 12&13	Day 14
Respiratory	Dyspnoea	-	-	-	-	-	-	-	-	-	-	-	-
	Apnoea	-	-	-	-	-	-	-	-	-	-	-	-
	Nostril discharges	-	-	-	-	-	-	-	-	-	-	-	-
Motor activity	Tremor	-	-	-	-	-	-	-	-	-	-	-	-
	Hyper activity	-	-	-	-	-	-	-	-	-	-	-	-
	Hypo activity	-	-	-	-	-	-	-	-	-	-	-	-
	Ataxia	-	-	-	-	-	-	-	-	-	-	-	-
	Jumping	-	-	-	-	-	-	-	-	-	-	-	-
	Catalepsy	-	-	-	-	-	-	-	-	-	-	-	-
	Locomotor activity	-	-	-	-	-	-	-	-	-	-	-	-
Reflexes	Corneal reflex	-	-	-	-	-	-	-	-	-	-	-	-
	Pinna reflex	-	-	-	-	-	-	-	-	-	-	-	-
	Righting reflex	-	-	-	-	-	-	-	-	-	-	-	-
Convulsion	Tonic and clonic convulsion	-	-	-	-	-	-	-	-	-	-	-	-
Muscle Tone	Hypertonia	-	-	-	-	-	-	-	-	-	-	-	-
	Hypotonia	-	-	-	-	-	-	-	-	-	-	-	-
Ocular sign	Lacrimation	-	-	-	-	-	-	-	-	-	-	-	-
	Miosis	-	-	-	-	-	-	-	-	-	-	-	-
	Mydriasis	-	-	-	-	-	-	-	-	-	-	-	-
	Ptosis	-	-	-	-	-	-	-	-	-	-	-	-
Skin	Edema	-	-	-	-	-	-	-	-	-	-	-	-
	Skin and fur	-	-	-	-	-	-	-	-	-	-	-	-
	Erythema	-	-	-	-	-	-	-	-	-	-	-	-
Cardiovascular signs	Bradycardia	-	-	-	-	-	-	-	-	-	-	-	-
	Tachycardia	-	-	-	-	-	-	-	-	-	-	-	-
Piloerection	Contraction of erectile tissue of hair	-	-	-	-	-	-	-	-	-	-	-	-
Gastro intestinal signs	Diarrhoea	-	-	-	-	-	-	-	-	-	-	-	-

Table 22 : MORTALITY RECORD FOR TEST COMPOUND IN ACUTE ORAL TOXICITY STUDY

Sighting study: 2000mg/kg		Main study: 2000 mg/kg							
No. of animals	1	2	3	4	5	6	7	8	9
Body weight (g)	180	185	180	190	185	180	185	185	190
Sex	Female	Female	Female	Female	Female	Female	Female	Female	Female
30 min	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil
1 h	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil
2 h	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil
3 h	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil
4 h	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil
Day 1	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil
Day 2	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil
Day 3	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil
Day 4	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil
Day 5	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil
Day 6	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil

Sighting study: 2000mg/kg		**Main study: 2000 mg/kg**							
No. of animals	1	2	3	4	5	6	7	8	9
Body weight (g)	180	185	180	190	185	180	185	185	190
Sex	Female	Female	Female	Female	Female	Female	Female	Female	Female
Day 7	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil
Day 8	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil
Day 9	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil
Day 10	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil
Day 11	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil
Day 12	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil
Day 13	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil
Day 14	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil
Mortality	0/1	0/1		0 0/1	0/1	0/1	0/1	0/1	0/1

Note: Acute toxicity study of compound was performed and it was non-toxic up to 2000 mg/kg dose.

***IN VIVO* ANTIDIABETIC ACTIVITY OF *Pterocarpus marsupium* silver nanoparticles**

Table 24 shows that the biological evaluation was carried out using 200mg/kg dose of aqueous bark extract of *Pterocarpus marsupium* silver nanoparticles. Table show the body weight of control and experimental animals on 0, 7, 14, 21, 28 days of treatment. There was significant reduction of body weight in diabetic control animals compared to normal control & test drug treated animals. On 7th day significant reduction of body weight was observed in diabetic control animals (159.85±3.95) when compared to control rats (193.14±2.47). Reduction in body weight indicates the induction of diabetes. The normal control (209.28±2.28) and drug treated rats *pterocarpus marsupium* aqueous extract, *Pterocarpus marsupium* silver nanoparticles, glibenclamide gained significant weight (172.42±9.19, 175.57±3.32, and 173±8.3) ($P<0.01$) on 28 days of treatment.

Table 23 shows the effect of *pterocarpus marsupium* aqueous extract, *Pterocarpus marsupium* silver nanoparticles, glibenclamide on streptozotocin and nicotinamide induced diabetes in rats. Initially it was found that a significant ($P<0.01$) increase in blood glucose level was observed in STZ-nicotinamide induced diabetic rats (243±4.601) compared to normal control (80.5±3.12). After the daily treatment for 14 days showed significant ($p<0.05$), ($P<0.01$) reduction in blood glucose 200 mg/kg p.o of aqueous extract of *Pterocarpus marsupium* (195±2.90), aqueous extract of *Pterocarpus marsupium* silver nanoparticles (179.25±1.493), 2.5 mg/kg, p.o of glibenclamide (165.25±3.78) as compared to diabetic control group. $(243 \pm 4.60)^{\#\#}$

Table 25 shows that the level of protein after 28 days of treatment in liver is given in Table. A decrease in protein level was found in diabetic control (101±5.323) rats compared with control rats (209±5.297). Administration of *Pterocarpus marsupium* aqueous extract, *Pterocarpus marsupium* silver nanoparticles (200 mg/kg), (133.5+5.33,163+3.742) and glibenclamide (2.5

mg/kg) treated rats (198.25±9.613) restored the protein level significantly near to normal levels. The results were found to be statistically significant (P<0.01).

A significant (P<0.01) increase was observed in the activities of SOD in the treatment groups administered with, *Pterocarpus marsupium* aqueous extract, *Pterocarpus marsupium* silver nanoparticles (200 mg/kg), glibenclamide (274.5±15.2, 330.75±7.95, 368.75 ± 5.97) CAT (154.5±3.12, 172.5±2.73, 176.75±4.47) GSSH (3.33±0.16, 4.52±0.14, 5.38±0.18) GPX (0.57±0.39, 0.84±0.003, 1.5±0.11) and GSH (1.09±0.09, 1.62±0.14, 1.51±0.16) in liver homogenates of diabetic rats compared to control rats.

Administration of *pterocarpus marsupium* silver nanoparticles (200mg/kg) (172.5±2.73) and standard glibenclamide (176.75±4.47) significantly (P<0.01) increased the activity of CAT enzymes compared to the negative control group (112±5.67)

The level of GSSH (4.52±0.14, 3.33±0.16, 5.38±0.18) and GPX (0.57±0.39, 0.84±0.03,1.5±0.11) level were also increased with the treatment of *Pterocarpus marsupium* aqueous extract *pterocarpus marsupium* silver nanoparticles
(200 mg/kg), glibenclamide (2.5mg/kg) compared with the negative control in liver. (112±5.67)

The non-enzymatic antioxidant, GSH level also decreased in diabetic control group compared with the control group. Treatment with *Pterocarpus marsupium* aqueous extract 200 mg/kg (1.09±0.09) *pterocarpus marsupium* silver nanoparticles 200 mg/kg (1.62±0.14) and glibenclamide (1.51±0.16) significantly (P<0.01) increases this enzymes level compared with the negative control group in liver

The effect of *Pterocarpus marsupium* bark extract & its silver nanoparticles on MDA shown in table. MDA level was found to be elevated in streptozotocin and nicotinamide-induced diabetic rat (22.5±1.84) compared to

control rats (6.64±0.3.80) in liver. This level was significantly ($P<0.01$) reduced in the diabetic rats treated with the, *Pterocarpus marsupium* bark extract & its silver nanoparticles 200 mg/kg (18.5±0.64, 13.17±0.86) and standard glibenclamide (11.52±0.46) treated groups.

Table 23 : Effect of aqueous extract of *Pterocarpus marsupium* silver nanoparticles on the blood glucose level in streptozotocin and nicotinamide-induced type 2 diabetes mellitus rats

GROUPS	DRUG TREATMENT	BLOOD GLUCOSE (mg/dL)		
		0 DAY	7th DAY	14th DAY
I	0.5% w/v CMC (1ml/ 200g b.w.)	82 ± 2.160	80.5± 3.12	84 ±1.291
II	Diabetic control (STZ-60mg/kg NIC120mg/kg)	243 ± 4.60$^{\#\#}$	259 ±3.81$^{\#\#}$	269.25± 5.10$^{\#\#}$
III	Diabetic + AEPM (200mg/kg)	238 ± 7.26^{ns}	223.75 ±5.25**	195± 2.90**
IV	Diabetic + PMAgNP (200mg/kg)	238.75 ± 3.750^{ns}	209.75± 3.40**	179.25 ±1.493**
V	Diabetic + glibenclamide (2.5mg/kg)	232.75 ± 7.20^{ns}	194.5±1.84**	165.25± 3.78**

One way ANOVA followed by dunnet's test

Each value represents the mean ± SEM, n= 8

In 0th day, Group II , # $P< 0.01$ Vs Group I ,

Groups III, IV, V Vs group II , ^{ns} $P < 0.05$.

On 7th & 14th day, Group II, # $P< 0.01$ Vs Group I,

Groups III, IV, V Vs group II , ** $P < 0.01$

Table 24 : Effect of aqueous extract of *Pterocarpus marsupium* leaves on the body weight in streptozotocin and nicotinamide-induced type 2 diabetes mellitus rats

Group	Drug Treatment	Average Body Weight (b.w./kg)		
		0th DAY	7th DAY	14th DAY
I	0.5% w/v CMC (1ml/ 200g b.w.)	181.71±4.49	193.14±2.47	209.28±2.28
II	Diabetic control (STZ-60mg/kg NIC120mg/kg)	164.14±5.30##	159.85±3.95##	154.28±2.29##
III	Diabetic + AEPM (200mg/ kg)	165.85±6.89**	168±9.38*	172.42±9.19**
IV	Diabetic + PMAgNP (200mg/kg)	164.28±8.71**	169.14±3.32**	175.57±3.32**
V	Diabetic + glibenclamide (2.5mg/kg)	167.42±4.72**	173±8.3**	173±8.3**

One way ANOVA followed by Dunnet's test

Each value represents the mean ± SEM, n= 8

On 7th & 14th day, Group II, # P< 0.01 Vs Group I,

Groups IV, V Vs group II, ** P < 0.01

Group III Vs Group II * P< 0.05

Table 25 : Effect of *Pterocarpus marsupium* silver nanoparticles on the serum in streptozotocin and nicotinamide-induced type 2 diabetes mellitus rats

DRUG TREATMENT	Total protein (mmoles/min/tissue)	MDA (nmoles/min/ mg protein)	SOD (nmoles/min/ mg protein)	CAT (µmoles/min/ mg protein)	GSSH (µmoles/min/ mg protein)	GPx (µmoles/min/ mg protein)	GSH (µmoles/min/ mg protein)
0.5% w/v CMC (1ml/ 200g b.w.)	209±5.297	6.64±3.80**	404±6.277	189.75±1.49	6.37±0.28	1.32±0.1	2.56±0.07
Diabetic control (STZ-60mg/kg NIC120mg/kg	101±5.323#	22.5±1.84**	176.5±4.40#	112±5.67#	2.34±0.18#	0.05±0.01#	0.63±0.05#
Diabetic + AEPM (200mg/kg)	133.5±5.33**	18.5±0.64**	274.5±15.2**	154.5±3.12**	3.33±0.16*	0.57±0.39**	1.09±0.09*
Diabetic + PMAgNP(200mg /kg)	163±3.742**	13.17±0.86**	330.75±7.95**	172.5±2.73**	4.52±0.14**	0.84±0.03**	1.62±0.14**
Diabetic + glibenclamide (2.5mg/kg)	198±9.613**	11.52±0.46**	368.75±5.97**	176.75±4.47**	5.38±0.18**	1.5±0.11**	1.51±0.16**

Each value represents the mean ± SEM, n= 8 In negative group, # P< 0.01 Vs control
Group IV & V ** P < 0.01 Vs Group II and Group III *P< 0.05 Vs group II
Data were analysed by one way ANNOVA followed by Dunnet's test.

IN VIVO RESULTS

Acute toxicity studies and selection of dose for in-vivo studies

The aqueous extract of bark of *Pterocarpus marsupium* silver nanoparticles were selected and used for further in vivo evaluation. Acute toxicity was carried out as per OECD guidelines 420 employing fixed dose procedure for selecting the dose for biological activity.

For acute toxicity studies female *wistar* rats weighing 180-195gms were taken and they were fasted overnight before the experimental day. Overnight fasted rats were weighed and body weight determined for dose calculation and test compound were administered orally.

2000 mg/kg dose of Pterocarpus marsupium silver nanoparticles and the rats were observed for signs of acute toxicity. No toxic effect was observed after sufficient interval of time (2-3days).

Signs and symptoms of toxicity and death if any were observed individually for each rat at 0, 0.5, 1, 2, 3 and 4h for first 24h and thereafter daily for 14 days. Diet was given to the animals after 4th hour of dosing. The animals were observed twice daily for 14 days and body weight changes, food and water consumption were noted.

In acute toxicity studies, it was found that the animals were safe up to a maximum dose of 2000mg/kg of body weight. There were no changes in normal behavioural pattern and no signs and symptoms of toxicity and mortality in rats. As per the OECD 420 guidelines *Pterocarpus marsupium* silver nanoparticles can be included in the category 5 or unclassified category of globally harmonized classification system (GHS).

Hence based on these results the *Pterocarpus marsupium* silver nanoparticles were considered non-toxic and 1/20th dose were used for the biological evaluation (antidiabetic activity) and the studies were conducted at dose

levels of 200 mg/kg body weight.

Table 21 : OBSERVATIONS DONE FOR THE ACUTE ORAL TOXICITY STUDY OF TEST COMPOUNDS

Parameters observed		0 h	0.5h	1 h	2 h	4 h	Day 2&3	Day 4&5	Day 6&7	Day 8&9	Day 10&11	Day 12&13	Day 14
Respiratory	Dyspnoea	-	-	-	-	-	-	-	-	-	-	-	-
	Apnoea	-	-	-	-	-	-	-	-	-	-	-	-
	Nostril discharges	-	-	-	-	-	-	-	-	-	-	-	-
Motor activity	Tremor	-	-	-	-	-	-	-	-	-	-	-	-
	Hyper activity	-	-	-	-	-	-	-	-	-	-	-	-
	Hypo activity	-	-	-	-	-	-	-	-	-	-	-	-
	Ataxia	-	-	-	-	-	-	-	-	-	-	-	-
	Jumping	-	-	-	-	-	-	-	-	-	-	-	-
	Catalepsy	-	-	-	-	-	-	-	-	-	-	-	-
	Locomotor activity	-	-	-	-	-	-	-	-	-	-	-	-
Reflexes	Corneal reflex	-	-	-	-	-	-	-	-	-	-	-	-
	Pinna reflex	-	-	-	-	-	-	-	-	-	-	-	-
	Righting reflex	-	-	-	-	-	-	-	-	-	-	-	-
Convulsion	Tonic and clonic convulsion	-	-	-	-	-	-	-	-	-	-	-	-
Muscle Tone	Hypertonia	-	-	-	-	-	-	-	-	-	-	-	-
	Hypotonia	-	-	-	-	-	-	-	-	-	-	-	-
Ocular sign	Lacrimation	-	-	-	-	-	-	-	-	-	-	-	-
	Miosis	-	-	-	-	-	-	-	-	-	-	-	-
	Mydriasis	-	-	-	-	-	-	-	-	-	-	-	-
	Ptosis	-	-	-	-	-	-	-	-	-	-	-	-
Skin	Edema	-	-	-	-	-	-	-	-	-	-	-	-
	Skin and fur	-	-	-	-	-	-	-	-	-	-	-	-
	Erythema	-	-	-	-	-	-	-	-	-	-	-	-
Cardiovascular signs	Bradycardia	-	-	-	-	-	-	-	-	-	-	-	-
	Tachycardia	-	-	-	-	-	-	-	-	-	-	-	-
Piloerection	Contraction of erectile tissue of hair	-	-	-	-	-	-	-	-	-	-	-	-
Gastro intestinal signs	Diarrhoea	-	-	-	-	-	-	-	-	-	-	-	-

Table 22 : MORTALITY RECORD FOR TEST COMPOUND IN ACUTE ORAL TOXICITY STUDY

Sighting study: 2000mg/kg		**Main study: 2000 mg/kg**							
No. of animals	1	2	3	4	5	6	7	8	9
Body weight (g)	180	185	180	190	185	180	185	185	190
Sex	Female	Female	Female	Female	Female	Female	Female	Female	Female
30 min	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil
1 h	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil
2 h	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil
3 h	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil
4 h	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil
Day 1	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil
Day 2	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil
Day 3	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil
Day 4	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil
Day 5	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil
Day 6	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil

Sighting study: 2000mg/kg		Main study: 2000 mg/kg							
No. of animals	1	2	3	4	5	6	7	8	9
Body weight (g)	180	185	180	190	185	180	185	185	190
Sex	Female	Female	Female	Female	Female	Female	Female	Female	Female
Day 7	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil
Day 8	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil
Day 9	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil
Day 10	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil
Day 11	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil
Day 12	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil
Day 13	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil
Day 14	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil
Mortality	0/1	0/1		0 0/1	0/1	0/1	0/1	0/1	0/1

Note: Acute toxicity study of compound was performed and it was non-toxic up to 2000 mg/kg dose.

***IN VIVO* ANTIDIABETIC ACTIVITY OF *Pterocarpus marsupium* silver nanoparticles**

Table 24 shows that the biological evaluation was carried out using 200mg/kg dose of aqueous bark extract of *Pterocarpus marsupium* silver nanoparticles. Table show the body weight of control and experimental animals on 0, 7, 14, 21, 28 days of treatment. There was significant reduction of body weight in diabetic control animals compared to normal control & test drug treated animals. On 7th day significant reduction of body weight was observed in diabetic control animals (159.85±3.95) when compared to control rats (193.14±2.47). Reduction in body weight indicates the induction of diabetes. The normal control (209.28±2.28) and drug treated rats *pterocarpus marsupium* aqueous extract, *Pterocarpus marsupium* silver nanoparticles, glibenclamide gained significant weight (172.42±9.19, 175.57±3.32, and 173±8.3) ($P<0.01$) on 28 days of treatment.

Table 23 shows the effect of *pterocarpus marsupium* aqueous extract, *Pterocarpus marsupium* silver nanoparticles, glibenclamide on streptozotocin and nicotinamide induced diabetes in rats. Initially it was found that a significant ($P<0.01$) increase in blood glucose level was observed in STZ-nicotinamide induced diabetic rats (243±4.601) compared to normal control (80.5±3.12). After the daily treatment for 14 days showed significant ($p<0.05$), ($P<0.01$) reduction in blood glucose 200 mg/kg p.o of aqueous extract of *Pterocarpus marsupium* (195±2.90), aqueous extract of *Pterocarpus marsupium* silver nanoparticles (179.25±1.493), 2.5 mg/kg, p.o of glibenclamide (165.25±3.78) as compared to diabetic control group. (243 ± 4.60)$^{\#\#}$

Table 25 shows that the level of protein after 28 days of treatment in liver is given in Table. A decrease in protein level was found in diabetic control (101±5.323) rats compared with control rats (209±5.297). Administration of *Pterocarpus marsupium* aqueous extract, *Pterocarpus marsupium* silver nanoparticles (200 mg/kg), (133.5+5.33,163+3.742) and glibenclamide (2.5

mg/kg) treated rats (198.25±9.613) restored the protein level significantly near to normal levels. The results were found to be statistically significant (P<0.01).

A significant (P<0.01) increase was observed in the activities of SOD in the treatment groups administered with, *Pterocarpus marsupium* aqueous extract, *Pterocarpus marsupium* silver nanoparticles (200 mg/kg), glibenclamide (274.5±15.2, 330.75±7.95, 368.75 ± 5.97) CAT (154.5±3.12, 172.5±2.73, 176.75±4.47) GSSH (3.33±0.16, 4.52±0.14, 5.38±0.18) GPX (0.57±0.39, 0.84±0.003, 1.5±0.11) and GSH (1.09±0.09, 1.62±0.14, 1.51±0.16) in liver homogenates of diabetic rats compared to control rats.

Administration of *pterocarpus marsupium* silver nanoparticles (200mg/kg) (172.5±2.73) and standard glibenclamide (176.75±4.47) significantly (P<0.01) increased the activity of CAT enzymes compared to the negative control group (112±5.67)

The level of GSSH (4.52±0.14, 3.33±0.16, 5.38±0.18) and GPX (0.57±0.39, 0.84±0.03,1.5±0.11) level were also increased with the treatment of *Pterocarpus marsupium* aqueous extract *pterocarpus marsupium* silver nanoparticles
(200 mg/kg), glibenclamide (2.5mg/kg) compared with the negative control in liver. (112±5.67)

The non-enzymatic antioxidant, GSH level also decreased in diabetic control group compared with the control group. Treatment with *Pterocarpus marsupium* aqueous extract 200 mg/kg (1.09±0.09) *pterocarpus marsupium* silver nanoparticles 200 mg/kg (1.62±0.14) and glibenclamide (1.51±0.16) significantly (P<0.01) increases this enzymes level compared with the negative control group in liver

The effect of *Pterocarpus marsupium* bark extract & its silver nanoparticles on MDA shown in table. MDA level was found to be elevated in streptozotocin and nicotinamide-induced diabetic rat (22.5±1.84) compared to

control rats (6.64±0.3.80) in liver. This level was significantly ($P<0.01$) reduced in the diabetic rats treated with the, *Pterocarpus marsupium* bark extract & its silver nanoparticles 200 mg/kg (18.5±0.64, 13.17±0.86) and standard glibenclamide (11.52±0.46) treated groups.

Table 23 : Effect of aqueous extract of *Pterocarpus marsupium* silver nanoparticles on the blood glucose level in streptozotocin and nicotinamide-induced type 2 diabetes mellitus rats

GROUPS	DRUG TREATMENT	BLOOD GLUCOSE (mg/dL)		
		0 DAY	7th DAY	14th DAY
I	0.5% w/v CMC (1ml/ 200g b.w.)	82 ± 2.160	80.5± 3.12	84 ±1.291
II	Diabetic control (STZ-60mg/kg NIC120mg/kg)	243 ± 4.60$^{\#\#}$	259 ±3.81$^{\#\#}$	269.25± 5.10$^{\#\#}$
III	Diabetic + AEPM (200mg/kg)	238 ± 7.26^{ns}	223.75 ±5.25**	195± 2.90**
IV	Diabetic + PMAgNP (200mg/kg)	238.75 ± 3.750^{ns}	209.75± 3.40**	179.25 ±1.493**
V	Diabetic + glibenclamide (2.5mg/kg)	232.75 ± 7.20^{ns}	194.5±1.84**	165.25± 3.78**

One way ANOVA followed by dunnet's test

Each value represents the mean ± SEM, n= 8

In 0th day, Group II , # $P< 0.01$ Vs Group I ,

Groups III, IV, V Vs group II , ^{ns} $P < 0.05$.

On 7th & 14th day, Group II, # $P< 0.01$ Vs Group I,

Groups III, IV, V Vs group II , ** $P < 0.01$

Table 24 : Effect of aqueous extract of *Pterocarpus marsupium* leaves on the body weight in streptozotocin and nicotinamide-induced type 2 diabetes mellitus rats

Group	Drug Treatment	Average Body Weight (b.w./kg)		
		0th DAY	7th DAY	14th DAY
I	0.5% w/v CMC (1ml/ 200g b.w.)	181.71±4.49	193.14±2.47	209.28±2.28
II	Diabetic control (STZ-60mg/kg NIC120mg/kg)	164.14±5.30##	159.85±3.95##	154.28±2.29##
III	Diabetic + AEPM (200mg/kg)	165.85±6.89**	168±9.38*	172.42±9.19**
IV	Diabetic + PMAgNP (200mg/kg)	164.28±8.71**	169.14±3.32**	175.57±3.32**
V	Diabetic + glibenclamide (2.5mg/kg)	167.42±4.72**	173±8.3**	173±8.3**

One way ANOVA followed by Dunnet's test

Each value represents the mean ± SEM, n= 8

On 7th & 14th day, Group II, # P< 0.01 Vs Group I,

Groups IV, V Vs group II, ** P < 0.01

Group III Vs Group II * P< 0.05

Table 25 : Effect of *Pterocarpus marsupium* silver nanoparticles on the serum in streptozotocin and nicotinamide-induced type 2 diabetes mellitus rats

DRUG TREATMENT	Total protein (mmoles/min/tissue)	MDA (nmoles/min/ mg protein)	SOD (nmoles/min/ mg protein)	CAT (µmoles/min/ mg protein)	GSSH (µmoles/min/ mg protein)	GPx (µmoles/min/ mg protein)	GSH (µmoles/min/ mg protein)
0.5% w/v CMC (1ml/ 200g b.w.)	209±5.297	6.64±3.80**	404±6.277	189.75±1.49	6.37±0.28	1.32±0.1	2.56±0.07
Diabetic control (STZ-60mg/kg NIC120mg/kg	101±5.323#	22.5±1.84**	176.5±4.40#	112±5.67#	2.34±0.18#	0.05±0.01#	0.63±0.05#
Diabetic + AEPM (200mg/kg)	133.5±5.33**	18.5±0.64**	274.5±15.2**	154.5±3.12**	3.33±0.16*	0.57±0.39**	1.09±0.09*
Diabetic + PMAgNP(200mg/kg)	163±3.742**	13.17±0.86**	330.75±7.95**	172.5±2.73**	4.52±0.14**	0.84±0.03**	1.62±0.14**
Diabetic + glibenclamide (2.5mg/kg)	198±9.613**	11.52±0.46**	368.75±5.97**	176.75±4.47**	5.38±0.18**	1.5±0.11**	1.51±0.16**

Each value represents the mean ± SEM, n= 8 In negative group, # P< 0.01 Vs control
Group IV & V ** P < 0.01 Vs Group II and Group III *P< 0.05 Vs group II
Data were analysed by one way ANNOVA followed by Dunnet's test.

Streptozotocin (STZ) is 1-methyl-l-nitrosourea attached to the carbon-2 position of glucose that causes β-cell necrosis and induces experimental diabetes in many animal models. It causes DNA strand breaks that induce the activation of poly-ADP-ribose synthetase followed by lethal nicotinamide adenine dinucleotide (NAD) depletion. Nicotinamide adenine dinucleotide causes activation of the poly ADP ribose synthase to repair the damaged DNA and protecting the decrease in the level of NAD and proinsulin thereby partially reversing the inhibition of insulin secretion to prevent the aggravation of experimental diabetes. This condition shows a number of features which are similar with type 2 diabetic mellitus (T2DM). Hence, based on this point of view, the hypoglycemic activity of *Pterocarpus marsupium* silver nanoparticles carried out on STZ and nicotinamide induced type 2 diabetic rats.

In streptozotocin and nicotinamide-induced type 2 diabetic mellitus there was a significant reduction in body weight in diabetic rats is due to excessive break down of tissue protein. Treatment with *Pterocarpus marsupium* silver nanoparticles & glibenclamide improved body weight significantly inducing prevention of muscle wasting due to hyperglycemic condition.

The difference in body weight is large in control group compared with negative control group. In treatment group also, there is an increase in the body weight compared with the diabetic control group.

Generation of free radicals in diabetes mellitus reacts with lipids causing lipid peroxidation, resulting in the release of products such as malondialdehyde, hydroperoxide and hydroxyl radicals. The oxidative stress in diabetes decreases the antioxidant status. SOD, CAT, GSSH and GPx are enzymatic antioxidants and non enzymatic antioxidant like GSH plays an important role in protecting cells from being exposed to oxidative damage by direct elimination of reactive oxygen species (ROS). CAT and SOD are considered primary enzymes since they are

involved in the direct elimination of ROS. SOD is an important defense enzyme which catalyzes the dismutation of superoxide radical and CAT is a hemoprotein which catalyzes the reduction of H_2O and protects the tissue from hydroxyl radicals. GPX, a selenium containing enzyme present in significant concentration detoxifies H_2O_2 to H2O through the oxidation of reduced glutathione. The reduced activity of SOD, CAT, GPX, GSSH, GSH in the liver during diabetes is a result of deleterious effects which results in the accumulation of superoxide anion radicals and H2O2. The activity of enzymatic and non enzymatic antioxidants are increased significantly in *Pterocarpus marsupium* silver nanoparticle treated animals ($P<0.01$)

Marked increase in the concentration of MDA was observed in the liver of diabetes rats. *Pterocarpus marsupium* silver nanoparticle and glibenclamide tends to bring the increased concentration of lipid peroxidation products to near normal level.

In conclusion it maybe stated that, there occurs a significant ($P<0.01$) decrease in the hyperglycemic state after the administration of *Pterocarpus marsupium* silver nanoparticle which reduce the severity of oxidative and acuity of hyperglycemia, a process that closely linked to glucose oxidation and formation of free radicals. Our results suggested that *Pterocarpus marsupium* silver nanoparticle has more favourable reduction in lipid level in STZ and nicotinamide - induced diabetic rats, compared with glibenclamide as well as regeneration of ß-cells of pancreas. The present study suggests that *Pterocarpus marsupium* silver nanoparticles can be successfully utilized for the management of diabetes due to their anti-hyperglycemic action.

SUMMARY & CONCLUSION

Nanoparticles can be defined as particulate dispersions or solid particles with a size in the range of 10-1000nm. In recent years, particularly green synthesis of silver nanoparticles, have been used as potential drug delivery system because of their ability to target a particular organ.

The input of today's nanotechnology is that it allows real progress to achieve temporal and spatial site-specific delivery. The market of nanotechnology and drug delivery systems based on this technology will be widely felt by the pharmaceutical industry. In recent years, the number of patents and products in this field is increasing significantly.

Diabetes mellitus refers to the group of diseases that lead to high blood glucose levels due to defects in either insulin secretion or insulin action. Diabetes develops due to a diminished production of insulin (in type 1) or resistance to its effects (in type 2 and gestational). Both lead to hyperglycaemia, which largely causes acute signs of diabetes: excessive urine production, resulting compensatory thirst and increased fluid intake, blurred vision, unexplained weight loss, lethargy, and changes in energy metabolism.

The main aim of this study is to formulate silver nanoparticles with the bark of aqueous extract of Pterocarpus marsupium by green synthesis method without the use of any chemicals to deliver drugs in a controlled manner and to evaluate the formulation against streptozotocin nicotinamide induced type 2 diabetes in *wistar* rats. The formulation has reduced side effects, thus minimized the dosing frequency when compared it with aqueous extract of *Pterocarpus marsupium.*

Preformulation studies were carried out to find out the solubility of *Pterocarpus marsupium* and solubility gave an idea that it is soluble in water, phosphate buffer, ethanol & dimethyl sulphoxide.

UV spectral studies authenticate the spectra obtained for Pterocarpus marsupium. UV spectra gave the maximum absorption peak at 337 nm

Then the calibration curve of Pterocarpus marsupium Roxb. bark extracts were plotted and R^2 value was found to be 0.99802 for the concentration between 40- 200 µg/ml.

The herbal *Pterocarpus marsupium* silver nanoparticles were formulated by green synthesis method. The silver nanoparticle formation is indicated by the color change of solution from yellow to brown where the reduction of silver ion occurs which leads to the formation of silver nanoparticles. This was further confirmed by Uv- visible spectral analysis of the sample at different time intervals and found to be 427 nm

Field Emission Scanning electron micrograph of the prepared nanoparticles at different magnification showed that the nanoparticles were smooth surface morphology and spherical shape.

Particle size and zeta potential were determined by Malvern Zeta sizer. The particle size analysis confirmed that the prepared sample were in the nanometer range. particle size obtained for the formulation 132.6 nm. Zeta potential values of nanoparticles indicated that the formulated nanoparticles are stable.

The amount of drug entrapped in the nanoparticle was calculated and it is of 93 % which possess high entrapment efficiency.

From the invitro release data, it has been observed that in various time intervals, invitro drug release for *Pterocarpus marsupium* silver nanoparticles

were checked using dialysis bag diffusion method to find the cumulative percentage of drug release. It is 18.53% for 1hr, 26.85% for 2 hrs, 41.98% for 3 hrs, 47.36% for 4 hrs, 73.56% for 5 hrs, 88.41% for 6 hrs and 92.8% for 24 hrs. the drug release increased from 1 to 1.5 fold during every one hour of time interval.

The *in-vitro* antidiabetic activity of the *Pterocarpus marsupium* silver nanoparticles have been evaluated by measuring its α-amylase inhibitory activity. α-amylase is an enzyme which converts starch to oligosaccharide. Inhibition of this enzyme can retard glucose absorption, and thereby can produce hypoglycemic action.

The results of *in-vitro* study with alpha amylase exhibited potential inhibitory activity for the aqueous extract of *Pterocarpus marsupium* silver nanoparticles and it was compared with standard acarbose. *pterocarpus marsupium* Roxb. silver nanoparticles percentage α amylase inhibition at lowest (0.2mg/ml) and highest (1mg/ml) concentration were found to be 25.68% and 86.15% respectively.

Streptozotocin (STZ) is 1-methyl-l-nitrosourea attached to the carbon-2 position of glucose that causes β-cell necrosis and induces experimental diabetes in many animal models. It causes DNA strand breaks that induce the activation of poly-ADP-ribose synthetase followed by lethal nicotinamide adenine dinucleotide (NAD) depletion. Nicotinamide adenine dinucleotide causes activation of the poly ADP ribose synthase to repair the damaged DNA and protecting the decrease in the level of NAD and proinsulin thereby partially reversing the inhibition of insulin secretion to prevent the aggravation of experimental diabetes. This condition shows a number of features which are similar with type 2 diabetic mellitus (T2DM). Hence, based on this point of view, the hypoglycemic activity of *Pterocarpus marsupium* silver nanoparticles carried out on STZ and nicotinamide induced type 2 diabetic rats.

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MINISTRY OF ENVIRONMENT, FOREST & CLIMATE CHANGE
भारतीय वनस्पति सर्वेक्षण
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सं. भा.व.स./द.क्षे.के./No.: BSI/SRC/5/23/2020/Tech./505

दिनांक/Date: 9th January 2020

पौधे प्रमाणीकरण प्रमाणपत्र / PLANT AUTHENTICATION CERTIFICATE

The plant specimen given by you for authentication is identified as *Pterocarpus marsupium* Roxb. - FABACEAE. The identified specimen is returned herewith for preservation in their College/ Department/ Institution Herbarium.

C. Murugan 09/01/2020

डॉ सी मुरुगन/Dr. C. Murugan
वैज्ञानिक 'ई' एवं प्रभारी/Scientist 'E'-in-Charge

वैज्ञानिक 'ई' एवं कार्यालय अध्यक्ष
SCIENTIST 'E' & HEAD OF OFFICE I/c
भारतीय वनस्पति सर्वेक्षण
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कोयम्बतूर / COIMBATORE - 641 003

सेवा में / To

Ms. Saikrishnapriya B
Semester IV (2nd Year) PG Student
Department of Pharmaceutics
Sri Ramakrishna Institute of Paramedical Sciences
Coimbatore – 641 046

9/1/2020
dc

INSTITUTIONAL ANIMAL ETHICS COMMITTEE

(CPSCEA Registration # 1559/PO/Re/S/11/CPCSEA)

College of Pharmacy

Sri Ramakrishna Institute of Paramedical Sciences

(Educational Service of M/s SNR Sons Charitable Trust)

Coimbatore – 641 044.

IAEC PROTOCOL APPROVAL CERTIFICATE

Date: 11/12/2019

Approval #:1559/PO/Re/S/11/CPCSEA

IAEC PROTOCOL#: COPSRIPMS/IAEC/PG/Pharmaceutics/001/2019-2020

IAEC PROTOCOL TITLE: Evaluation of antidiabetic activity of aqueous extract of bark of *Pterocarpus marsupium* silver nanoparticles against streptozotocin and nicotinamide induced type 2 diabetes in rats.

Dear Dr. J. Bagyalakshmi,

This is to certify that above mentioned animal study protocol has been approved in IAEC meeting held on 11/12/2019 with following conditions:

PI : Ms. Sai Krishna Priya B

Duration of Study : months/years (From 11/12/2019 to11/12/2020)

Animal Sanctioned : 49 *Wistar* Rats

Species : Rats

Strain : *Wistar*

Sex/Age : Male (40 Rats) and Female (09 Rats) / 12 Weeks

Total No. : 49

It is requested to get prior approval of IAEC in case of any deviation/changes in submitted protocol. Please maintain the Form D & provide the photocopy to IAEC along with project report at defined interval.

11/12/19		11/12/19
Member Secretary	**Chairman**	**Main nominee**
IAEC, COP, SRIPMS	**IAEC, COP, SRIPMS**	**CPCSEA**
Dr. K. Asok Kumar, M.Pharm., Ph.D. Professor & Head Department of Pharmacology College of Pharmacy Sri Ramakrishna Institute of Paramedical Sciences Coimbatore - 641 044.	CHAIRMAN IAEC	MAIN NOMINEE CPCSEA