# EVALUATION OF ANTI DIABETIC ACTIVITY OF ETHANOLIC ROOT EXTRACT OF *Premna corymbosa rottl* AGAINST STREPTOZOTOCIN AND NICOTINAMIDE IN DIABETIC RATS

Dissertation submitted to

THE TAMILNADU Dr.M.G.R.MEDICAL UNIVERSITY, CHENNAI,

In partial fulfillment of the requirement for the award of the degree of

**MASTER OF PHARMACY**

(PHARMACOLOGY)

Submitted By

Reg.No:261625403

Under the guidance of

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DEPARTMENT OF PHARMACOLOGY

**SEPTEMBER 2018**

**NANDHA COLLEGE OF PHARMACY AND RESEARCH INSTITUTE**

**ERODE- 638 052, TAMILNADU**

# *CERTIFICATES*

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## CERTIFICATE

This is to certify that the work embodied in this thesis entitled **"EVALUATION OF ANTI-DIABETIC ACTIVITY OF ETHANOLIC ROOT EXTRACT OF *Premna Corymbosa Rottl* AGAINST STREPTOZOTOCIN AND NICOTINAMIDE IN DIABETIC RATS"** submitted to The Tamilnadu Dr. M.G.R. Medical University, Chennai, was carried out by **Mr. MUHAMMED RASHID.V.P.** in the Department of Pharmacology, Nandha College of Pharmacy, Erode-52 for the partial fulfillment for the degree of **MASTER OF PHARMACY** in Pharmacology under the supervision of **Dr.S.HAJASHERIEF, M.Pharm., Ph.D.,** Professor, Department of Pharmacology, Nandha College of Pharmacy, Erode.

The work is original and has not been previously formed the basis for the award of any other Degree, Diploma, Associateship, Fellowship or any other similar title and the dissertation represent entirely an independent work on the part of the candidate.

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This work is original and has not been submitted in part or full for any other degree or diploma of this or any other university.

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## EVALUATION CERTIFICATE

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**Committee for the Purpose of control and Supervision of Experiments on Animals (CPCSEA)**
**Institutional Animal Ethics Committee (IAEC)**
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| | | |
|---|---|---|
| Title of the project | : | Evaluation of Anti-Diabetic Activity of Ethanolic Root Extract of *Premna corymbosa rottl* against Streptozotacine and Nicotinamide in diabetic rats |
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## DECLARATION

The work presented in this thesis entitled "**EVALUATION OF ANTIDIABETIC ACTIVITY OF ETHANOLIC ROOT EXTRACT OF *Premna corymbosa rottl* AGAINST STREPTOZOTOCIN AND NICOTINAMIDE IN DIABETIC RATS**" was carried out by me in the Department of Pharmacology, Nandha College of Pharmacy, Erode -52 under the direct supervision of **Dr. S. HAJA SHERIEF,** Department of Pharmacology, Nandha College of Pharmacy, Erode-52.

This work is original and as not been submitted in part of full for the award of any other degree or diploma of any other University.

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# *ACKNOWLEDGEMENT*

## ACKNOWLEDGEMENT

" Develop an attitude of gratitude, and give thanks for everything that happens to you, knowing that every step forward is a step toward achieving something bigger and better than your current situation. Success of any project depends solely on support, guidance and encouragement received from the guide and well wishers**"**

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*DEDICATEDB TO MY FAMILY &FRIENDS*

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# *ABBREVIATIONS*

# ABBREVIATIONS

| | | |
|---|---|---|
| 1. | IDDM | Insulin dependent diabetes mellitus |
| 2. | NIDDM | Non-insulin dependent diabetes mellitus |
| 3. | IRS | Insulin reagent substrate |
| 4. | PC1&PC2 | Pro hormone convertase |
| 5. | GLUT2 | Type 2 glucose transportase |
| 6 | ATP | Adenosine triphosphate |
| 7. | OGTT | Oral glucose tolerance test |
| 8. | HbA1c | Glycated hemoglobin levels |
| 9. | SOD | Superoxide dismutase |
| 10. | CAT | Catalase |
| 11. | GPx | Glutathione peroxidase |
| 12. | GST | Glutathione -S-transferase |
| 13. | PPO | Polyphenol oxidase |
| 14. | EEPC | Ethanolic extract of *Premnacorymbosarottl* |
| 15. | CMC | Carboxy methyl cellulose |
| 16. | PCEE | *Premna corymbosa* ethanolic extract |
| 17. | CFA | Complete freund's adjuvant |
| 18. | SGOT | Serum glutamate oxaloacetate transaminase |
| 19. | SGPT | Serum glutamate pyruvate transaminase |
| 20. | ALP | Alkaline phosphatase |
| 21. | ANOVA | One-way analysis of variance |
| 22. | IAEC | Institutional animal ethical commite |
| 23. | STZ | Streptozotocin |
| 24. | AST | Aspartate aminotransferase |
| 25. | ALT | Alanine aminotransferase |
| 26. | HDL | High density lipoprotein |
| 27. | LDL | Low density lipoprotein |
| 28. | VLDL | Very low density lipoprotein |

| | | |
|---|---|---|
| 29. | NAD | Nicotinamide adenine dinucleotide |
| 30. | NADH | Nicotinamide adenine dinucleotide-hydrogen |
| 31. | OECD | Organization for economic co-operation and evelopment |
| 32. | P.O | Per oral |
| 33. | SEM | Standard error mean |
| 34. | P | Probability |
| 35. | mg | Microgram |
| 36. | kg | Kilogram |
| 37. | b.w | Body weight |
| 38. | ml | Millilitre |
| 39. | WHO | World health organization |
| 40. | Mol.wt | Molecular weight |

# *INTRODUCTION*

# 1. INTRODUCTION

## 1.1 DIABETES MELLITUS

The term diabetes mellitus describes a metabolic disorder of multiple etiology characterized by chronic hyperglycemia with disturbances of carbohydrates, fat and protein metabolism resulting from defects in insulin secretion, insulin action or both. Diabetes means that your blood sugar is too high. Your blood always has some sugar in it because the body needs sugar of energy to keep you going. But too much sugar in the blood is not good for your health. Diabetes is the disorder of metabolism the way our body use the digested food for growth and energy. Most of the food we eat is broken down by the Digestive juices into a simple sugar called glucose. which is the main source of fuel for the body(Vigneri R *et al.*, 1987).

Diabetes mellitus is a group of syndromes characterized by hyperglycemia. The World Health Organization (WHO) has designated diabetes mellitus as an epidemic, although it is a non-infectious disease. Nearly 203% of the world's population is estimated to be suffering from this disease, with an increasing trend of 4-5% every year (Allison DB *et al*., 1999).

After digestion, the glucose passes into our bloodstream where it is available for body cells to use for growth and energy. For the glucose to get into the cells, insulin must be present. Insulin is a hormone produced by the pancreas, a large gland behind the stomach. When we eat, the pancreas is supposed to automatically produce the right amount of insulin to move the glucose from our blood into our cells. If your body doesn't make enough insulin or the insulin doesn't work right, the sugar cannot get into the cells. It stays in the blood; this makes your blood sugar level high and causing you to have diabetes. As a result, glucose builds up in the blood, overflow into the urine, and passes out of the body, Thus the body loses its main source of fuel even through the blood contains large amounts of glucose(Vigneri R *et al*., 1987).

Diabetes is a metabolic disorder and metabolism is the way by which the body uses digested food for growth and energy. Glucose is the end product of our food after all the processes of ingestion, digestion, assimilation and absorption which means anything that is consumed, finally breaks down into glucose, once digestion is over, next comes the process known as the assimilation. After the digestion process, the glucose that passes into the bloodstream needs to be utilized by cells for growth and energy. To aid the glucose getting into

the cells, we require a hormone called the insulin a hormone produced by the pancreas of islets of Langerhans (Narayan KM *et al*., 2003).

## 1.2 CLASSIFICATION

The first widely accepted classification of diabetes mellitus was published by WHO in 1980 and modified from in 1995.

- Type 1 or Insulin Dependent Diabetic Mellitus (IDDM) or juvenile onset diabetes.
- Type II or Non Insulin Dependent Diabetes Mellitus (NIDDM) or maturity onset diabetes.
- Gestational Diabetes (diabetes of pregnancy)

### 1.2.1 Type 1 or Insulin Dependent Diabetic Mellitus (IDDM) or juvenile onset diabetes:

Type-I or Insulin dependent diabetes mellitus or juvenile onset diabetes mellitus which is due to the destruction of pancreatic b cells, it causes are unknown but in some cases due to viral infection and current research indicates that it may also be due to auto immunity. Islets cell auto antibodies are present in the serum of 90% newly diagnosed cases. Such antibodies are directed against several cell components including cytoplasm and membrane antigens or against insulin itself (Daneman D *et al.,* 2006).

### 1.2.2 Type II or Non Insulin Dependent Diabetes Mellitus (NIDDM) or maturity onset diabetes:

Type-II or Non insulin Dependent Diabetes Mellitus or Adult onset Diabetes Mellitus which is due to the impairment of insulin release and basal secretion of insulin. In this case, insulin resistance occurs due to the defect in the tissue response to insulin caused by the defective insulin receptors on the target cells. Type-II diabetes is the most common form of diabetes accounting for 90-95% of cases. In Type-II diabetes, the body does not respond properly to insulin, a condition known as insulin resistance (Goldfine A *et al.,* 2001).

### 1.2.3Gestational Diabetes:

Gestational diabetes is a condition in which some women develop gestational diabetes in late pregnancy. Although this form of diabetes usually disappear after the delivery, women who have had gestational diabetes have a 40 to 60 percent chance of developing Type-II diabetes within 5 to 10 years. Maintaining a reasonable body weight and being physically active may help prevent development of Type-II diabetes. About 5% of pregnant women develop a form of Type-II diabetes, usually temporary, in their third trimester called gestational diabetes (American Diabetes Association, 2005).

## 1.3 EPIDEMIOLOGY

Diabetes mellitus became the common non-communicable disease in both developing and developed countries compared to developing countries like India and it became epidemic disease. On the basis of number of epidemiological studies, it has been reported that in India more than fifty million of people suffering with diabetes mellitus next follows China with nearly forty four million people.India leads the world with highest number of diabetic subjects earning the dubious distinction of being termed them 'Diabetes capital of the world'.

Diabetes Atlas 2006 published by the international Diabetes federation, the number of people with diabetes mellitus in India currently around 409 million and is expected to rise to 69.9 million by 2035 unless essential preventive steps are taken. They so called ' Asian India Phenotype' refers to certain unique clinical and biochemical abnormalities in Indians which include increased insulin resistance, greater abdominal adiposity like higher waist circumference a despite lower body mass index, and higher high sensitive C reactive protein, lower adiponectin levels.

The microvascular and macrovascular impediment caused by the diabetes mellitus like diabetic neuropathy, nephropathy, retinopathy, cardiovascular diseases and foot amputation are also became the major health in 21$^{st}$ century. WHO estimated that, in 1995 the number of people with diabetes anticipates rising from current estimate of 150-220 million in 2010 and 300 million in 2025.

## 1.4 PATHOPHYSIOLOGY

### 1.4.1 Type-I Diabetes:

Type I diabetes mellitus (Insulin-Dependent Diabetes Mellitus, IDDM ) is due to destruction of pancreatic beta cells. The cause of beta cells destruction in type-I diabetes is unknown. A few cases have followed viral infections, most commonly with coxsakie virus B or mumps virus. Autoimmunity is believed to be the major mechanism involved. Sensitized T lymphocytes with activity against beta cells have also been demonstrated in some patients.

The etiology of Type-II diabetes mellitus (Non-Insulin-Dependent Diabetes Mellitus, NIDDM) is even less clearly understood. Two factors have been identified:

a) Impaired insulin release-basal secretion of insulin is often normal, but the rapid release of insulin follows a meal is greatly impaired, resulting in failure of normal handling of a carbohydrates load.

b) Insulin resistance-a defect in the tissue response to insulin is believed to play a major role.

This phenomenon is called insulin resistance and is caused by defective insulin receptors on the target cells. Insulin resistance occurs in association with obesity and pregnancy.

### 1.4.2 Type-II Diabetes

#### 1.4.2.1 Pancreas

The pancreas is a pale grey gland weighing about 60 grams and is about 12 to 15 cm long and situated in the epigastric and left hypochondriac regions of the abdominal cavity. It consists of a broad head, a body and a narrow tail. The head lies in the curve of the duodenum, the body behind the stomach and the tail in front of the left kidney and just reaches the spleen. The pancreas is both an exocrine and endocrine gland.

### 1.4.2.2 Functions of Pancreas

- The enzymes secreted by the exocrine tissue in the pancreas help in breaking down carbohydrates, proteins, fats and acids, in the duodenum. When they enter into the duodenum, they get activated, the exocrine tissue also secretes bicarbonates to neutralize stomach in the duodenum.
- The functions of the endocrine tissue in the pancreas is to secrete the hormones insulin and glucagon.

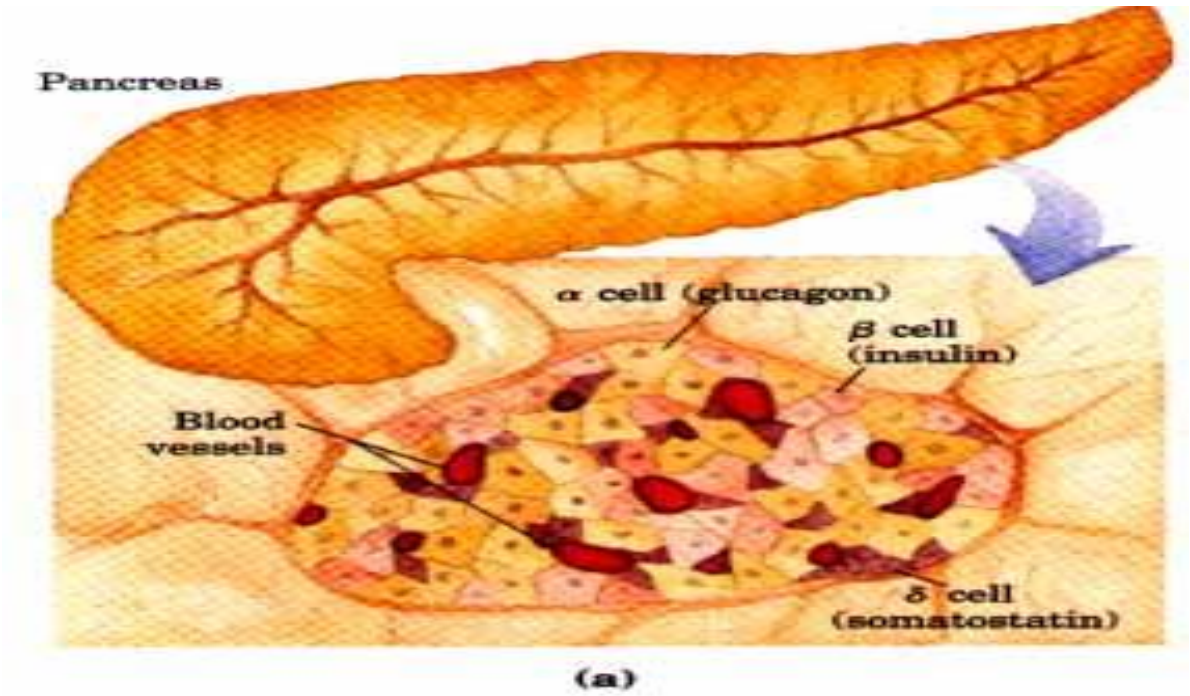


**Figure No.1. Pancreas and islet of langerhans**

### 1.4.2.3 Islets of Langerhans

Islets of Langerhans is the insulin-producing tissue which consists of groups of specialized cells in the pancreas that make secrete hormone named after the German pathologist Paul Langerhans (1847- 1888), who discovered in 1869.These cells sit in group that Langerhans likened to little islands in the pancreas the pancreatic islet includes four types of hormones secreting cells :

- **Alpha cells: -** composing about 20% of pancreatic islet cells, it secretes glucagon which rises blood glucose level.
- **Beta cells: -** These secretes insulin which reduces the blood glucose level, constitute about 70% pancreatic islet cells (Tortora GJ *et al.*, 2005).
- **Delta cells: -** constitute the 5% of pancreatic islets cells, these secretes somatostatin which provides local inhibitory regulation of insulin and glucagon release within the islet.
- **F cells: -** constitute the remainder of pancreatic islet cells, it secrete pancreatic polypeptide, which inhibit the secretion of somatostatin and pancreatic digestive enzyme (Tortora GJ *et al*., 2005).

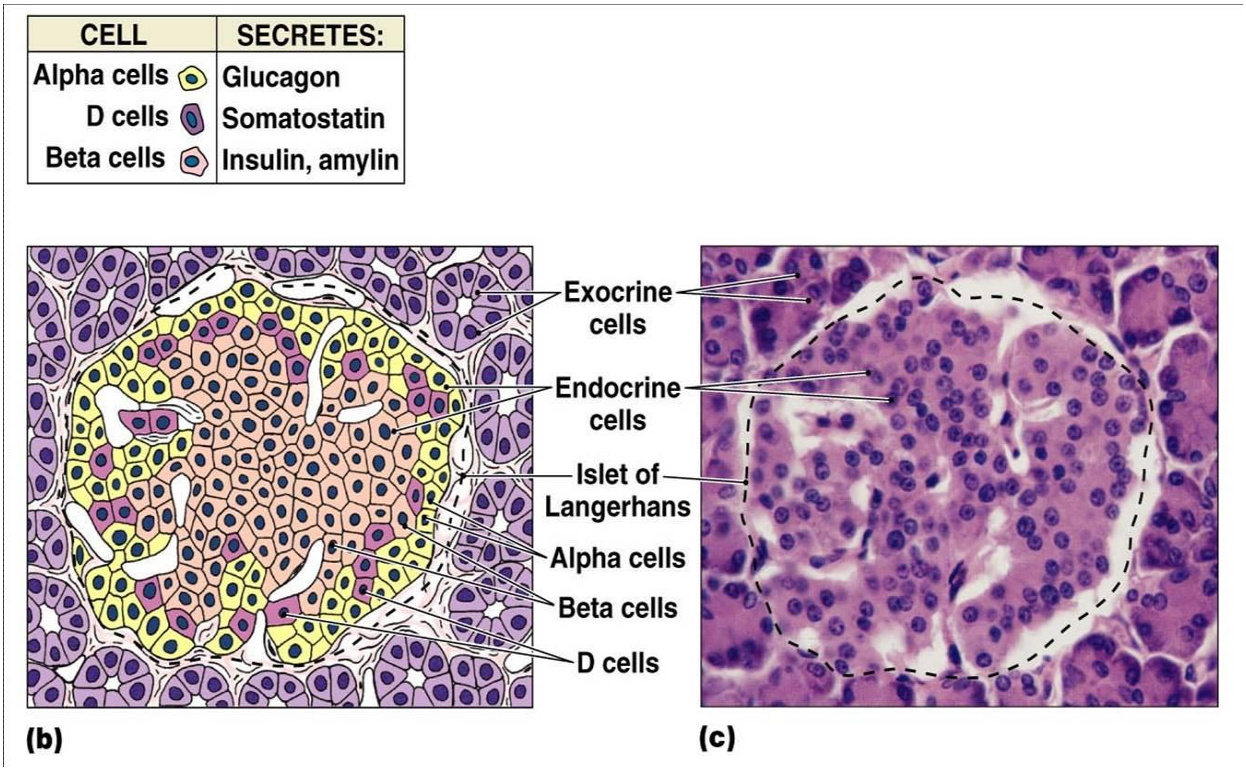


**Figure No.2. Structure of islet of langerhans of pancreas**

### 1.4.2.4 Insulin

Insulin is a polypeptide hormone produced by the b cells of islets of Langerhans of pancreas. It has profound influences on the metabolism of carbohydrate, fat and protein. Insulin is considered as anabolic hormone, as it promotes the synthesis of glycogen, triacylglycerol and proteins. This hormone has been implicated in the development of diabetes mellitus. Human insulin (Mol. wt. 5,734) contains 51 amino acids, arranged in to polypeptides chains. The chain A has 21 amino acids while B has 30 amino acids. Both are held together by two inter chain disulfide bridges. The synthesis of insulin involves two precursors, namely pre proinsulin with 108 amino acids and pro insulin with 86 amino acids.

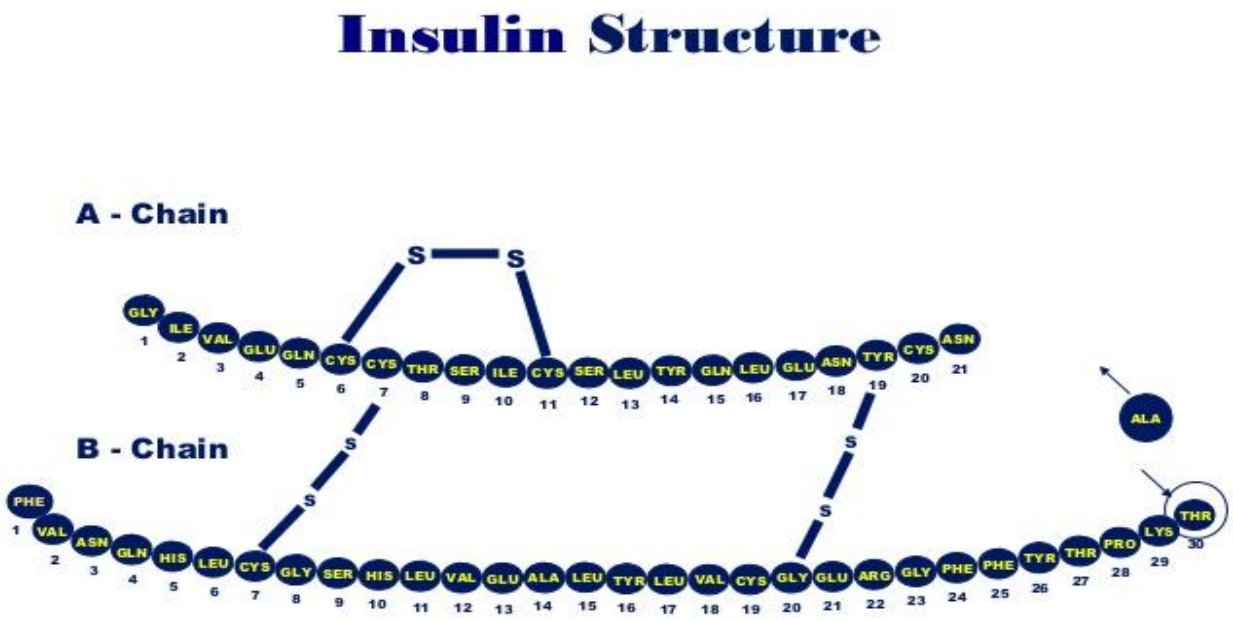


**Figure No.3. Structure of Insulin**

**Insulin Mechanism of Action**

Insulin binds to specific receptors on the surface of its target cells. The receptors are a large transmembrane glycoprotein complex consisting of two alpha and beta subunits. The alpha sub units are entirely extra cellular and each carries an insulin-binding site, where as the beta subunits are membrane proteins with tyrosine kinase activity. This activity is suppressed by the alpha sub units, but insulin binding causes a conformational change that depresses (activates) the tyrosine kinase activity of the beta subunits, which act on each other (auto phosphorylation) and on other target proteins.

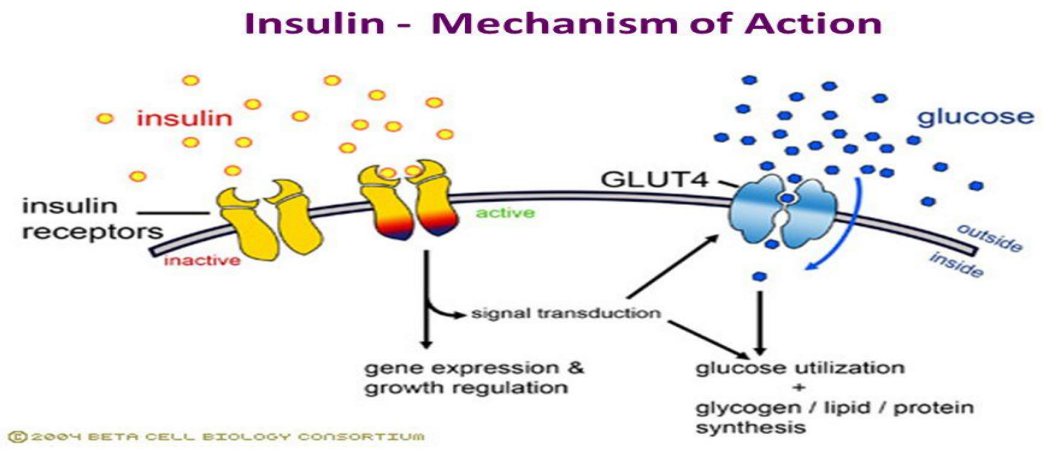


**Figure No.4. Mechanism of action of insulin**

Insulin reagent substrate (IRS) also auto phosphorylation takes place, further which will activates the protein kinase enzyme. This enzyme responsible for all cellular functions. At concentration of insulin that produces maximum effects, less than 10% of the receptors are occupied. Occupied receptors aggregate in to clusters, which are subsequently internalized in vesicles, resulting in down regulation. Internalized insulin is degraded in lysosomes, but the receptors are recycled to the plasma membrane (Rang HP *et al.*, 2006).

### a) Production of insulin

Insulin is a hormone which is synthesized in the beta cells of the islets of Langerhans. Its signal peptide is removed in the endoplasmic reticulum and then packed into secretory vesicles in the Golgi. It is folded in its nature and locked in this confirmation by the formation of two disulfide bonds. In normal persons, insulin is produced by the body in response to the rice in blood glucose level.

Apart this, spurts of insulin produced throughout the day and night, to look after the body resting needs for insulin and ensure that the cells can take up glucose. There are numerous hyperglycemic hormones untreated disorders associated with insulin, generally leads to severe hyperglycemia and a shortened life span.

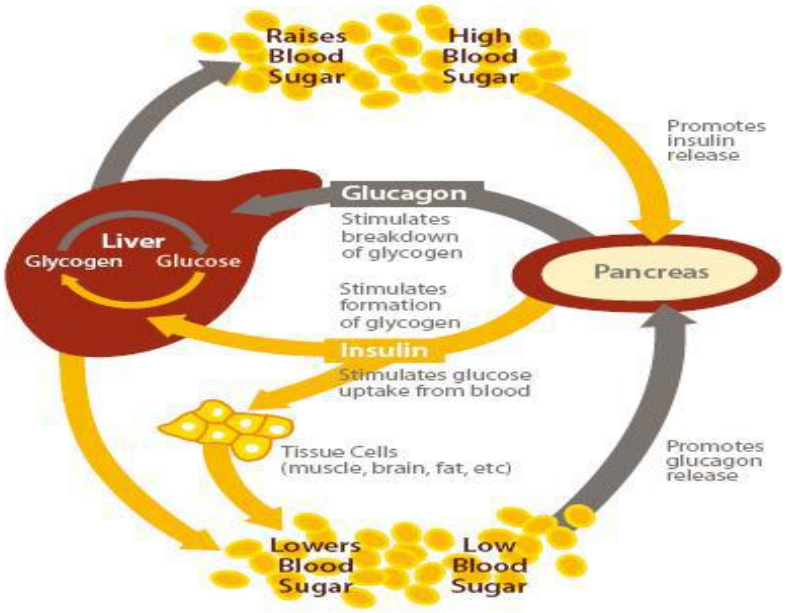


**Figure No.5. Production of insulin**

Insulin is synthesized from the proinsulin molecule by the proteolytic enzymes, known as prohormone convertase (PC1 and PC2), as well as the Exo protease carboxypeptidase E. these modification of proinsulin remove the center part of the molecule from the C- and N- terminal ends of proinsulin. The remaining Polypeptides (51 amino acids), B and A chains are bound each other by disulfide bond.

**b) Insulin secretion**

Rising of glucose levels inside the pancreatic beta cells trigger the release of insulin. Glucose is transported in to the beta cell by Type 2 glucose transporters (GLUT2). The initial step in glucose metabolism in the phosphor relation of glucose to produce glucose 6-phosphate. This is catalyzed by glucokinase, which is the rate limiting step in glycolysis, and it effectively traps glucose inside the cell.

The glucose 6- phosphate is subsequently undergoing oxidation to form adenosine triphosphate (ATP), It is produced in the mitochondria. The increase in the ATP than ADP ratio closed ATP gated potassium channels in the beta cell membrane. Positively charged potassium ions ($K^+$) are now prevented from leaving in the beta cell.

This rise in positive charge inside in the beta cell causes the depolarization. Voltage-gated calcium channels open and allowing calcium ions ($Ca^{2+}$) to pass in to the cell. The increase in intracellular calcium ion concentration triggers the insulin secretion via exocytosis.

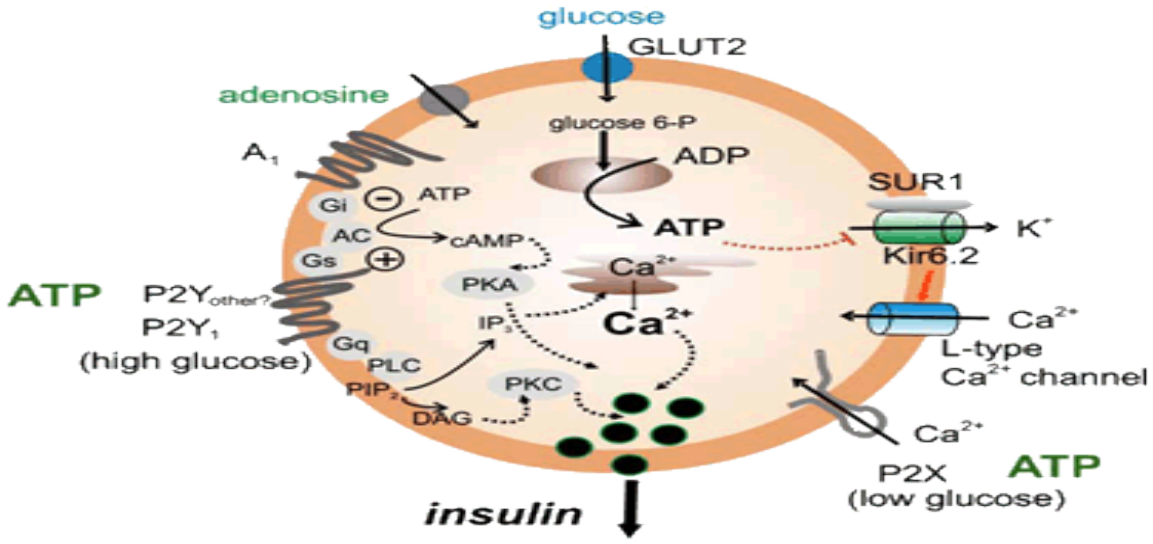


**Figure No.6. Insulin Secretion.**

**Factors affecting insulin secretion:-**

- ❖ Epinephrine is the highly effective inhibitor of insulin secretion.
- ❖ Starvation reduces insulin secretion.
- ❖ Magnesium also inhibits insulin secretion.
- ❖ Vagotomy reduces insulin secretion.

### c) Insulin resistance

Insulin resistance is a condition at which body does not able to utilize the insulin due to lack of insulin receptors. Then normally body produced insulin is not sufficient to maintain blood glucose level at normal range. So extra insulin may be needed to break down glucose in order to release energy. In about 1/3 of the cases blood cells resist to even high level of insulin. High triglycerides and low HDL, cardiovascular diseases hypertension and other such abnormalities are stimulant the insulin resistance. It is thus believed that diabetes and other problems go hand in hand.

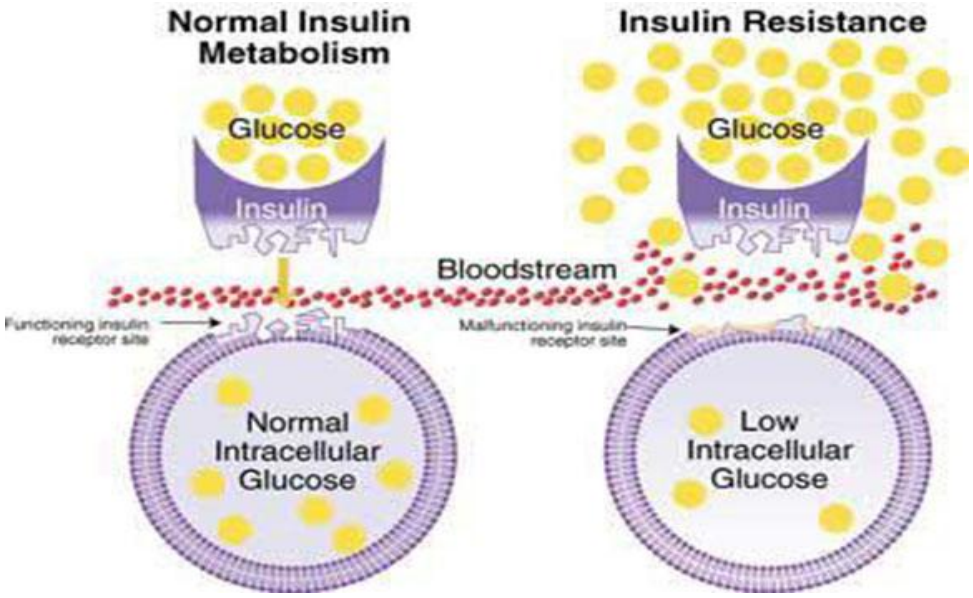


**Figure No.7. Insulin Resistance**

Insulin resistance can also appear in the following conditions like the obesity, metabolic syndrome, severe illness, pregnancy, infection and stress during steroid use. Insulin resistance initially starts with hyperglycemia and over time, hyperglycemia leads to diabetes Type-II (Eva toams*et al*., 2002).

**1.5. Risk factors of diabetes.**

a) **Heredity or inherit traits:** it is due to passing of genes from one generation to another, a person can inherit diabetes. It depends up on closeness of blood relationship as mother is diabetic, risk is 2 to 3% father is diabetic, then the risk is more than the previous case and if both the parents are diabetic, the child has higher risk for diabetes.

b) **Age:** increased age is a factor which gives more possibility than in younger age. This disease may occur at any age, but 80% of cases occur after 50 year, incidence increase with the age factor.

c) **Poor diet (malnutrition related diabetes):** improper nutrition, low protein and fiber intake, high energy intake of refined products are the expected reasons for developing diabetes mellitus.

d) **Obesity and fat distribution:** being overweight indicates increased insulin resistance that is if body fat more than 30%, BMI 25+, waist grith 35 inches in women or 40 inches in males.

e) **Sedentary lifestyle:** people with more sedentary lifestyle are prone to diabetes, when compared to those who exercise thrice a week, are at low risk of falling prey to diabetes.

f) **Stress:** either physical injury or emotional disturbance is frequently blamed as the initial cause of the diabetic. Any disturbance in corticosteroid or ACTH therapy may lead to clinical signs of the diabetes.

g) **Drug induced:** clozapine (Clozaril), olanzapine (Zyprexa), risperidone (Risperdal), quetiapine (Seroquel) and ziprasidone (Geodon) are known to induce this lethal disease.

h) **Infection:** some of the staphylococci is suppose to be responsible factor for infection in pancreas.

i) **Gender:** diabetes is commonly seen in elder persons particularly males but, strongly in women and those females with multiple pregnancy or suffering from (PCOS) polycystic ovarian syndrome.

j) **Hypertension:** hypertension had been reported in many studies that there is direct relationship between high systolic pressure and diabetes.

### 1.6 Signs and symptoms of diabetes

- The most common symptoms of DM are those of hyperglycemia an osmotic diuresis caused by glycosuria leading to urinary frequency, polyuria, and polydipsia that may progress to orthostatic hypotension and dehydration. Severe dehydration causes weakness, fatigue, and mental status changes.

- Hyperglycemia can also cause weight loss, nausea and vomiting, and blurred vision, and it may predispose to bacterial or fungal infections.

- Patients with Type I DM typically present with symptomatic hyperglycemia and sometimes with diabetic ketoacidosis.

- In some patients, hyperosmotic coma occurs initially, especially during a period of stress or when glucose metabolism is further impaired by drugs such as corticosteroids (King H *et al.,* 1998).

#### 1.6.1 Symptoms of Type I diabetes may include:

- Increased thirst and urination
- Constant hunger
- Weight loss
- Blurred vision
- Extreme tiredness

### 1.6.2 Symptoms of Type II diabetes may include:

- Feeling tired or ill
- Frequent urination (especially at night )
- Unusual thirst
- Weight loss
- Blurred vision
- Slow healing of sores
- Having dry itchy skin
- Losing feeling in the feet or having tingling in the feet

## 1.7 Drugs for Diabetes Mellitus

1) **Sulfonyl Ureas:**

**First generation:**

- Tolbutamide
- Chloropropamide

**Second generation:**

- Glibenclamide
- Glipizide
- Gliclazide
- Glimepride

2) **Biguanides:**

- ❖ Phenformin
- ❖ Metformin

3) **Meglitinide analogues:**

- ❖ Repaglinide
- ❖ Nateglinide

4) **Thiazolidinediones:**

- ❖ Rosiglitazone
- ❖ Pioglitazone

5) **Glucosidase inhibitors:**

- ❖ Acarbose
- ❖ Miglitol

The following are the 2006 WHO recommendation for the diagnostic criteria and intermediate hyperglycemia.

1. Measurement of blood glucose levels
2. Oral glucose tolerance test (OGTT)
3. Glycated hemoglobin levels (HbAlc)

## 1.8 Diabetic Complications

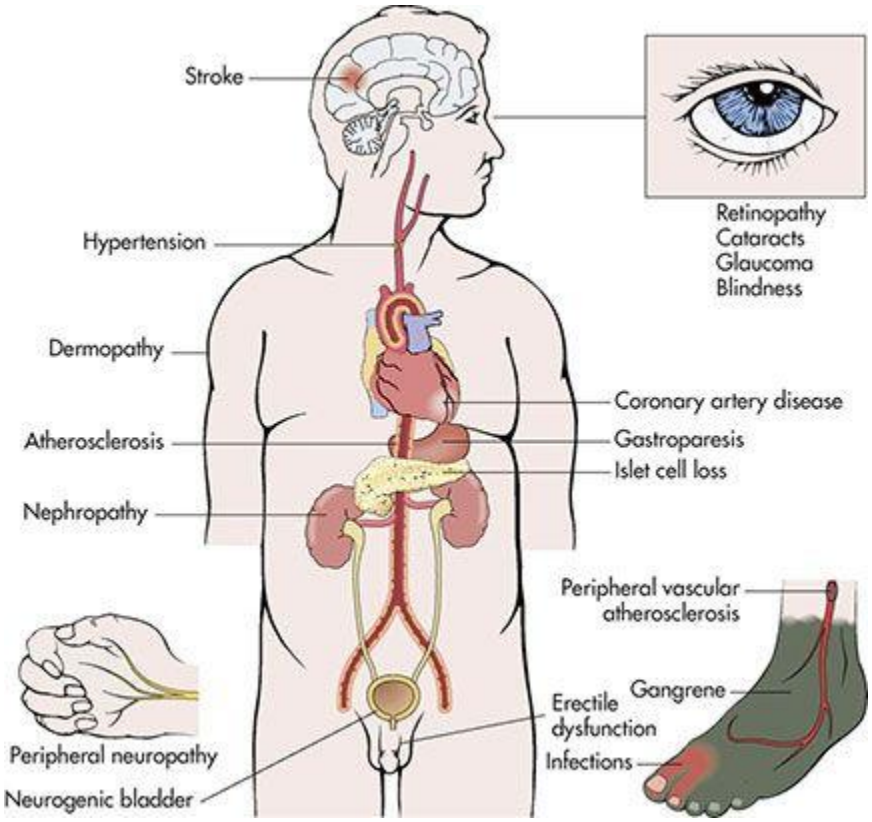


**Figure No.8. Complication of Diabetes**

Microvascular complications of diabetes are a significant are a cause of morbidity, persistent hyperglycemia is the important cause of the microvascular complications which are highly specific for diabetes.

- ❖ Retinopathy with potential loss of vision.
- ❖ Nephropathy leading to kidney failure.
- ❖ Peripheral neuropathy leading to pain, foot ulcers and limb ampulsion.
- ❖ Autonomic neuropathy causing gastrointestinal, genitourinary, cardiovascular symptoms and sexual dysfunction.

**Diabetic Retinopathy**

Diabetic retinopathy occurs in $3/4^{th}$ of persons with more than 15 years of diabetes. Diabetic retinopathy is diagnosed by the appearance of retinal lesions of eye which increasing severity, Culminating in the growth of new vessels. Retinal vessels of eye can leak at any stage of retinopathy and produce macular edema with potentially irreversible loss of central vision.

Excessive retinal revascularization, vitreous hemorrhage, and increased levels of VEGF can lead to fibrosis and retinal detachment. Application of pan-retinal laser photocoagulation has dramatically reduced the rate of blindness in patients with diabetic retinopathy.

**Diabetic Neuropathy**

About 50% of diabetic patients have some degree of neuropathy which present in either polyneuropathy or a mono neuropathy. Diabetic neuropathy is thought to occur both from direct hyperglycemia-induced damage to the nerve parenchyma and from neuronal ischemia brought about indirectly by hyperglycemia induced decreases in neurovascular flow. Diabetic neuropathy and decreases in neuronal function were prevented by oxygen supplementation and by the administration of vasodilator agents.

**Diabetic Nephropathy**

Diabetic nephropathy is characterized by glomerular hemodynamic abnormalities in kidney that result in glomerular hyper filtration, leading to glomerular damage as evidenced by micro-albuminurea. As glomerular function of kidney continuous to abnormal. Over proteinuria, decreased glomerular filtration rate, and at the end stage renal failure will result.

Angiotensin-converting enzyme inhibitors and angiotensin receptors blockers is effective in delaying the progression of clinical diabetic nephropathy.

**Macrovascular**

Macrovascular complications are the main cause of mortality. Persistent hyperglycemia may contribute to macrovascular complications of diabetes.

- Coronary heart disease is the major cause of death for patients with diabetes.
- Peripheral vascular disease
- Cerebrovascular disease.

# *PLANT PROFILE*

# 2. PLANT PROFILE

**Figure.No.9 Whole plant of *Premna corymbosa*** **Figure.No.10 Roots of *Premna corymbosa***

## 2.1 Distribution:

Throughout india in the plains.

## 2.2 The plant:

A large shrub or a small tree up to 9cm in height with yellowish lenticellate bark, spinous large branches and yellowish brown woody aromatic root; leaves simple, opposite, sometimes whorled, elliptic-ovate, membranous when young, coriaceous when mature, entire or irregularly toothed, primary lateral nerves 4-6 pairs; flowers small, greenish yellow or greenish white with a strong disagreeable odour in corymbiform cymose panicles; fruits globose drupes, black when ripe with the persistent saucer-shaped calyx surrounding its base.

## 2.3 Taxonamy:

Scientific Name : *Premna corymbosa Rottl.*

Synonym : *Premna serratifolia Linn.*

Family : Verbenaceae.

Common names : Malayalam: Munna.

Tamil : Munnai, pasumunnai.

Telugu : Padmaka.

## 2.4 Parts used:

Roots and leaves.

## 2.5 Properties and uses:

The roots are astringent, bitter, acrid, sweet, thermogenitic, anodyne, anti-inflammatory, alexeteric, alterant, expectorant, depurative, diagestive, carminative, stomachic, laxative, febrifuge, antibacterial and tonic.

They are useful in vitiated conditions of *vata* and *kapha*, neuralgia, inflammations, cardiac disorders, hepatopathy, cough, asthma, bronchitis, leprosy, skin diseases, dyspepsia, flatulence, colic, anorexia, constipation, haemorrhoids, fever, diabetes and general debility.

The leaves are stomachic, carminative and galactagogue, and are useful in rheumatalgia, neuralgia, haemorrhoids and tumours.

## 2.6 Chemical constituents:

The plant is reported to contain Alkaloids, Tannins, Cardiac glycosides and traces of flavonoids etc.

# *REVIEW OF LITERATURE*

# 3. REVIEW OF LITERATURE

- **Karthikeyan M *et al.*, (2008)** reported that the *Premna corymbosa* is a common plant, it is used in various ayurvedic preparations. The aim of the present study is to evaluate the anti hyperlipidemic activity of *Premna corymbosa* leaves in CCl4 induced hepatic damaged rats. Liver was damaged in wistar rats of both sex by intraperitoneal injection of CCl4 in the concentration of 1 ml/kg body weight for every 72 h. The ethanolic extract of *Premna corymbosa* leaves were administrated at thedoses of 200 & 400 mg/kg body weight, p.o., daily for 14 days. The anti hyperlipidemic effect of the ethanolic extract was assessed by the assay of the biochemical parameters like serum triglycerides (STG), serum total cholesterol (STC) and Very low density lipoprotein cholesterol (VLDL-c). The ethanolic extract, significantly ($p<0.01$) restoredthe levels of biochemical parameters as compared to normal. The results obtained shows that the ethanolic extract of *Premna corymbosa* has anti hyperlipidemic property.

- **Jayakar B *et al.*, (2010)** evaluated that the ethanolic and aqueous extracts of *Premna corymbosa* (Burm.F.) Rottl (Verbenacea) in normal and streptozotocin (STZ) induced diabetic rats. Diabetes was induced by intra peritoneal (i.p) injection of streptozotocin (50 mg/kg) in adult male albino Wistar rats. Blood glucose levels were determined after oral administration of a dose of *P. corymbosa* (400 mg/kg b. wt) in diabetic groups. Blood glucose levels were determined on 0, 7th, 14th and 21st day after oral administration of ethanolic and aqueous extracts of *P. corymbosa* (400 mg/kg) respectively and standard drug (glibenclamide) exhibited (500 µg/kg) in diabetic rats. The effect of extracts of *P. corymbosa* on blood glucose levels and serum lipid profile like Total cholesterol, triglycerides, phospholipids, low density, very low density and high density lipoprotein were measured in the diabetic and non diabetic rats. There was significant reduction in Total cholesterol, LDL cholesterol, VLDL cholesterol and improvement in HDL cholesterol in diabetic rats. These results indicate that *P. corymbosa* possesses a hypoglycemic effect.

- **Deepa k *et al.*, (2010)** investigated that the hepatotoxicity was induced in wistar rats of both sexes by intraperitoneal injection of $CCl_4$, 1 mL/kg body weight for every 72 h. The ethanolic extract of *Premna corymbosa* leaves were administrated at doses of 200 & 400 mL/kg body weight, p.o. daily for 14 days. The hepatotoxicity and its prevention was assessed by serum markers like serum alkaline phosphatase (SALP), serum triglycerides (STG), serum total protein (STP), serum cholesterol (SC), and liver wet weight and histopathological studies of the liver. Results in treatment with the ethanolic extract, the toxic effect of $CCl_4$was controlled significantly ($P < 0.01$) by restoration of the levels of biochemical parameters as compared to normal and standard drug silymarin treated groups. The liver weight was reduced by the ethanolic extract treated groups. The histopathology of the liver sections evidenced the hepatoprotective activity.

- **M Karthikeyan*et al.*, (2011**) reported that the acute toxicity study, a single dose of PCEE of 2000 mg/kg body weight, p.o. was administered and observed for 48 h. In acute models as egg albumin induced paw edema and chronic model as cotton pellet methods was followed. Results in acute models, egg albumin induced paw edema PCEE significantly ($P<0.01$) inhibited the edema formation. In chronic model, cotton pellet induced granuloma formation in rats PCEE significantly ($P<0.01$) reduced the granuloma formation with percentage inhibition of 35.17% and 50.38 % respectively.

- **ThamizhSelvam N *et al.*, (2015)** evaluated that the hyperlipidemia is one of the greatest risk factors contributing to atherosclerosis and occurrence of coronary heart diseases. Hence hypolipidemic drugs are extensively used as prophylactic agents for preventing such atherosclerosis induced disorders. As synthetic drugs have lot of side effects, the focus on herbal drugs is increasing present day. SPHAG is a poly herbal formulation developed by the combination of aqueous extracts of plants *Solanum nigrum*, *Premna corymbosa*, *Holarrhena pubescens*, *Alstonia scholaris* and *Gymnema sylvestre*. The experiment was carried out in Wistar albino rats comprised of five groups such as Healthy Control, Disease Control, Drug Control, SPHAG Low Dose and SPHAG High Dose. The study was conducted for a period of 25 days by daily single dose of test extract through oral administration. At the end of the experiment, lipid profile, Biochemical

profiles were evaluated. The study demonstrated the Hypolipidemic activity of SPHAG and the efficacy was dose dependent. The phytochemical studies showed the presence of phytoconstituents like alkaloids, flavonoids and phenols.

- **NarmadhaRajasekaran *et al.*, (2012)** investigated that the in-vivo antioxidant activity of ethanolic extract *Premna corymbosa* (Rottl.) root against streptozotocin induced oxidative stress in different organs (liver, kidney, brain, heart and pancreas) of rats. Ethanolic extract of *Premna corymbosa* (Rottl.) root was administered orally (200 mg/kg body weight) and the effect of extract on enzymatic antioxidants like superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), glutathione-S-transferase (GST) and polyphenol oxidase (PPO), non enzymatic like vitamin C, vitamin E and glutathione. Lipid peroxidation likeasal, ascorbate and peroxide induced lipid peroxidation were also estimated. Glibenclamide was used as standard reference drug. A significant increase in the activities of superoxide dismutase, catalase, glutathione peroxidase, glutathione-Stransferase and reduced glutathione were observed in different organs of diabetic rats on treatment with 200 mg/kg body weight of *Premna corymbosa* (Rottl.) root extract and glibenclamide for 30 days treatment. Both the treated groups showed significant decrease in lipid peroxidation, suggesting its role in protection against lipid peroxidation induced membrane damage. *Premna corymbosa* (Rottl.) possesses antioxidant potential which may be used for therapeutic purposes mainly in the prevention of oxidative damage that occur during diabetes.

- **Rajni Yadav *et al.*, (2016)** reported that the Ethanolic extract of *Premna corymbosa* Rottl. Leaves (EEPC) were evaluated for anti-cancer activity against Ehrlich ascites carcinoma (EAC) bearing swiss albino mice and for the present anticancer study we used two concentration of ethanolic extract of *Premna corymbosa* Rottl. (EEPC) in the dose of 100 mg/kg and 200 mg/kg in 1% carboxy methyl cellulose (CMC) and were given orally.Result: The leaf of Premna corymbosa Rottl was selected for the study, on the basis of ethano botanical information which reveals its uses against one of the most hazardous disease cancer which shown significant response. Conclusion: The

leaves of *Premna corymbosa* Rottl. Has been studied to compare and give detailed reports on pharmacological studied on it.

- **M Karthikeyan *et a*l., (2010)** evaluated that the acute toxicity and anti nociceptive activity of an ethanol extract of *Premna corymbosa* in animal models. In the acute toxicity study, the ethanolic extract showed no clinical signs and mortality of the animals and was found to be safe. In the acetic acid-induced writhing model, the ethanolic extract at a dose of 200 or 400 mg kg(-1) body weight significantly ($p < .01$) inhibited the writhing response by 42.57% and 54.67%, respectively. In the hot plate test, the extract produced a significant ($p < .01$) increase in latency with 34.50% and 51.08% of protection in a dose-related manner. This study has established the analgesic properties of *Premna corymbosa* (Burm.f.) Rottl. And Willd. The beta-sitosterol or luteolin present in the ethanolic extract may contribute the antinociceptive activity of *Premna corymbosa*.

- **M Karthikeyan *et al.*, (2010)** *Premna corymbosa* (Burm.f.) Rottl. & Willd. (Verbenaceae) is a small tree used in traditional medicine for rheumatic disorders and it is the one of the ingredient in many ayurvedic preparations available in the market for inflammatory disorder uses. The aim of this work was to evaluate the acute toxicity and anti-arthritic activity of *Premna corymbosa* ethanolic extract (PCEE) in experimental animals. In the acute toxicity study, a single dose of PCEE, 2000 mg kg (-1) body weight p.o., was administered. The animals observed for 48 h showed no clinical signs, no mortality, and the extract was found to be safe. To evaluate the anti-arthritic activity of PCEE in Complete Freund's Adjuvant (CFA)-induced arthritis in rats were conducted. The results indicated that the long-term treatment significantly ($p<.01$) suppressed the development of chronic arthritis induced by CFA. This study established the anti-arthritic activity of *Premna corymbosa* leaves.

- **Mahmud ZA *et al*.,** *Premna esculenta* Roxb. (family-Verbenaceae) is a shrub used by the ethnic people of Chittagong Hill Tracts of Bangladesh for the treatment of hepatocellular jaundice. The present study was done to evaluate the hepatoprotective and the in vivo antioxidant activity of ethanolic extracts of leaves of the plant in carbon tetrachloride-induced liver damage in rats. Hepatotoxicity was induced in rats by i.p. injection of CCl4 diluted with olive oil (1:1 v/v; 1 mL/kg body weight) on alternate days for 7 days. After 7 days of pretreatment of test extracts, the biochemical markers such as Serum Glutamate Oxaloacetate Transaminase (SGOT), Serum Glutamate Pyruvate Transaminase (SGPT), Alkaline Phosphatase (ALP), total protein, and albumin were estimated followed by the measurement of liver cytosolic antioxidant enzymes such as superoxide dismutase, catalase, and peroxidase.The data were analyzed using one-way analysis of variance (ANOVA) followed by Dunnett's t-test. The extract both at the doses of 200 and 400 mg/kg p.o. significantly ($P < 0.001$) reduced the elevated levels of SGPT, SGOT, ALP and increased the reduced levels of total protein and albumin compared to the CCl4 -treated animals. The extracts also showed a significant ($P < 0.001$) increase in the reduced levels of superoxide dismutase (SOD), catalase, and peroxidase. The effects of the extracts on these parameters were comparable with those of the standard, silymarin. The findings of the study indicate that the leaf extract of *Premna esculenta* showed a potential hepatoprotective activity and the protective action might have manifested by restoring the hepatic SOD, catalase, and peroxidase levels. The results justify the traditional use of this plant in liver disorders.

# *SCOPE AND PLAN OF WORK*

# 4. SCOPE AND PLAN OF WORK

Management of diabetes mellitus is a global problem. Successful treatment is very important for preventing or at least delaying the onset of long-term complications. Through nature in the form of herbal medicines or drugs with very minimal adverse effect when compared to the available synthetic drugs to treat such chronic disease and disorder. Such herbal drugs as therapeutic agents are a nature's boon when compared to the severe adverse effects of the allopathic medical practice for diabetes, despite the fact that the search for a complete and permanent cure for the disease is being pursued uncompromisingly by eluding physicians and researchers.

These herbal remedies which exemplifies the process of symbiosis still remains unfamiliar to the up to data technical advances, which has fashioned a marvelous scope for folk lore or traditional medicine. it is supposed that the traditional medicines used for the treatment of diabetes mellitus satisfy the sequence of complications of the disease.

Even through the traditional medicinal plant are used to cure the disease from human origin, scientific validation of such medicinal plants are necessary and also a scientific research to prove its pharmacological and therapeutic efficacy is became vital part. The rummage around for the efficient herbal drugs for the treatment of diabetes based on ethno medical clues still continuous and in the extended spirit it has yield us very useful herbal remedies on such basis to prove the ethno medicinal use of such traditional systems of medicine in India for the treatment of diabetes mellitus. So we wish to establish its anti-diabetic property.

# PLAN OF WORK

The following in-vitro and in-vivo biochemical markers studies and histopathology of tissue were done with of ethanolic root extract of *Premna corymbosa Rottl.*

- Collection of plant materials.
- Authentication of plant materials.
- Extraction of plant material using Soxhlet apparatus.
- Determination of phytochemical constituents present in the extract.
- Pharmacological activities.
- **Anti-diabetic screening models:-**
  - ✓ Streptozotocin - Nicotinamide induced diabetes in experimental animals.
- **Parameters observed and biochemical evaluation**
  - ✓ Blood Glucose Level
  - ✓ Liver function tests
  - ✓ Renal function tests
  - ✓ Lipid Profile

# *MATERIALS AND METHODS*

# 5. MATERIALS AND METHODS

## 5.1 Plant materials

Roots of *Premna corymbosa* was collected from Kolli hills, Namakkal District, Tamil Nadu, India and authenticated by Dr R. Duraisami, Professor and Head, Department of Pharmacognosy, Nandha College of Pharmacy, Erode, Tamil Nadu, India. Voucher specimens (PC/0220/06) were deposited at our College Museum for future reference.

## 5.2 Preparation of the extract

The powdered material of roots of *Premna corymbosa* was extracted separately using 70% ethanol by Soxhlet apparatus (Harold varley *et al.*, 1983). The extracts were dried under reduced pressure. The dried extract (24.8 g) was stored in desiccator and was subjected to various chemical tests to detect the presence of different phyto-constituents like alkaloids, tannins, cardiacglycosides and traces of flavonoids etc.

## 5.3 Preliminary phytochemical screening

The ethanolic extract of roots of *Premna corymbosa Rottl* were subjected to preliminary screening for various active phytochemical constituents (Evans WC. 1989) by the following tests.

1) **Test for Alkaloids:-**

The extract was treated with dilute hydrochloric acid and filtered. The filtrate is used for the following tests.

a) **Mayer's reagent (Potassium Mercuric Solution)**

0.5 ml of the extract was treated with Mayer's reagent and the appearance of cream color indicates the presence of alkaloid.

b) **Dragendroff's test (Potassium Bismuth Iodide)**

0.5 ml of the extract was treated with Dragendroff's reagent and the appearance of reddish brown color precipitate indicates the presence of alkaloid.

c)**Wagner's test(Iodine-Potassium Iodide Solution)**

0.5ml of the extract was treated with Wagner's test and the appearance of brown color precipitate indicates the presence of alkaloid.

d**) Hager's test (Saturated solution of picric acid)**

0.5ml of the extracts was treated with Hager's test and the appearance of yellow color precipitate indicates the presence of alkaloid.

2) **Test for Tannin's:-**

a) The extract was treated with 10% lead acetate solution. The appearance of white precipitate indicates the presence of tannins.

b) The extract was treated with aqueous bromine solution. The appearance of white precipitate indicates the presence of tannins.

3) **Test for Flavonoid's:-**

a)**Alkaline Reagent Test**

The extract was added few drops of NaOH solution. Intense of yellow color was formed which turns to colourless on addition of few drops of dilute acid indicated the presence of flavonoids.

**b) Zinc Hydrochloride Test**

The extract solution added mixture of zinc dust and concentrated Hcl it gives red colour after few minute indicates the presence of flavonoids.

c) **Shinoda's Test**

The extracts were dissolved in alcohol, to that one piece of magnesium is added followed by concentrated hydrochloric acid along the sides of the test tube drop wise. It is heated in a boiling water bath for few minutes. The appearance of magenta colour indicates the presence of flavanoids.

4) **Test for cardiac glycosides:-**

Crude extract (2-5 ml) was mixed with 2 ml of glacial acid containing 1-2 drops of 2% solution of ferric chloride; the mixture was then poured in to another test tube containing 2 ml of concentrated sulfuric acid. A brown ring of a deoxy-sugar, characteristic of alcoholic cardinolides at the interface indicated the presence of cardiac glycosides.

**5.4 Pharmacological Screening**

**5.4.1 Experimental Animals & Ethical Consideration**

Male wistar albino rats, 9-12 weeks old with average weight of 150-180 g were purchased fromthe animal house, Nandha college of pharmacy, Erode, Tamil Nadu, India and used for the study. They were housed in polypropylene cages and fed with standard chow diet and water *ad libitum.* The animals were exposed to alternate cycle of 12 h of darkness and light each. Before each experiment, the animals were fasted for at least18h. The experimental protocols were approved by Institutional Animal Ethics Committee (Regd. No. 688/PO/re/S/02/CPCSEA) and were in accordance with IAEC.

### 5.5 Drug Treatment

#### 5.5.1Acute toxicity studies

The animals were divided into six groups separately and were treated orally with ethanolic extracts of roots of *Premna corymbosa rottl* at a doses of 50, 300, 500, 1000 and 2000 mg/kg b.w. The animals were continuously observed for 1 hr., then frequently for 14 days. The animals were observed continuously for the initial 4 h and intermittently for the next six hour and then again at 24h and 48h following drug administration. The parameters observed were grooming, hyperactivity, sedation, loss of righting reflex, respiratory rate and convulsion (Ghosh M. 1994).

#### 5.5.2 Estimation of blood glucose levels:

Blood was collected from tip of the tail vein and fasting blood glucose levels were measured using a commercial glucometer and glucose-oxidase strips (One touch glucometer).

#### 5.5.3 Streptozotocin-nicotinamide induced diabetic rats

This model uses concurrent administration of nicotinamide to afford partial protection of β-cells against STZ (Masiello et al., 1998). It is based on the work of Junod *et al.,* (1969), who systematically investigated the early demonstration (Schein *et al.,* 1967) that nicotinamide protected against the diabetogenic effect of STZ. This regimen produces a model of insulin-deficient, but not insulin-resistant, T2D, characterized by stable, moderate hyperglycemia, associated with 60% loss of β-cell function (Masiello *et al.,* 1998; Ghasemi *et al.,* 2014).

**Requirements and Chemicals**

Wistar albino rats: 150 to 200 g, 8 to 10 weeks old;

Nicotinamide (Sigma)

0.9% (w/v) Sodium chloride

50 mM Sodium citrate buffer (enzyme grade; Fisher), pH 4.5: prepared immediately before use

Streptozotocin (STZ; Sigma)

1-ml syringes

23- and 25-G needles

One Touch Basic blood glucose monitoring system (Lifescan)

**Prepare Animals**

1. At least 5 days prior to the start of the experiment, house two to five male rats per cage at 24°C ± 1°C and 55% ± 5% humidity, with a 12-hour light-dark cycle (light on at 8:00 and off at 20:00). Allow the rats to have free access to food and water. (Males are generally preferred for these studies, as female rats are less sensitive to STZ. While the protocol is designed to minimize variability, it is recommended that group sizes number 6 animals each. This allows for the morbidity and variance generally associated with these studies. Usually >80% of STZ-injected rats develop diabetes under this protocol).

2. Weigh all rats accurately to 1 g, and randomly divide them into control and experimental groups. (The number of rats should be the same in each group).

3. On experimental day 1, fast all rats for 6 to 8 hr (from 7:00 to 13:00-15:00) prior to STZ treatment. Provide water as normal.

**Induction of Diabetes with STZ and Nicotinamide**

Dissolve nicotinamide in 0.9% sodium chloride solution to a concentration of 230mg/ml. Weigh 32.5 mg STZ into a 1.5 ml micro centrifuge tube and cover the tube with aluminium foil; use one tube for each rat. Prepare the citrate buffer.

Using a 1-ml syringe and a 23-G needle, inject nicotinamide i.p. (Donovan and Brown, 2006 a) at a dose of 230 mg/kg (1.0 ml/kg).

(The nicotinamide injection must be made 15 min before the i.v. administration of streptozotocin).

Immediately prior to injection, dissolve STZ in the 50 mM sodium citrate buffer, (pH 4.5 (see step 5), to a final concentration of 32.5 mg/ml.

(The STZ solution should be prepared fresh for each injection and administered within 5min of dissolution).

Using a 1-ml syringe and 25-G needle, inject the STZ solution i.p. (Donovan and Brown, 2006 a) at 65 mg/kg (2.0 ml/kg) for the experimental group. The control animals receive an injection of an equal volume of citrate buffer (pH 4.5) only and return the rats to their cages. Provide normal food and drinking water. At around 8:00 a.m. on experimental day 10, test the blood glucose level from a tailvein blood sample (Donovan and Brown, 2006 b) using a One Touch Basic blood glucose monitoring system (Brian L.Furman 2015) after injection the animals had free access to food and water and were given 5% glucose in their 48h after streptozotocin administration, blood samples were drawn from tail and glucose levels determined to confirm diabetes.

The rats were divided into 5 groups as follows, Group I served as normal control which received vehicle, Group II served as diabetic control, Group III & IVserved as tests group, received 200 & 400 mg/kg b.w. of ethanolic extract of *Premna corymbosa rottl* respectively whereas Group V served as standard which received Glibenclamide (5mg/kgb.w.). The treatment was continued daily for 21 days and the blood was collected from the tail for blood glucose estimation.

Group I- Normal control animals treated with 1% CMC.

Group II - Diabetic control animals treated with Streptozotocin (65 mg/kg, i.p)

Group III –Test animals treated with Streptozotocin (65 mg/kg, i.p) and treated with low dose of EEPC 200mg/kg b.w/ p.o.

Group IV -Test animals treated with Streptozotocin(65 mg/kg, i.p) and treated with low dose of EEPC 400mg/kg b.w/ p.o.

Group V- Standard animals treated with streptozotocin (65mg/kg, i.p) and treated with Glibenclamide 0.5mg/kg b.w/p.o.

The above mentioned treatment schedule was followed for the respective group of animals for 21 days. Blood samples were collected from tail vein in animals on 0, 3, 7, 14 & 21$^{st}$ day to estimate blood glucose levels using a commercial glucometer and glucose-oxidase strips. (One touch glucometer).

**5.6 BIOCHEMICAL STUDIES:**

At the end of the study, the blood samples were collected by bleeding of retero-orbital plexus using micro capillary technique from all the groups of rats and serum was separated to study biochemical parameters such as liver parameters (AST,ALP, ALT), Renal function tests (serum creatinine and blood urea) and lipid profile (Total cholesterol, Triglycerides, HDL, LDL,VLDL).

**5.6.1 Biochemical parameters:**

**a) Estimation of serum triglycerides.**

**Principle:**

Lipoprotein lipase hydrolyses triglycerides to glycerol and free fatty acids. The glycerol formed with ATP in the presence of glycerol kinase forms glycerol 3 phosphates which is oxidized by the enzyme glycerol phosphate oxidase to form hydrogen peroxide. The hydrogen peroxide further reacts with phenolic compound and 4-aminoantipyrine by the catalytic action of

peroxidase to form a red colored quinoneimine dye complex. Intensity of the color formed is directly proportional to the amount of triglycerides present in the sample.

Triglycerides kit consists of LI (triglyceride reagent) and S (triglyceride standard) 200mg/dl.

**Procedure:**

0.01ml of sample was mixed with 1.0 ml of working reagent and incubated at $35^0$C for 5min. measured the absorbance of the standard and test sample at 505nm at a light path of 1cm against the blank,within 60 min.

**Triglycerides in mg/dl=Abs.T/Abs.S X 200**

**b) Estimation of Serum total cholesterol.**

**Principle:**

Cholesterol esterase hydrolysis esterified cholesterol to free cholesterol. The free cholesterol is oxidized to from hydrogen peroxide which further reacts with phenol and 4-aminoantipyrine by the catalytic action of peroxidase to form a red colored quinoneimine dye complex. Intensity of the color formed is directly proportional to the amount of cholesterol present in the sample.

Cholesterol kit has LI reagent(cholesterol reagent) and Standard

Cholesterol(200mg/dl)

**Procedure:**

0.01ml sample was mixed with 1.0 ml of cholesterol reagent and incubated at $35^0$C 5min. measured the absorbance of the standard and test at 505nm at a light path of 1cm against the blank,within 60 min.

**Calculation:**

Serum total cholesterol (mg/dl)=absorbance of test × conc. of standard.

absorbance of standard

**c) Estimation of serum High-Density Lipoprotein Cholesterol.**

**Principle:**

The principle behind the HDL estimation lies in precipitating the serum VLD and LDL. When the serum is reacted with the polyethylene glycol contained in the precipitating reagent, all the VLDL and LDL are precipitated. The HDL remains in the supernatant and is then assayed as a sample of cholesterol using the cholesterol reagent.HDL kit has LI, L2 (enzyme reagent), L3 (precipitating reagent) and HDL cholesterol standard (25mg/dl)

Working reagent:L1 (4 parts): L2 (1 part)

**Procedure:**

**Precipitation of VLDL and LDL:**

0.1ml of sample was mixed with 0.1 ml of precipitating reagent. Incubated at room temperature for 5 min. centrifuged at 2500-3000 rpm to obtain a clear supernatant.

**HDL assay:**

0.05 ml of sample was mixed with 1.0 ml of working reagent and incubate at $35^0$ C for 5min. measure the absorbance of the Standard and test sample at 505nm at a light path of 1cm against the blank, within 60 min.

**HDL Cholesterol in mg/dl=Abs.T/Abs. S X 25 X2**

(Where 2 is the dilution factor due to the deproteinization step)

Calculation of LDL cholesterol (mg/dl) = total cholesterol – (Triglycerides/5)- HDL cholesterol

d) **Estimation of serum Low- Density Lipoprotein Cholesterol (LDL-C)**

Using the data obtained including total cholesterol, HDL cholesterol and VDL, the LDL cholesterol levels were calculated using the empirical equation of Friede Wald

**Calculation:**

**Serum LDL cholesterol = total cholesterol- (Triglyceride-HDL cholesterol)**

**5**

**e) Estimation of Serum Glutamate Oxaloacetate Transaminase- SGOT**

**Principle:**

Between L-Aspartate and SGOT catalyzes the transfer of amino group between L-Aspartate and alpha Ketoglutarate to form oxaloacetate and glutamate. The oxaloacetate formed reacts with NADH in the presence of malate Dehydrogenase to form NAD. The rate of oxidation of NADH to NAD is measured as a SGOT kit contains L1 (enzyme reagent) and L2 (Starter reagent).

**Procedure:**

**Substrate start assay:**

0.1ml of sample was mixed with 0.8 ml of enzyme reagent and incubated at $35^0$ C for 1 min and added 0.2 ml of starter reagent. Mixed well and read the initial absorbance change per minute (ΔA/min).

**Sample start assay:**

1.0ml of working reagent is incubated at $35^0$ C for 1 min and added 0.1 ml of starter reagent. Mixed well and read the initial absorbance $A_0$ and repeat the absorbance reading after every 1,2 and 3 min. calculate the mean absorbance changer per minute (ΔA/min).

**SGOT (ASAT) activity in U/L =ΔA/min X 1546**

**f) Estimation of serum SGPT.**

**Principle:**

SGPT catalyzes the transfer of amino group between L-Alanine and α Ketoglutarate to form pyruvate and glutamate. The pyruvate formed reacts with NADH in the presence of lactate Dehydrogenase to form NAD. The rate of oxidation of NADH to NAD is measured as a decrease in absorbance which is proportional to the SGPT activity in the sample.

SGPT kit contains L1 (Enzyme reagent) L2 (Starter reagent)

**Procedure:**

**Substrate start assay:**

0.1ml of sample was mixed with 0.8 ml of enzyme reagent and incubated at $35^0$ C for 1 min and added 0.2ml of starter reagent. Mixed well and read the initial absorbance $A_0$ and repeat the absorbance reading after every 1, 2 and 3 min. calculate the mean absorbance change per minute (ΔA/min).

**Sample start assay:**

1.0ml of working reagent is incubated at $35^0$ C for 1 min and adds 0.1 ml of starter reagent. Mix well and read the initial absorbance $A_0$ and repeat the absorbance reading after every 1, 2, and 3 min. calculate the mean absorbance change per minute(ΔA/min).

**SGPT (ALAT) activity in U/L = ΔA/min X 1546**

**g) Estimation of creatinine ( Modifiedjaffe's Kinetic method)**

Serum creatinine levels were estimated by using test kit.

h) **Estimation of Urea:**

Serum Urea levels were estimated by using test kit.

# *RESULTS*

# 6. RESULTS

## 6.1 PHARMACOGNOSTICAL STUDY

### 6.1.1 Qualitative Determination

The qualitative determinations of the ethanolic extract of root of *Premna corymbosa rottl* were shown in Table No.1. In qualitative determination, the Phytoconstituents analysis was performed and which confirms the presence of alkaloids, glycosides, flavonoids and tannins.

**Table No. 1. Phytoconstituents analysis of ethanolic root extract of *Premna corymbosa rottl***

| S.No. | Plant constituents | *Premna corymbosa rottl* |
|---|---|---|
| 1 | Test for alkaloids | + |
| 2 | Test for glycosides | + |
| 3 | Test for carbohydrates | - |
| 4 | Test for phytosterols | - |
| 5 | Test for sterols | - |
| 6 | Test for flavonoids | + |
| 7 | Test for saponins | - |
| 8 | Test for tannins | + |
| 9 | Test for terpenoids | - |
| 10 | Test for fixed oils and fats | - |

'-' Absent, '+' Present

### 6.2. Acute Toxicity Studies

This study helps us to determine the therapeutic index and the extract was confirmed to be safe. Acute toxicity study was performed as per the OECD guidelines. The extracts were administered orally at a doses of 50, 300, 500, 1000 and 2000mg/kg b.w. in 0.5% carboxy methyl cellulose (CMC). No gross observational changes were recorded during the period of 14 days observation.

**Table No. 2. The effects of ethanolic root extract of *Premna corymbosa rottl* on general behavioral observation in acute toxicity studies**

| S.No. | General Behaviour | Observation after drug administration |
|---|---|---|
| 1 | Sedation | + |
| 2 | Hypnosis | - |
| 3 | Convulsion | - |
| 4 | Ptosis | - |
| 5 | Analgesia | - |
| 6 | Stupar reaction | - |
| 7 | Motor activity | - |
| 8 | Muscle relaxant | - |
| 9 | CNS stimulant | + |
| 10 | CNS depressant | - |
| 11 | Pilo erection | - |
| 12 | Skin colour | - |
| 13 | Lacrimation | - |
| 14 | Stool consistency | - |

'-' Absent, '+' Present

### 6.3 Pharmacological Studies

#### 6.3.1 Antidiabetic Activity

The blood glucose level increased significantly in STZ and nicotinamide treated group when compared to control group. The STZ and nicotinamide induced rats were treated with the ethanolic root extract of *Premna corymbosa rottl* 200mg/kg /p.o and 400mg/kg/p.o for the duration of 21 days. Treatment with ethanolic root extract of *Premna corymbosa rottl* at the dose of 200 mg/kg/p.o shows marginal reduction in the blood glucose level at second week. Treatment with ethanolic root extract of *Premna corymbosa rottl* at the dose of 400mg/kg/p.o. showed significant decrease in the blood glucose level at first week ($p<0.01$), which further reduced in the second, third and fourth weeks ($p<0.001$), respectively. Treatment with glibenclamide (0.5mg/kg b.w/ p.o) produced a significant ($p<0.001$) decrease in blood glucose level from first week to fourth week.

**Table No.3 The effect of ethanolic root extract of *Premna corymbosa rottl* on blood glucose levels in STZ - nicotinamide induced diabetic rats**

| Groups Treatment/ Dose | 0 day (mg/dL) | After 3days (mg/dL) | After 7 days (mg/dL) | After 14 days (mg/dL) | After 21days (mg/dL) |
|---|---|---|---|---|---|
| Normal control | 96.32± 1.12 | 95.71± 0.96*** | 96.08± 1.35*** | 96.54± 1.24*** | 98.92± 0.97*** |
| Diabetic control | 264.18± 2.64 | 281.06± 1.98 | 271.12± 2.54 | 219.37± 2.36 | 209.14± 1.97 |
| EEPC (200mg/kg b.w.) | 263.16± 2.91 | 247.74± 2.23** | 208.17± 3.06** | 176.51± 3.27** | 130.36± 2.97** |
| EEPC (400mg/kg b.w.) | 261.49± 3.82 | 221.12± 3.54** | 160.75± 2.59*** | 130.50± 2.89*** | 110.69± 3.06*** |
| Standard \|Glibenclamide (5mg/kg b.w.) | 256.13± 3.29 | 200.12± 3.07*** | 152.43± 2.99*** | 118.74± 2.86*** | 98.16± 3.04*** |

Values are expressed as mean ± SEM (n=6). *$p<0.05$,**$p<0.01$,***$p<0.001$Vs control. Statistical significance test for comparision was done by one way ANOVA followed by Dunnet's't' test

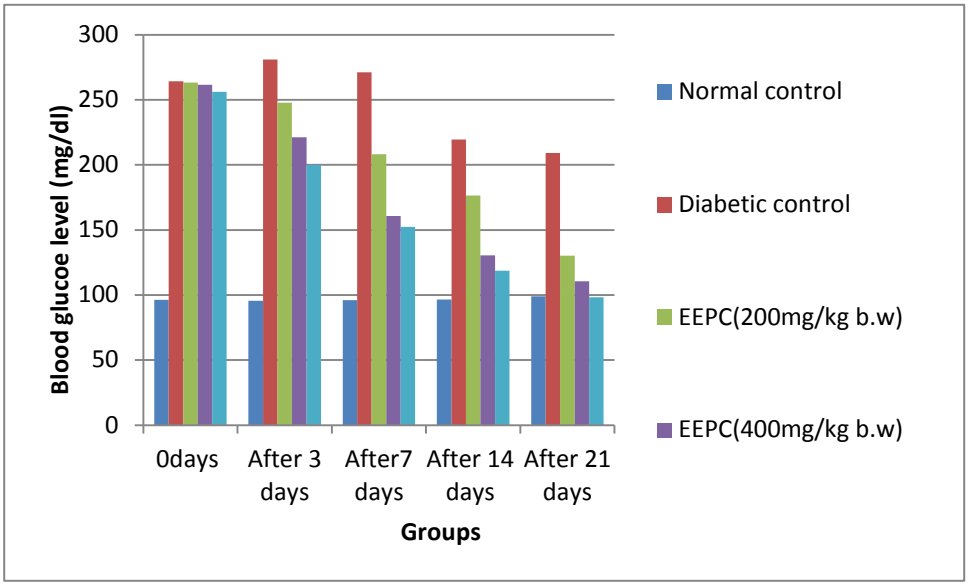


**Figure No.11. Effect of ethanolic root extract of *Premna corymbosa rottl* on blood glucose level in STZ and nicotinamide induced diabetic rats**

### 6.3.2 Estimation of liver enzymes

The effect of ethanolic root extract of *Premna corymbosa rottl* at doses of 200 & 400 mg/kg b.w. on liver enzymes. It was found that the ethanolic root extract of *Premna corymbosa rottl* showed significant ($p<0.001$) protection on liver parameters. The ethanolic root extract of *Premna corymbosa rottl* has not elevated the normal liver profiles (ALP, AST, and ALT) on dose dependent manner. The standard drug glibenclamide (0.5mg/kg/p.o) showed significant ($p<0.001$) protection in ALP,AST,ALT when compared STZ-nicotinamide induced diabetic animals.

**Table No.4.** Effect of Ethanolic root extract of *Premna corymbosa* on liver profile on STZ-nicotinamide induced diabetic rats.

| Groups Treatment/Dose | ALP (IU/L) | AST (IU/L) | ALT (IU/L) |
|---|---|---|---|
| Normal control | 89.35± 7.12*** | 83.75± 7.36*** | 25.52± 1.65*** |
| Diabetic control | 168.96± 9.02 | 161.92± 9.07 | 128.37± 7.92 |
| EEPC (200mg/kg b.w.) | 121.66± 8.12* | 87.31± 7.10** | 44.91± 2.36* |
| EEPC (400mg/kg b.w.) | 100.99± 7.96** | 89.18± 6.87*** | 34.68± 2.72** |
| Standard \|Glibenclamide (5mg/kg b.w.) | 85.94± 6.06*** | 89.85± 6.84*** | 28.96± 1.82*** |

Values are expressed as mean ± SEM (n=6). *p<0.05,**p<0.01,***p<0.001Vs control. Statistical significance test for comparision was done by one way ANOVA followed by Dunnet's 't' test.

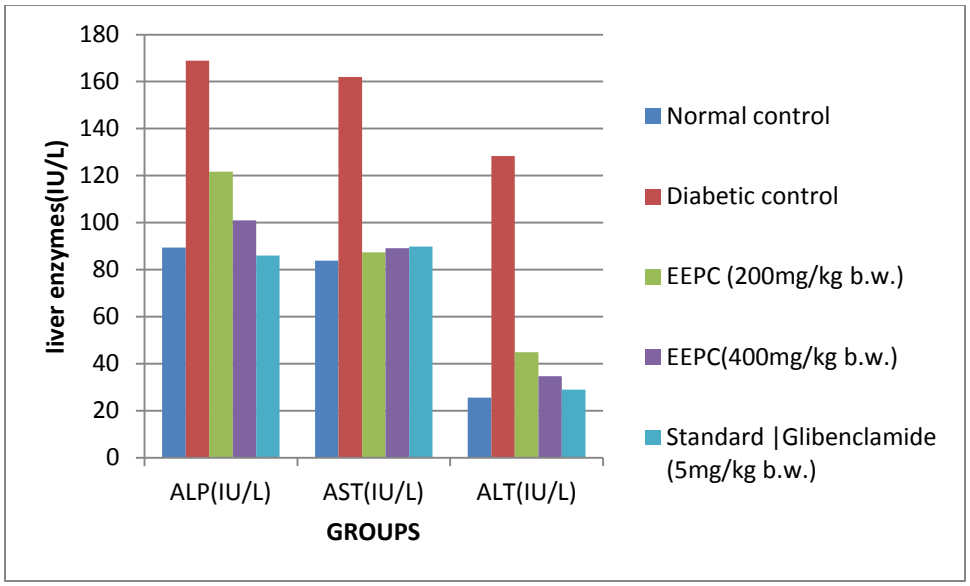


**Figure No.12. Effect of ethanolic root extract of *Premna corymbosa* on liver profile in STZ-nicotinamide induced diabetic rats**

### 6.3.3. Estimation of renal profile

The serum urea level was significantly ($p<0.001$) increased in STZ-nicotinamide induced diabetic rats when compared to control rats. Serum urea level of diabetic rat treated with EEPC 200mg/kg/p.o and 400mg/kg/p.o showed significant decrease ($p<0.001$) and ($p<0.001$) in serum urea level when compared to STZ- nicotinamide induced diabetic rat. Glibenclamide (0.5mg/kg b.w/p.o) treatment showed significant ($p<0.001$) decrease in serum urea when compared to STZ-nicotinamide induced diabetic animals.

**Table No.5.** Effect of ethanolic root extract of *Premna corymbosa* on renal profile in STZ-nicotinamide in diabetes induced rat

| Groups Treatment/Dose | Serum Creatinine (mg/dl) | Blood Urea (mg/dl) |
|---|---|---|
| Normal control | 1.18± 0.08*** | 25.73± 2.26*** |
| Diabetic control | 2.58± 0.16 | 41.14± 2.61 |
| EEPC (200mg/kg b.w.) | 1.42± 0.12** | 30.61± 2.84* |
| EEPC (400mg/kg b.w.) | 1.32± 0.09*** | 27.86± 2.51** |
| Standard \|Glibenclamide (5mg/kg b.w.) | 1.27± 0.10*** | 23.94± 2.08*** |

Values are expressed as mean ± SEM (n=6). *p<0.05,**p<0.01,***p<0.001Vs control. Statistical significance test for comparision was done by one way ANOVA followed by Dunnet's 't' test.

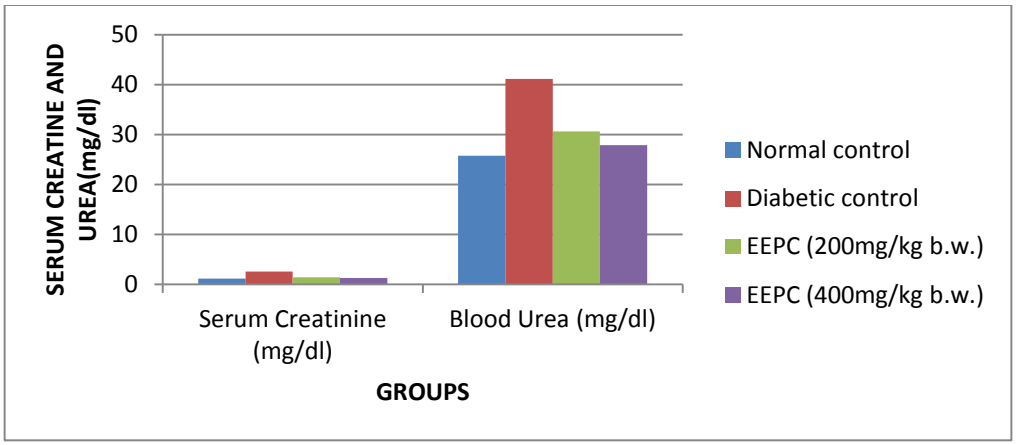


**Figure No.13. Effect of ethanolic root extract of *Premna corymbosa* on renal profile in STZ-nicotinamide induced diabetic rats**

### 6.3.4 Estimation of lipid profile

The serum total cholesterol, triglyceride,LDL,VLDL level was significantly increased whereas HDL was significantly decreased in STZ-nicotinamide induced diabetic rat when compared to control rats. Serum total cholesterol, triglyceride, LDL, VLDL level of diabetic animal treated with EEPC 200mg/kg/p.o and 400mg/kg/p.o showed significant decrease ($p<0.001$) and HDL level of diabetic animal treated with EEPC showed significant increase ($p<0.01$), when compared to STZ-nicotinamide induced diabetic animals. Glibenclamide(0.5mg/kg/p.o) also showed a significant decrease ($p<0.001$) in serum total cholesterol ,triglyceride, LDL,VLDL level and HDL was significantly increased when compared to STZ-nicotinamide induced diabetic rat.

**Table No 6**: Effect of ethanolic root extract of *Premna corymbosa* on lipid profile in STZ-nicotinamide induced diabetic rats\

| Groups Treatment/ Dose | Total cholesterol (mg/dl) | Triglycerides (mg/dl) | HDL (mg/dl) | LDL (mg/dl) | VLDL (mg/dl) |
|---|---|---|---|---|---|
| Normal control | 66.68± 1.95*** | 34.32± 1.72*** | 13.75± 0.82*** | 59.62± 3.16*** | 6.85± 0.22*** |
| Diabetic control | 77.61± 1.56 | 44.11± 2.16 | 12.21± 0.95 | 74.25± 2.94 | 9.49± 0.30 |
| EEPC (200mg/kg b.w.) | 71.74± 1.47** | 36.33± 1.94** | 15.16± 0.82* | 64.35± 3.32** | 7.88± 0.28* |
| EEPC (400mg/kg b.w.) | 68.18± 1.78*** | 35.53± 2.21*** | 14.08± 0.91*** | 63.35± 2.68*** | 7.09± 0.32** |
| Standard \|Glibenclamide (5mg/kg b.w.) | 67.41± 3.23*** | 35.64± 2.62*** | 14.30± 0.76*** | 63.25± 2.94*** | 7.50± 0.28*** |

Values are expressed as mean ± SEM (n=6). *p<0.05,**p<0.01,***p<0.001Vs control. Statistical significance test for comparision was done by one way ANOVA followed by Dunnet's 't' test.

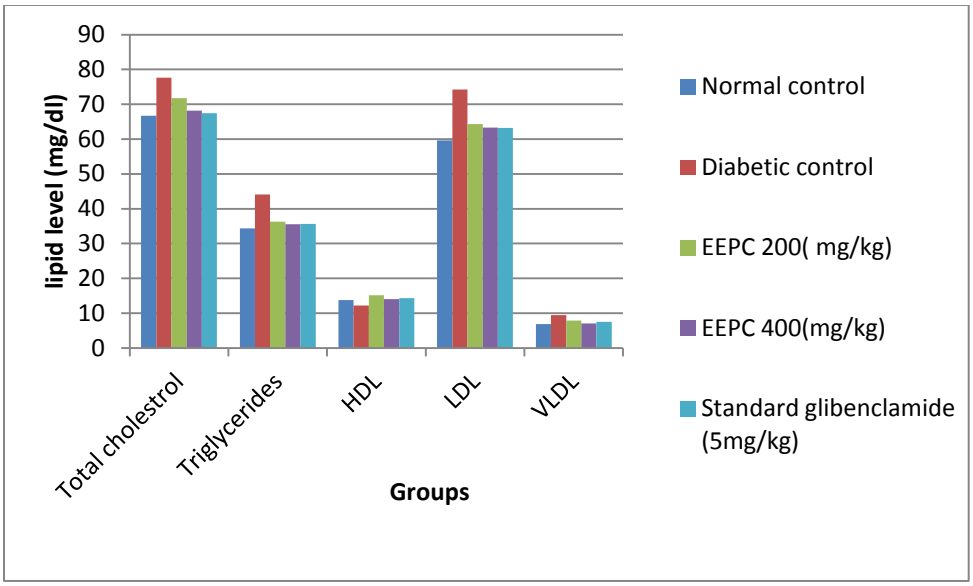


**Figure No.14. Effect of ethanolic root extract of *Premna corymbosa* on serum total cholesterol, triglycerides,LDL,HDL,VLDL in STZ- nicotinamide induced diabetic rats.**

# *DISCUSSION*

# 7. DISCUSSION

Medicinal plants due to their natural origin plays a major role in treatment of diabetes, especially who have limited resources. Report of ethnobotany revealed that about 800 medicinal plants have anti diabetic activity(I-4) and their respective phytochemical constituents have alkaloids, glycosides, terpenoids, flavonoids etc are very effective in both preclinical and clinical studies (I-5,6). The present investigation showed that the ethanolic extract of *premna corymbosa* is nontoxic by oral route in wistar albino rats, up to a maximum of 2000mg/kg b.w acutely.

Literature review and phytochemical evaluation showed that presence of flavonoids, alkaloids, glycosides, sterols.It is possible that the anti diabetic property of ethanolic extract of *Premna corymbosa* could be mediated by the synergistic effect of these phytochemicals.The STZ induced diabetic model is one of the best model in which Streptozotocin is an alkylating agent which causes DNA damage which results in the activation of poly(ADP-ribose) synthetase that leads to the depletion of NAD and ATP virtually causes beta cell necrosis in the experimental rats. It leads to a reduction in insulin release there by a drastic reduction in plasma insulin concentration leading to stable hyper glycemic state. STZ is the most commonly used chemical for the induction of experimental diabetes for both IDDM and NIDDM.

The present study was conducted to evaluate the anti diabetic potential of *Premna corymbosa* in normal as well as STZ-nicotinamide induced diabetic rat. The study indicated that the ethanolic extract of *Premna corymbosa* possess blood glucose lowering property in STZ –nicotinamide induced diabetic rats. Oral administration of ethanolic extract of *Premna corymbosa* protected against STZ –nicotinamide induced diabetes mellitus.

The normal level blood urea and serum creatinine indicates that the ethanolic extract of *Premna corymbosa* did not interfere with the renal function and renal integrity was preserved and also there were no significant abnormalities.The study also revealed that there were no significant changes inliver function tests. The ethanolic extract of *Premna corymbosa* does not affect the normal values of ALP, AST, ALT and it confirmsthat the extract doesn’t have any hepatotoxic nature.The study also reveals that the ethanolic extract of *Premna corymbosa* does not affect the lipid profile such as triglycerides, total cholesterol, HDL, LDL, and VLDL.

# *SUMMARY AND CONCLUSION*

# 8. SUMMARY AND CONCLUSION

Several factors underlie the growing popularity of herbal treatments for a variety of chronic conditions. Interestingly, people who utilize alternative therapies are not necessarily uniformed. Many people using herbal medicines find the health care alternatives are more congruent with their own values, beliefs and philosophical orientation towards health and life.

The root of *Premna corymbosa* was selected for the evaluation of antidiabetic potential in STZ-nicotinamide induced diabetic rat model.The root extract was extracted by hot percolation using ethanol as a solvent in soxhlet apparatus and the preliminary phytochemical screening was performed and it shows that the ethanolic root extract of *Premna corymbosa* contains alkaloids, glycosides, flavonoids and tannins.

Safety profile is essential for the drugs obtained from the plant origin. The level of toxicity can be evaluated by toxicological studies.From the results it was concluded that the ethanolic root extract of *Premna corymbosa* showed significant anti diabetic activity in a dose dependant manner compared to the standard drug glibenclamide.

The result also showed significantdecrease in the liver Alkaline phosphatase(ALP), Aspartate amino transferase(AST), Alanine amino transferase(ALT), serum urea level, cholesterol, triglycerides, VLDL and LDL was in STZ-nicotinamide induced diabetic animals when compared to control group. It is concluded that ethanolic root extract of *Premna corymbosa* showed significant effect in STZ-nicotinamide induced diabetic rats. Further studies are necessary to examine the underlying mechanism of hypoglycemic effect and to isolate the active compound (s) responsible for antidiabetic activities.

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