ANTIDIABETIC ACTIVITY OF *Moringa Pterygosperma* AGAINST STREPTOZOTOCIN AND NICOTINAMIDE-INDUCED TYPE 2 DIABETES

A Dissertation submitted to
THE TAMIL NADU Dr.M.G.R. MEDICAL UNIVERSITY
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In partial fulfillment of the requirements for the award of the Degree of
MASTER OF PHARMACY IN
BRANCH-IV- PHARMACOLOGY

Submitted by
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COIMBATORE - 641 044

May 2019

CERTIFICATE

This is to certify that the M. Pharm., dissertation entitled **"ANTIDIABETIC ACTIVITY OF *Moringa pterygosperma* AGAINST STREPTOZOTOCIN AND NICOTINAMIDE-INDUCED TYPE 2 DIABETES"** being submitted to The Tamil Nadu Dr. M.G.R. Medical University, Chennai, in partial fulfillment of **Master of Pharmacy** programme in Pharmacology, carried out **Register No. 261725103** in the Department of Pharmacology, College of Pharmacy, Sri Ramakrishna Institute of Paramedical Sciences, Coimbatore, under my direct supervision and guidance to my full satisfaction.

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LIST OF ABBREVIATIONS

ANOVA	-	Analysis of Variance
ADP	-	Adenosine diphosphate
ALT	-	Alanine transaminase
ALP	-	Alkaline phosphatise
AST	-	Aspartate transaminase
CAT	-	Catalase
GSH	-	Reduced Glutathione
GSSH	-	Glutathione reductase
HDL	-	High Density Lipoproteins
H_2O_2	-	Hydrogen peroxide
IC_{50}	-	Half Maximal Inhibitory Concentration
IV	-	Intravenous
LDH	-	Lactate dehydrogenase
LDL	-	Low Density Lipoproteins
MDA	-	Malondialdehyde
NAD^+	-	Nicotinamide Adenine Dinucleotide oxidized
NADH	-	Nicotinamide Adenine Dinucleotide reduced
NADPH	-	Nicotinamide Adenine Dinucleotide Phosphate

OD	-	Optical Density
ROS	-	Reactive Oxygen Species
SEM	-	Standard Error of Mean
SOD	-	Superoxide dismutase
STD	-	Standard
STZ	-	Streptozotocin
TBA	-	Thiobarbituric Acid
TC	-	Total Cholesterol
TCA	-	Trichloroacetic Acid
TG	-	Triglyceride
UV	-	Ultra Violet
VLDL	-	Very Low Density Lipoprotein
WHO	-	World Health Organisation

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1. INTRODUCTION

Diabetes was considered a disease of the wealthy in ancient India, and was known as *Madhumeha* (sweet urine disease); it was observed that ants were attracted to the urine. The ancient Greeks coined the term "diabetes", meaning excessive urination with dehydration, but neither they nor the Romans appreciated that the urine contained sugar; "diabetes" was considered a kidney disease until the 18th century. The word diabetes derived from Greek-meaning to “to pass through” was first used by Aretaeus of Cappadocia in the 2nd century-AD as a generic description for conditions causing increased urine output. An English physician John Rollo (1809) introduced the adjective word “mellitus”[1].

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 type1) or resistance to its effects (in type 2 and gestational) both of which leads to hyperglycaemia. 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 (glycosuria) and causes an osmotic dieresis (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 1diabetes. Type 2 is managed with combination of dietary treatment, exercise, medications and insulin[2].

1.1 Symptoms of diabetes

The classic symptoms of untreated diabetes are weight loss, polyuria (frequent urination), polydipsia (increased thirst), and polyphagia (increased hunger). Symptoms may develop rapidly (weeks or months) in type 1 diabetes, while they usually develop much more slowly and may be subtle or absent in type 2 diabetes.

Several other signs and symptoms can mark the onset of diabetes, although they are not specific to the disease. In addition to the known ones above, they include blurry vision, headache, fatigue, slow healing of cuts, and itchy skin. Prolonged high blood glucose can cause gNlucose absorption in the lens of the eye, which leads to changes in its shape, resulting in vision changes. A number of skin rashes that can occur in diabetes are collectively known as diabetic dermadromes. People (usually with type 1 diabetes) 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 type 2 diabetes and is mainly the result of dehydration[3,4].

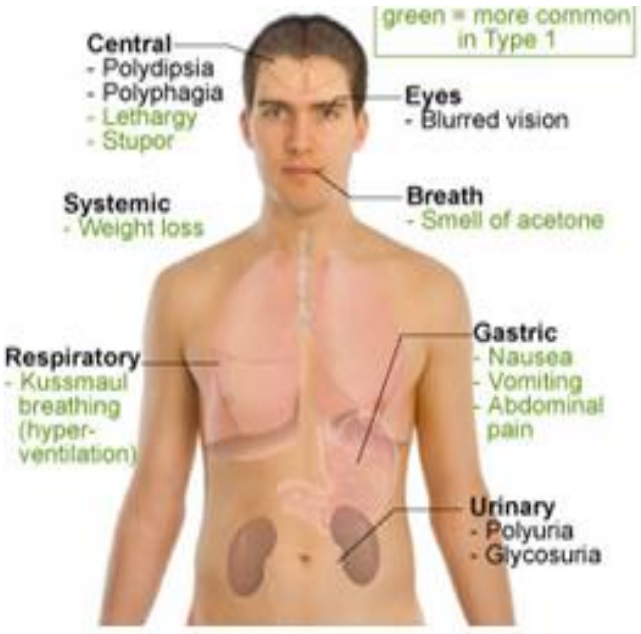

Fig: 1 Main symptoms of diabetes

1.2 Mechanism of induction diabetes mellitus[5]

1. β- cell destruction (Type 1 diabetes - IDDM)
    (a) Immune mediated
    (b) Idiopathy
2. Insulin resistance (Type 2 diabetes - NIDDM)
3. Genetic defects of β- cell function
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    (c) Insulin promoter factor
    (d) Mitochondrial DNA
    (e) Proinsulin or insulin conversion
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6. Endocrinopathy
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1.3 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 a universal consensus on the common classification[6]

- Type 1 diabetes (absolute insulin deficiency)
    - Immune mediated
    - Idiopathic
- Type 2 diabetes (predominantly insulin resistance with relative insulin deficiency
- Other specific types
- Gestational diabetes mellitus (GDM)

1.3.1 Type 1 diabetes

Insulin-dependent (Type I) 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.

Insulin secretory function is limited and cell membrane binding is not primarily involved. The goal of treatment is to relieve symptoms and to achieve blood glucose levels as close to normal as possible without severe hypoglycemia. However, even with education and self monitoring of the blood glucose level, attaining recommended target values (plasma glucose level less than 8.0 mmol/L before main meals for adults) remains difficult. Therapy with one or two injections per day of mixed short-acting or intermediate acting insulin preparations is a compromise between convenience and the potential for achieving target plasma glucose levels[7,8].

Intensive insulin therapy with multiple daily injections or continuous infusion with an insulin pump improves mean glycated hemoglobin levels; however, it increases rates of severe hypoglycemia and has not been shown to decrease the incidence of clinically significant renal, retinal or neurologic dysfunction. Future prospects include automated techniques of insulin delivery, immunosuppressant to preserve endogenous insulin secretion and islets transplantation.

Type 1 diabetes (IDDM) is characterized by loss of the insulin producing beta cells of the islet of langerhans in the pancreas leading to deficiency. In type 1 diabetes, the body does not produce insulin, and daily insulin injections are required. Type 1 diabetes is usually diagnosed during childhood or early adolescence and it affects about 1 in every 600 children[9].

a) **Immune mediated diabetes**

This form was previously called as insulin dependent diabetes (IDDM). Type 1 diabetes or Juvenile onset diabetes results from a cellular mediated autoimmune destruction of the beta cell of the pancreas. In this form of diabetes, the rate of beta cell destruction is quite variable being rapid in some individuals (mainly infants and children) and slow in others (mainly adults)[7].

b) Idiopathic diabetes

Some forms of type 1 diabetes have no known etiologies. 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. However most of those who fall into this category are of the African or Asian origin.

Individuals with this form of diabetes suffer from episodic ketoacidosis and exhibit varying degrees of insulin deficiency between episodes[7].

Symptoms of type1 diabetes

Type 1 diabetes signs and symptoms appears quickly and may include[10]

- Increased thirst and frequent urination
- Extreme hunger
- Weight loss
- Fatigue
- Blurred vision

Causes of type 1 diabetes

The exact cause of type 1 diabetes is unknown. In most people with type 1 diabetes, the body's own immune system which normally fights harmful bacteria and viruses mistakenly destroys the insulin-producing (islet) cells in the pancreas. Genetics may play a role in this process, and exposure to certain viruses may trigger the disease[11].

- **A family history**: Anyone with a parent or sibling with type 1 diabetes has a slightly increased risk of developing the condition.
- **Genetics**. The presence of certain genes indicates an increased risk of developing type 1diabetes. In some cases-usually through a clinical trial genetic testing can be done to determine if someone who has a family history of type 1diabetes is at increased risk of developing the condition.
- **Geography**: The incidence of type 1 diabetes tends to increase as you travel away from the equator. People living in Finland and Sardinia have the highest incidence of type 1 diabetes — about two to three times higher than rates in the United States and 400 times that of people living in Venezuela.
- **Viral exposure**: Exposure to Epstein-Barr virus, coxsackie virus, mumps virus or cytomegalovirus may trigger the autoimmune destruction of the islet cells, or the virus may directly infect the islet cells.
- **Early vitamin D**: Research suggests that vitamin D may be protective against type 1 diabetes. However, early drinking of cow's milk — a common source of vitamin D — has been linked to an increased risk of type 1 diabetes.
- **Other dietary factors**: Omega-3 fatty acids may offer some protection against type 1 diabetes. Drinking water that contains nitrates may increase the risk. Consuming dairy products, particularly cow's milk, may increase infants' risk of the disease.

Additionally, the timing of the introduction of cereal into a baby's diet may affect risk. One clinical trial found that between ages 3 and 7 months appears to be the optimal time for introducing cereal.

Complications of type1 diabetes mellitus

Type 1 diabetes can affect major organs in the body, including heart, blood vessels, nerves, eyes and kidneys. Keeping the blood sugar level close to normal most of the time can dramatically reduce the risk of many complications.

Long-term complications of type 1 diabetes develop gradually, over years. The diabetes developed earlier - and the less controlled blood sugar may causes higher the risk of complications. Eventually, diabetes complications may be disabling or even life-threatening[12].

1) Heart and blood vessel disease: Diabetes dramatically increases your risk of various cardiovascular problems, including coronary artery disease with chest pain (angina), heart attack, stroke, narrowing of the arteries (atherosclerosis) and high blood pressure.

2) Nerve damage (neuropathy): Excess sugar can injure the walls of the tiny blood vessels (capillaries) that nourish your nerves, especially in the legs. This can cause tingling, numbness, burning or pain that usually begins at the tips of the toes or fingers and gradually spreads upwards. Poorly controlled blood sugar could cause you to eventually lose all sense of feeling in the affected limbs. Damage to the nerves that affect the gastrointestinal tract can cause problems with nausea, vomiting, diarrhoea or constipation. For men, erectile dysfunction may be an issue.

3) Kidney damage (nephropathy): The kidneys contain millions of tiny blood vessel clusters that filter waste from your blood. Diabetes can damage this delicate filtering system. Severe damage can lead to kidney failure or irreversible end-stage kidney disease, which requires dialysis or a kidney transplant.

4) Eye damage: Diabetes can damage the blood vessels of the retina (diabetic retinopathy), potentially leading to blindness. Diabetes also increases the risk of other serious vision problems, such as cataract and glaucoma.

5) Foot damage: Nerve damage in the feet or poor blood flow to the feet increases the risk of various foot complications. Cuts and blisters when left untreated can become serious infections. Severe damage might require toe, foot or even leg amputation.

6) Skin and mouth conditions: Diabetes patients may be more susceptible to skin problems, including bacterial and fungal infections. Gum infections also may be a concern, especially if they have a history of poor dental hygiene.

7) Osteoporosis: Diabetes may lead to lower than normal bone mineral density, increasing the risk of osteoporosis.

8) Pregnancy complications: High blood sugar levels can be dangerous for both the mother and the baby. The risk of miscarriage, stillbirth and birth defects are increased when diabetes is not well controlled. For the mother, diabetes increases the risk of diabetic ketoacidosis, diabetic eye problems (retinopathy), pregnancy-induced high blood pressure and preeclampsia.

9) Hearing problems: Hearing impairments occur more often in people with diabetes.

Insulin

Insulin is a peptide hormone, produced by the beta cells of the islets of langerhans of the pancreas and is central to regulate the blood glucose level in the body. Like most of the other hormones, insulin is a protein comprising of 2 polypeptide chains A (with 21 amino acid residues and B (with 30 amino acid residues).Chains A and B are linked by disulphide bridges. In addition A-chain contains an intra-chain disulphide bridge linking residue 6 and 11.C-chain, which connect A and B chains is liberated along with insulin after breakdown of pro-insulin. Insulin monomers aggregate to form dimmers and hexamers. Zn hexamer is composed of three insulin dimmers associated in threefold symmetrical pattern. Patient with type 1 diabetic insulin cannot be secreted in their body (pancreas). So it (insulin) must be injected externally[13].

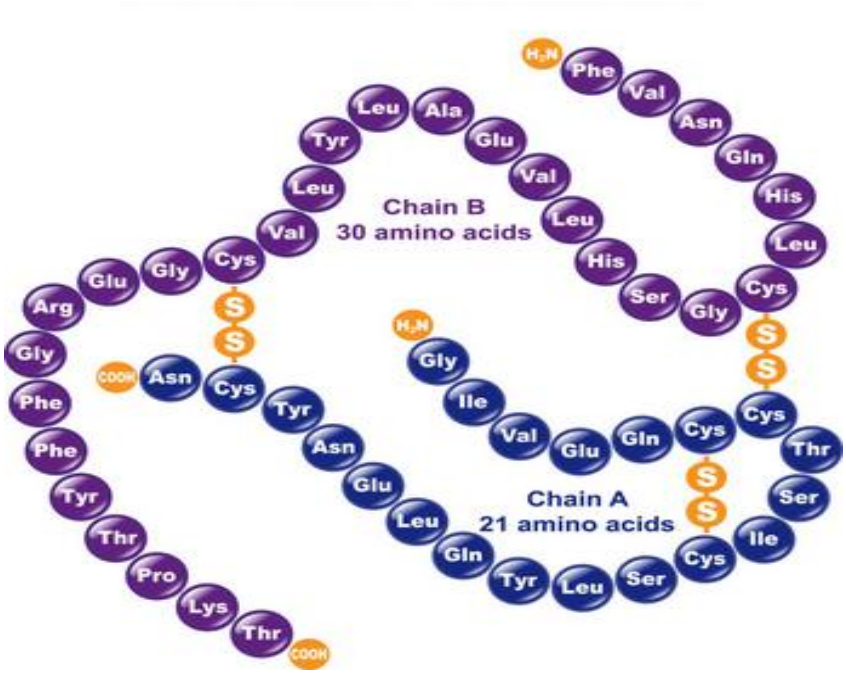


Fig : 2 structure of insulin [14]

Table:1 Classification of insulin preparations[15,16,17]

TYPE	BRAND NAME	GENERIC NAME	ONSET	PEAK	DURATION
Rapid-acting	Novolog	Insulin aspart	15minutes	30-90 minutes	3-5hours
	Apidra	Glulisine	15minutes	30-90 Minutes	3-5hours
	Humalog	Insulin lispro	15minutes	30-90 Minutes	3-5hours
Short-acting	Humulin R	Regular R	30-60 minutes	2-4hurs	5-8hours
	Novolin R				
Intermediate acting	Humulin N	NPH(H)	1-3hours	8hours	12-16hours
	Novolin N				
Long acting	Levamir	Insulin detemir	1hour	Peakless	20-26hour
	Lantus	Insulin glargine			
Pre-mixed NPH(intermediate acting)& regular(short)	Humulin70/30 Novolin 70/30	70%NPH & 30% regular	30-60 minutes	Varies	10-16hours
Pre-mixed insulin lispro protamine suspension (intermediate acting) & insulin lispro(rapid acting)	Humalog mix 75/25	75% insulin lispro protamine and 25% insulin lispro	10-15 minutes	Varies	10-16hours
	Humalog mix 50/50	50% lispro protamine and 50% insulin lispro	10-15 minutes	Varies	10-16 hours
Pre-mixed insulin aspart protamine suspension	NovoLog Mix 70/30	70% insulin aspart protamine &30% insulin aspart	5-15 minutes	varies	10-16 hours

Cellular actions of insulin

Key insulin target tissues for regulation of glucose homeostasis are liver, muscle, and fat, but insulin also exerts potent regulatory effects on other cell types. Insulin stimulates intracellular use and storage of glucose, amino acids, and fatty acids and inhibits catabolic processes such as the breakdown of glycogen, fat, and protein. It does this by stimulating the transport of substrates and ions into cells, promoting the translocation of proteins between cellular compartments, activating and inactivating specific enzymes, and changing the amounts of proteins by altering the rates of transcription and mRNA translation[18]

1.3.2 Type 2 diabetes

Type 2 diabetes, once known as adult-onset or noninsulin-dependent diabetes, is a chronic condition that affects the way the body metabolizes glucose, main source of fuel.

In type 2 diabetes, the body either resists the effects of insulin-a hormone that regulates the movement of glucose into the cells-or does not produce enough insulin to maintain a normal glucose level. Untreated, type 2 diabetes 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 [19].

Symptoms of type 2 diabetes

Type 2 diabetes symptoms may develop slowly [20]

- Increased thirst and frequent urination
- Increased hunger.
- Weight loss.
- Fatigue
- Blurred vision.
- Slow-healing sores or frequent infections.

Causes of type 2 diabetes

- **Weight**; Being overweight is a primary risk factor for type 2 diabetes. The more fatty tissue the cells become more insulin resistant.
- **Fat distribution;** If the body stores fat primarily in the abdomen, the risk of type 2 diabetes is greater than if the body stores fat elsewhere, such as the hips and thighs.
- **Inactivity;** The less active you are, the greater your risk of type 2 diabetes. Physical activity helps to control the weight, uses up glucose as energy and makes cells more sensitive to insulin.

- **Family history;** The risk of type 2 diabetes increases if the parent or sibling has type 2 diabetes.
- **Race;** Although it's unclear why, people of certain races — including blacks, Hispanics, American Indians and Asian-Americans — are more likely to develop type 2 diabetes than whites are.
- **Age;** The risk of type 2 diabetes increases as you get older, especially after age 45. That's probably because people tend to exercise less, lose muscle mass and gain weight as they age. But type 2 diabetes is also increasing dramatically among children, adolescents and younger adults.
- **Prediabetes;** Prediabetes is a condition in which your blood sugar level is higher than normal, but not high enough to be classified as diabetes. Left untreated, prediabetes often progresses to type 2 diabetes[21].

Complications of type 2 diabetes

1) Heart and blood vessel disease; Diabetes dramatically increases the risk of various cardiovascular problems, including coronary artery disease with chest pain (angina), heart attack, stroke, narrowing of arteries (atherosclerosis) and high blood pressure. The risk of stroke is two to four times higher for people with diabetes, and the death rate from heart disease is two to four times higher for people with diabetes than for people without the disease, according to the American Heart Association.

2) Alzheimer's disease; Type 2 diabetes may increase the risk of Alzheimer's disease and vascular dementia. The poorer your blood sugar control, the greater the risk appears to be. So what connects the two conditions? One theory is that cardiovascular problems caused by diabetes could contribute to dementia by blocking blood flow to the brain or causing strokes. Other possibilities are that too much insulin in the blood leads to brain-damaging inflammation, or lack of insulin in the brain deprives brain cells of glucose.

3) Nerve damage (neuropathy); Excess sugar can injure the walls of the tiny blood vessels (capillaries) that nourish your nerves, especially in the legs. This can cause tingling, numbness, burning or pain that usually begins at the tips of the toes or fingers and gradually spreads upward. Poorly controlled blood sugar can eventually cause you to lose all sense of feeling in the affected limbs.

4) Kidney damage (nephropathy); The kidneys contain millions of tiny blood vessel clusters that filter waste from your blood. Diabetes can damage this delicate filtering system. Severe damage can lead to kidney failure or irreversible end-stage kidney disease, requiring dialysis or a kidney transplant.

5) Foot damage; Nerve damage in the feet or poor blood flow to the feet increases the risk of various foot complications. Left untreated, cuts and blisters can become serious infections. Severe damage might require toe, foot or even leg amputation.

6) Skin and mouth conditions; Diabetes may leave you more susceptible to skin problems, including bacterial and fungal infections. Gum infections also may be a concern, especially if you have a history of poor dental hygiene[22].

1.3.3 Gestational diabetes mellitus (GDM)

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. Diagnosis of gestational diabetes is made during pregnancy. 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. Undiagnosed or uncontrolled gestational diabetes can raise the risk of complications during childbirth. The baby may be bigger than he/she should be [23]

Complications of gestational diabetes

- Increased risk of prenatal mortality and morbidity.
- Obesity or impaired glucose intolerance in the offspring accompanied by macrosomia
- Neural tube defects
- Prematurity syndromes[24]

Causes of gestational diabetes

Age > 30 years, obesity (BMI > 27.3 kg/m2), family history of diabetes, glycosuria, previous macrosomia, previous congenital malformation, previous stillbirth, past history of Gestational diabetes mellitus

1.4.4 Other forms of diabetes

a. Genetic defects in insulin action.

b. Diseases of the exocrine pancreas-includes fibrocalculous

c. Pancreatopathy

d. Endocrinopathies

e. Drugs or chemicals induced diabetes

f. Infection

e. Uncommon forms of immune mediated diabetes

h. Other genetic syndromes associated with diabetes.

Table 2: signs and symptoms observed in different types of diabetes

Type	Type 1	Type 2	Gestational
Characteristics	Sudden onset, pronounced thirst and hunger, frequent urination, fatique, nausea and vomiting, weight loss.	Slow, difficult to detect onset, pronounced thirst, frequent urination, fatigue, slow wound healing, tingling hands or feet; frequent infections, weight loss	Pronounced thirst, frequent urination, fatigue and other symptoms similar to those of type 2
Age at onset	Usually 20 or younger	Usually 40 or elder, although rates are escalating among younger people	Child-bearing years
Physical condition	Usually lean or normal weight	Usually over weight	Pregnant
Cause	Immune system destroys the pancreas cells that produce insulin	Genetics, lack of exercise, poor diet and resulting obesity	Hormones produced in the placenta hinder the function of insulin
Mainstay of treatment	Insulin injections	Life style changes, possibly augmented by insulin and oral hypoglycaemic agents	Lifestyle changes, possibly augmented by insulin injections

Special types of diabetes in India

The WHO classification (1985) had malnutrition related diabetes as a separate class protein deficient diabetes mellitus (PDDM) and fibrocalculous pancreatic diabetes mellitus (FCPD). In recent American Diabetic Association classification, FCPD has been included in other specific types and protein deficient diabetes mellitus (PDDM) has been deleted. However, this type of diabetes which is modulated by malnutrition is specially seen in India.

1.4 Etiology

- Insulin resistance in the hepatic and skeletal muscle, increased hepatic glucose synthesis, over production of free fatty acids and relative insulin deficiency.
- Beta cells failure.
- Contributing factors[25].
    - Obesity
    - Racial/ ethnic background.
    - Age (onset of puberty is associated with increased insulin resistance).
    - Sedentary lifestyle.
    - Genetic predisposition.
    - Conditions associated with insulin resistance, (e.g.polycystic ovary syndrome)

1.5 Risk factors of diabetes

The risk factors for diabetes may be categorized as modifiable risk factors and non-modifiable risk factors.

1.5.1 Modifiable risk factors

- ✓ **Obesity**

  via BMI-Body mass index

  WHR-Waist-hip ratio
- ✓ **Physical Inactivity:** The protective effect of physical activity in subjects with an excessive BMI and elevated glucose levels; Diabetes can be prevented by physical activity and weight control in peoples with both normal and impaired glycemic control.
- ✓ **Plasma Lipids and Lipoproteins Level:** There are reports like the blood level of LDL, VLDL, TGL are high and that of HDL is low in Diabetic people. According to American Diabetes Association LDL Cholesterol should be <100 mg/dl; HDL Cholesterol: >60 mg/dL; and Triglycerides: <150 mg/dl.

- ✓ **Hypertension:** It has been reported that the incident of diabetes for hypertensive patient is greater.
- ✓ **Dietary Habits:** It is suggested that whole grains are rich resources of dietary fiber, fat, vitamin, antioxidant nutrients, minerals, lignans, starch, and phenolic compounds that have been linked to the reduced risk of insulin resistance, dyslipidemia, obesity, T2DM,heart diseases and dietary fructose specifically increases de novo lipogenesis, promotes dyslipidemia, decreases insulin sensitivity,and increases visceral adiposity in overweight/obese adults[26].

1.5.2 Non-modifiable risk factors

- ✓ **Family History:** First degree family history of diabetes was found in an approximate of 75% of the T2DM patients.
- ✓ **Genetic factors:** Genome-wide association studies show a strong association between genetic factor and Diabetes.
- ✓ **Low/High Birth Weight:** Both low and high birth weight are risk factors of Diabetes[26,27].

1.6 Complications of diabetes

All forms of diabetes increase the risk of long-term complications. These typically develop after many years (10–20), but may be the first symptom in those who have otherwise not received a diagnosis before that time.

The major long-term complications relate to damage to blood vessels. Diabetes doubles the risk of cardiovascular disease and about 75% of deaths in diabetics are due to coronary artery disease. Other "macrovascular" diseases are stroke, and peripheral vascular disease.

The primary micro vascular complications of diabetes include damage to the eyes, kidneys, and nerves. Damage to the eyes, known as diabetic retinopathy, is caused by damage to the blood vessels in the retina of the eye, and can result in gradual vision loss and potentially blindness. Damage to the kidneys, known as diabetic nephropathy, can lead to tissue scarring, urine protein loss, and eventually chronic kidney disease, sometimes requiring dialysis or kidney transplant. Damage to the nerves of the body, known as diabetic neuropathy, is the most common complication of diabetes. The symptoms can include numbness, tingling, pain, and altered pain sensation, which can lead to damage to the skin. Diabetes-related foot problems (such as diabetic foot ulcers) may occur, and can be difficult to treat, occasionally requiring amputation. Additionally, proximal diabetic neuropathy causes painful muscle wasting and weakness. There is a link between cognitive deficit and

diabetes. Compared to those without diabetes, those with the disease have a 1.2 to 1.5-fold greater rate of decline in cognitive function[28].

1.7 Diagnosis

Diabetes mellitus is characterized by recurrent or persistent hyperglycemia, and 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/l (200 mg/dl) two hours after a 75 g 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 A1C) ≥ 6.5%.

A positive result, in the absence of unequivocal hyperglycaemia, 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 two 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.
Per the World Health Organization 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), two hours after a 75 g 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 (*insipidus* means "without taste" in Latin) and does not involve the same disease mechanisms[29].

Table: 3 WHO diagnosis criteria [30]

WHO diagnosis criteria			
Condition	**2 hour glucose mmmol/1(mg/dl)**	**Fasting glucose mmmol/1(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

1.8 PREVALANCE 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 indeveloping countries is 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[31].

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, and 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 for 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 [32].

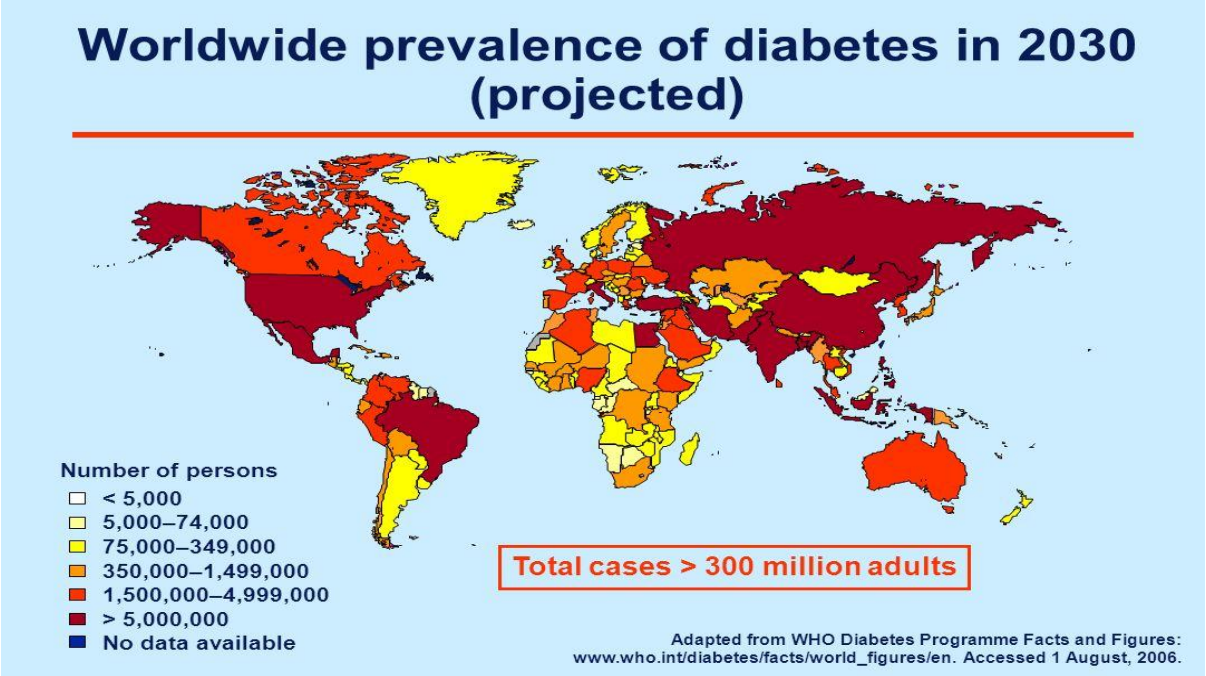


Fig ; 3 World wide prevalence of diabetes in 2030

The prevalence estimates were applied to population estimates for individual countries for 2000 and 2030, which were produced by the United Nations Population Division. In keeping with previous estimates, prevalence of diabetes was assumed to be similar in urban and rural areas of developed countries (Europe including former socialist economies, North America, Japan, Australia, and New Zealand). For developing countries, urbanization was used as a proxy measure of the increased risk of diabetes associated with altered diet, obesity, decreased physical activity, and other factors such as stress, which are assumed to differ between urban and rural populations. For most developing countries, the prevalence of diabetes in rural areas was assumed to be one-half that of urban areas, based on the ratio observed in a number of population studies and as used in previous estimates. For some populations in developing countries (small islands and populations for which prevalence data were derived from studies combining urban and rural populations), a single estimate of diabetes prevalence was used. In the current estimates, on the advice of local experts, the prevalence of diabetes in rural areas was assumed to be one-quarter that of urban areas for Bangladesh, Bhutan, India, the Maldives, Nepal, and Sri Lanka .

Table: 4 - 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	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	Pakistan	13.9
6	Pakistan	5.2	Brazil	11.3
7	Russian	4.6	Bangladesh	11.1
	Federation		Japan	8.9
8	Brazil	4.6	Philippines	7.8
9	Italy	4.3	Egypt	6.7
10	Bangladesh	3.2		

1.9 Drugs used in the induction of experimental diabetes

1.9.1 Alloxan

Alloxan is most prominent chemical compound used in diabetogenic research. In research it is used for induction of Type 1 diabetes. Alloxan is a urea derivative which causes selective necrosis of the β- 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. The chemical name of alloxan is 2,4,5,6 tetraoxypyrimidine; 2, 4, 5, 6- pyrimidinetetrone, which is an oxygenated pyrimidine derivative which is present as alloxan hydrate in aqueous solution.

Mechanism

Alloxan treatment evokes a sudden rise in insulin secretion in the presence or absence of glucose and this insulin release occurs for short duration followed by the complete suppression of the islet response to glucose even when high concentrations of glucose were used .Further, important feature of alloxan action in pancreas is preceded by its rapid uptake by pancreatic beta cells. Moreover, in pancreatic beta cells, the reduction process occurs in

the presence of reducing agents like reduced glutathione (GSH), cysteine, ascorbate and protein-bound sulfhydryl (-SH) groups. Alloxan reacts with two -SH groups in the sugar binding site of glucokinase and results in inactivation of the enzyme. As a result dialuric acid is formed which is then re-oxidized back to alloxan establishing a redox cycle and generates reactive oxygen species (ROS) and superoxide radicals .The superoxide radicals liberate ferric ions from ferritin and reduce them to ferrous and ferric ions and also undergo dismutation to yield hydrogen peroxide (H_20_2). As a result, highly reactive hydroxyl radicals are formed in the presence of ferrous and H_20_2. Another mechanism that has been reported is the effect of ROS on the DNA of pancreatic islets. In the beta cells alloxan causes DNA fragmentation and damage. Antioxidants like superoxide dismutase, catalase and the non enzymatic scavengers of hydroxyl radicals have been found to protect against alloxan toxicity. In addition cytosolic free elevated Ca^{2+} has also been reported to constitute an important step in the diabetogenic action of alloxan. The calcium influx results from the ability of alloxan to open voltage dependent calcium channels and enhances calcium entry into pancreatic cells. The increased concentration of Ca^{2+} ion further contributes to supra physiological insulin release that along with ROS eventually causes damage of beta cells of pancreatic islets[33].

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

Streptozotocin is a permanent diabetes inducing drug. It is synthesized by a strain of the soil microbe *Streptomyces achromogenes* (gram positive bacterium) with broad spectrum of antibacterial properties . Streptozotocin 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[34].

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 40 mg/kg b.w. may be ineffective. NIDDM can easily be induced in rats by intravenous or intraperitoneal treatment with 100 mg/kg b.w. STZ on the day of birth. Streptozotocin action in B cells is accompanied by characteristic alterations in blood insulin and glucose concentrations. Two hours after injection, the hyperglycaemia is observed with a concomitant drop in blood insulin. About six hours later, hypoglycaemia occurs with high levels of blood

insulin. Finally, hyperglycemia develops and blood insulin levels 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[35].

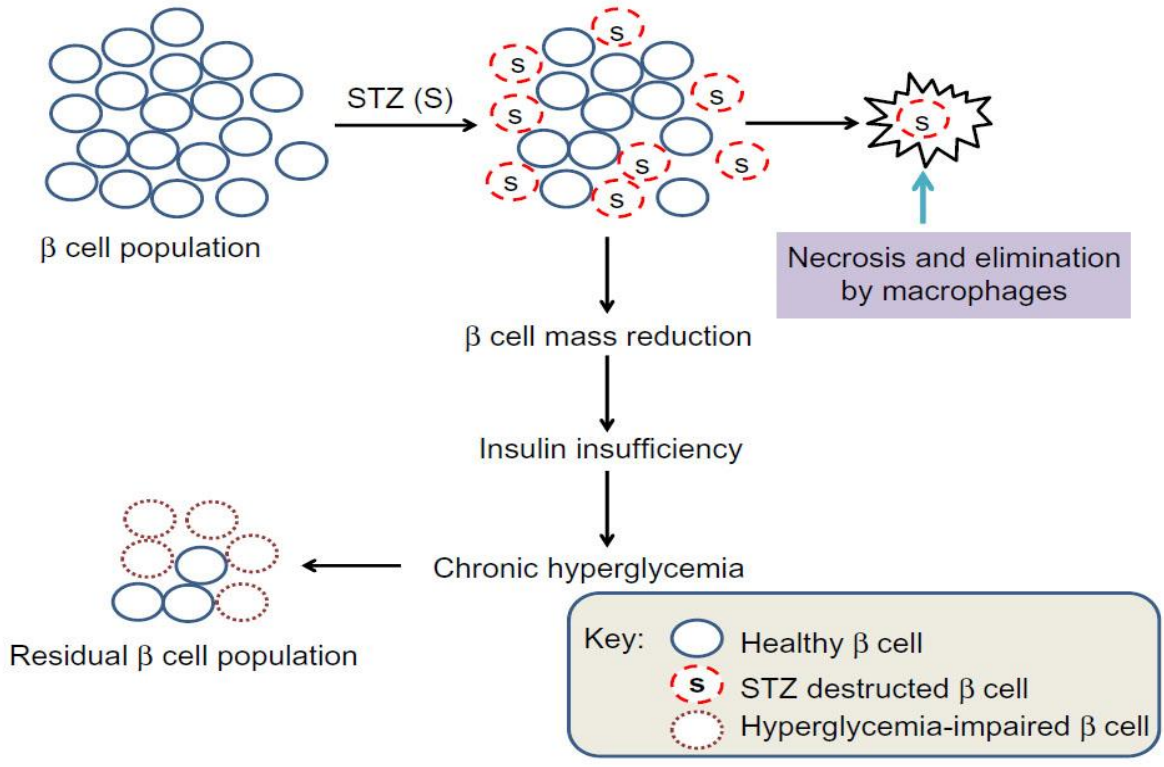


Fig: 4 Mechanism of action of streptozotocin on beta cells of rat pancreas

1.10 Effect of free radicals in diabetes

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 pathologic states, especially chronic diseases, the increased production and/or ineffective scavenging of reactive oxygen species (ROs) may play a critical role. High reactivity of ROS determines chemical changes in virtually all cellular components, leading to lipid peroxidation. Production of ROS and disturbed capacity of antioxidant defence in diabetic subjects have been reported. It has been suggested that enhanced production of free radicals and oxidative stress is central event to the development of diabetic complications. This suggestion has been supported by demonstration of increased levels of indicators of oxidative stress in diabetic individuals suffering from complications[36]

Our body possess defence 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 defences 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[37].

Hyperglycaemia 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 and aging and age-related diseases by an increase in oxidative chemical modification of lipids, DNA and proteins. In particular, long-lived proteins such as lens crystallines, collagens and haemoglobin 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 hyperglycaemia. Glutathione (gamma- glutamylcysteinyl glycine [GSHI) is thought to be an important factor in cellular function and defence against oxidative stress[38].

1.10.1 Biogenesis of free radicals

- Free radicals are the natural by products of many biochemical process within the cells an 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, ultraviolet 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, supper oxides and hydrochlorides. Hydroxyl radical is the most dangerous among all reactive oxygen intermediate species[39].

Table : 5 Types of free radicals

S.no	Free radicals	Structure
1	Superoxides	$O_2^{\bullet}$
2	Nitric oxide	$NO^{\bullet}$
3	Peroxy radical	$RCOO^{\bullet}$
4	Hydrogen	$H^{\bullet}$
5	Alkoxy	$RO^{\bullet}$
6	Hydroxyl	$OH^{\bullet}$
7	Singlet oxygen	$O^{\bullet}$
8	Hydrogen peroxide	$H_2O_2^{\bullet}$
9	Lipid peroxy radical	$LO_2^{\bullet}$
10	Hypochlorous acid	$HOCl^{\bullet}$
11	Peroxy nitrite	$ONOO^{\bullet}$
12	Carbon centered free radicals	$CCl_3^{\bullet}$

1.10.2 Diseases caused by free radicals

- Diabetes mellitus-diabetic retinopathy, nephropathy
- Cardiovascular disease-atherosclerosis, alcohol induced cardiomyopathy, heart attack , hypertension, coronary artery disease
- Gastrointestinal tract-pancreatitis, liver injury, ulcers
- Eye-cataract, glaucoma, degenerative retinal disease, ocular degeneration
- Kidney-kidney failure, nephrotic syndrome, nephrotoxicity, glomerulonephritis
- Nervous system-alzheimers disease, parkinsons disease, senile dementia, memory loss, multiple sclerosis
- Lungs-lungs cancer, emphysema[40]

1.11 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. Antioxidants 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 and especially weight reduction, can effectively prevent and even reverse the effects of diabetes, in part by reducing oxidative stress. Various hypoglycaemic agents reduce oxidative stress, indirectly by lowering blood glucose levels (prevents hyperinsulinemia) and directly acts as free radical scavengers. For example, gliclazide, a sulfonylurea normally used to augment insulin release, is an effective scavenger of superoide 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[41].

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.

However, certain antioxidants are of particular benefit with regard to the prevention and treatment of diabetic complications Primary among these are vitamin E (α-tocopherol) and lipoic acid (thioctic acid). Vitamin E is a fat-soluble vitamin that effectively scavenges the peroxyl radical in cell membranes, thereby inhibiting lipid peroxidation. Lipoic acid, an essential cofactor of alpha-oxoacid dehydrogenase complexes, is also a potent lipophilic free radical scavengers.

Other antioxidant agents are found in foods, such as dark green leafy vegetables. Items high in vitamin A, vitamin C, beta-carotene and flavanoids are believed to be the most beneficial-Dietary supplements are also available for those that do not consume enough antioxidant- producing foods.

People with diabetes have elevated levels of free radicals and lower levels of antioxidants. Therefore, it seems 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[42].

There are 3 types of antioxidants and it includes,

Primary antioxidants

This group prevent 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

- 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[43,44].

Seconday antioxidants

- This antioxidant 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, vitamine C, beta carotene, uric acid and albumin[43,44].

Tertiary antioxidant

They repair bio-molecules damaged by free radicals eg DNA repair enzymes, methionine sulphoxide reductase[45]

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) [46].

Treatment of type 2 diabetes

- **Oral hypoglycaemic drugs**

✓ **Sulphonyl ureas** –

First generation	**Second generation**
Tolbutamide	Glibenglamide
Chlorpropamide	Glipizide
	Gliclazide and Glimiperide

✓ **Biguanides**

Metformin

✓ **Meglitinides**

Repaglinide

Nateglinide

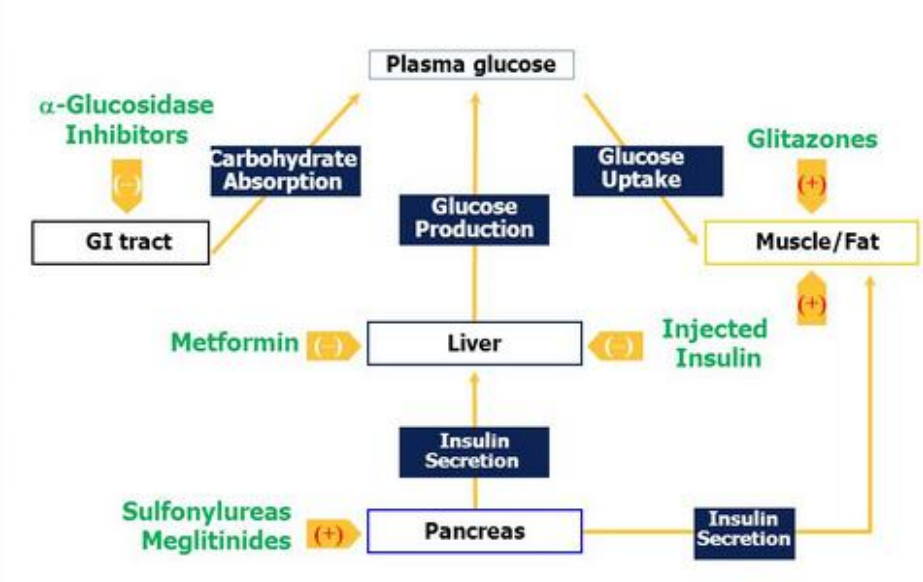
✓ **Thiazolidine diones** –

Rosiglitazone

Pioglitazone

✓ **Alpha glucosidase inhibitors**

Acarbose, Miglitol



Fig;5 Treatment of diabetes mellitus

Sulfonylurea's

Sulfonylurea's bind to (KATP) channel on the cell membrane of pancreatic this inhibits a tonic, hyperpolarizing efflux of potassium, thus causing the electric potential over the membrane to become more positive. This opens voltage-gated channels. The rises in intracellular calcium leads to increased fusion of granulate with the cell membrane, and therefore increased secretion of (pro) insulin.

Tolbutamide

Tolbutamide is a first generation This drug may be used in the management of if diet alone is not effective. Tolbutamide stimulates the secretion of by the. Since the pancreas must synthesize insulin in order for this drug to work, it is not effective in the management of. It is not routinely used due to a higher incidence of adverse effects compared to newer second generation sulfonylurea's, such as it generally has a short duration of action due to its rapid metabolism, and is therefore safe for use in elderly diabetics.

Glimiperide

The primary mechanism of action of glimepiride in lowering blood glucose appears to be dependent on stimulating the release of insulin from functioning pancreatic beta cells. In addition, extrapancreatic effects may also play a role in the activity of sulfonylureas such as glimepiride. This is supported by both preclinical and clinical studies demonstrating that glimepiride administration can lead to increased sensitivity of peripheral tissues to insulin. These findings are consistent with the results of a long-term, randomized, and placebo-controlled trial in which glimiperide therapy improved postprandial insulin/C-peptide responses and overall glycemic control without producing clinically meaningful increases in

fasting insulin/C-peptide levels. However, as with other sulfonylureas, the mechanism by which glimepiride lowers blood glucose during long-term administration has not been clearly established.

Glibenclamide

The drug works by binding to and activating the regulatory subunit of the (KATP). This inhibition causes cell membrane opening. This results in an increase in intracellular in the and subsequent stimulation insulin release.

After a cerebral ischemic insult is broken and glibenclamide can reach the central nervous system. Glibenclamide has been shown to bind more efficiently to the ischemic hemisphere. Moreover, under ischemic conditions SUR1, the regulatory subunit of the KATP- and the NCCa-ATP-channels, is expressed in neurons, atrocities, oligodendrocytes, endothelial cells and by reactive microglia.

Gliclazide

Gliclazide selectively binds to sulfonylurea receptors on the surface of the pancreatic beta-cells. It was shown to provide cardiovascular protection as it does not bind to sulfonylurea receptors (n the heart This binding effectively closes the K+ ion channels. This decreases the efflux of potassium from the cell which leads to the depolarization of the cell. This causes voltage dependent Ca++ ion channels to open increasing the Ca++ influx. The calcium can then bind to and activate calmodulin which in turn leads to exocytose of insulin vesicles leading to insulin release

Metformin

Metformin activates AMP activated protein kinase (AMPK) a liver enzyme that play an important role in signaling whole body energy balance and the glucose and fats activation of AMPK is required for Metformin inhibitory effects on the production of liver glucose.

Repaglinide

Repaglinide acts by stimulating release of insulin from the cells of the islets of pancreas inhibiting ATP-sensitive K+ channels, thereby activating the Ca++ channels with increase in intracellular calcium to release insulin. However, repaglinide acts on a different binding site than the sulphonylureas. Repaglinide is not effective in the absence of functioning beta-cells. Repaglinide increases the amount of insulin released in a natural and physiological pulsatile pattern the activity of repaglinide is dose-dependent. Mean insulin levels begin to rise approximately 1.5 hours after the pre-prandial dose of repaglinide and declines towards baseline levels between meal-time the rapid onset of action and the short duration of hypoglycemic effect of repaglinide makes this agent suitable for pre-prandial

administration. The main advantage of pre-prandial administration is that patients can miss or postpone a meal (and the corresponding repaglinide dose) without increasing the risk of hypoglycemia or compromising glycaemic control.

Pioglitazone

Pioglitazone is an oral drug that reduces the amount of glucose (sugar) in the blood. It is in a class of anti-diabetic drugs called thiazolidinediones that are used in the treatment. Another member of this class, troglitazone or Rezulin, was removed from the market because of side effect. Patients with type 2 diabetes cannot make enough insulin, and the cells of their body respond less to the insulin that is produced. Since insulin is the hormone that stimulates cells to remove glucose from the blood, the reduced amount of insulin and its reduced effect cause cells to take up less glucose from the blood and the level of glucose in the blood to rise. Pioglitazone often is referred to as an "insulin sensitizer" because it attaches to the insulin receptors on cells throughout the body and causes the cells to become more sensitive (more responsive) to insulin. As a result, more glucose is removed from the blood, and the level of glucose in the blood falls. At least some insulin must be produced by the pancreas in order for pioglitazone to work. Pioglitazone also lowers the level of glucose in the blood by reducing the production and secretion of glucose into the blood by the liver. In addition, pioglitazone may alter the blood concentrations of lipids (fats) in the blood. Specifically, it decreases and increases the "good" (HDL).

Acarbose

Acarbose inhibits enzymes, specifically, enzymes in the brush border of the small intestines and pancreatic alpha-amylase hydrolyzes complex starches to in the lumen of the small intestine, whereas the membrane-bound intestinal alpha-glucosidase in the small intestine. Inhibition of these enzyme systems reduces the rate of digestion of complex carbohydrates. Less glucose is absorbed because the carbohydrates are not broken down into glucose molecule[47,48,49].

Table - 6 Actions of antidiabetic drugs and side effect profile

Medication	Action	Advantages	Side effects
Meglitinides repaglinide(prandin) nateglinid(starlix)	Stimulate the release of insulin	Works quickly	Hypoglycaemia, weight gain, nausea, back pain, head ache
Biguanides Metformin(Fortamet,	Inhibit the release of glucose from the	May promote modest weight loss	Well tolerated weight loss, nausea,

Glucophage)	liver;improve sensitivity to insulin	and modest decline in LDL cholesterol	diarrhoea, rarely lactic acidosis
Thiazolidinediones Rosiglitazone(avandia) piogltazone (actos)	improve sensitivity to insulin; Inhibit the release of glucose from the liver	May slightly increase the HDL level	Heart failure, heart attack, stroke, 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

1.12 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 indispensible to man, for his life. A nest of other useful products are supplied to him by the plant kingdom. Nature has provides a complete range of remedies to came an ailments of mankind. The knowledge of drugs has accumulated thoughts of years of a result of meaning inquisitive nature so that today we possess many affective of causing health care. Archaeological evidence indicates that the use of medical plants data of least the paleotic, approximately 60,000 years age. In India, medicinal plants are widely used in traditional systems of medicine like Ayurvedic, Unani, Siddha and Homeopathy. India with it's valuable resources of natural flora has always been one of the richest sources of medicinal plants in the world[50].

Importance of herbal drugs

Antidiabetic allopathic drugs have their own side effect & adverse events like hypoglycaemia, nausea, vomiting, hyponatremia, flatulence, diarrhoea or constipation, alcohol flush, headache, weight gain, lactic acidosis, pernicious anaemia, 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 benefits patients with higher base line HbA1c value, warranting further research[50].

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 World Health Organization (WHO) there are about 21,000 plants, which are used for medicinal purposes around the world. Among these 2500 species are found in India. India is called as 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 and 343 plants from different parts of the world. Some plants like *Allium cepa* (Onion,piyaj), *Allium sativum* (garlic, lasun), *Syzygium cumini* (Syn.*Eugenia jambolana*; (black plum;jamun), *Momordica charantia* (bitter gourd; karela) *Gymema sylvestre* (Gurmar), *Pterocarpus marsupium* etc are well noticed by scientists as well as laymen, in recent years[51].

Biological actions of the plant products used against diabetes are related to their phyto chemistry. Herbal products or plant products are rich in phenolic compounds, flavonoids, terpenoids, coumarins, and other constituents which reduces the blood glucose levels.

Our Vedic literatures like Charak Samhita already report the use of herbs and herbal derivatives for treatment of diabetes mellitus. According to Charak Samhitsa more than 400 plants are used in 700 recipes which are used to treat diabetes mellitus in almost two thirds of the world population. A large number of in vivo studies have been conducted on animals to test the claimed activity have demonstrated the hypoglycaemic property of many plants, already reported in various literatures. The plant families, including the species most studied for their confirmed hypoglycemic effects include, Leguminoseae, Lamiaceae, Liliaceae, Cucurbitaceae, Asteraceae, Moraceae, Rosaceae, Euphorbiaceae and Araliaceae[51].

PLANT PROFILE

Scientific classification

Kingdom: Plantae

Division: Magnoliophyta

Class: Magnoliopsida

Order: VioleS

Family: Moringaceae

Genus: *Moringa*

Species: *Pterygosperma*

Different species in the genus of moringa family

Moringa pterygosperma, Moringa oleifera, Moringa arborea, Moringa borziana, Moringa concanensis, Moringa drouhadii, Moringa hildebrandtii, Moringa longituba, Moringa ovalifolia, Moringa peregrine, Moringa pygmaea, Moringa rivae, Moringa ruspoliana, Moringa stenopetala

Vernacular names of *Moringa pterygosperma* gaertn

English: Drumstick tree, horseradish tree, oil of beentree.

Hindi : Mungna, sahjan, saijna, sanjna, Shajna, Soanjana, Soajna, Sohajna.

Malayalam: Moringa, Murinna, Sigru.

Tamil : Moringa, murungai.

Description

A small or medium-sized tree up to 10 m tall, with thick, soft, corky, deeply fisured bark and tomentose twigs.

Roots: Acrid, bitter, pungent, thermogenic

Leaves: Usually tripinnate, 45 cm long; pinnate and pinnules opposite, deciduous; leaflets 1.2-2 cm long and 0.6-1 cm. wide. The lateral elliptic, the terminal obvates.

Flowers: White, fragrant, in large panicles.

Fruits: (Pods) Pendulous, green, 22-50 cm or more in length, triangular, 9-ribbed.

Seeds: Trigonous, the wings angled. Flowers and fruits once or twice each year, dpending on locality; in central India, where trees remain leafless between December-January and January-February, flowering occurs mainly between November and March, and fruiting from February to June.

Distribution

Moringa is native to the Himalayan foothills. As a commercial crop, it is cultivated extensively in India and Africa. Moringa is most commonly found in area with South and

Southeast Asia population. Today it is widely cultivated in Africa, Sri Lanka, India, Mexico, Malaysia. It is one of the most useful tree, every parts of Moringa can be use for various purpose.

Parts used

Leaves

Uses

External sores/ulcers, Malaria/Fever, Anti-hypertensive, Diabetes mellitus, Colitis, Gastritis/ulcers, Syphilis, Flu ,Asthma, Heart burn, Skin disease, Stress, Lactation enhancer, Anti-septic, Bronchiasis[52]

Reported activities

Anti-inflammatory, local anaesthetic[53], nephroprotective activity[54], hepatoprotective effect[55],cardiovascular effect[56].

Chemical constituents

Carotene, nicotinic acid ,ascorbic acid, oxidase sulphur, prolamin and essential amino acids, vitamine A ,B and C, calcium, iron, alpha-tocopherol.

Fig; 5 *Moringa pterygosperma* plant

REVIEW OF LITERATURE

Ibrahim *et al.*,(2019) evaluated the antidiabetic and haematological effects of *Chrysophyllum albidum* supplemented diet on streptozotocin induced diabetic rats. Diabetes mellitus was induced by a single intraperitoneal injection of 50 mg/kg streptozotocin (STZ) and 70g/kg CAFS supplemented diet was used on STZ-induced diabetic rats to test its antidiabetic efficacy with some biochemical parameters and histological evaluation of liver and pancreatic tissues for a treatment period of twenty-eight days. CAFS was significantly ($p<0.05$) effective in inhibiting hyperglycemia by 68%, decreased glycosylated haemoglobin by 20%, plasma and liver lipid profile except HDL-c and white blood cell count but increased the body weight by 17%, insulin, hepatic glycogen and red blood cell levels in comparison with diabetic untreated group. This study has indicated that CAFS possesses antihyperglycemic, antihyperlipidemic and ameliorative effect on diabetic induced abnormalities in haematological parameters, β-cell and liver tissue. The findings suggest that CAFS may be used as therapeutic adjunct in the management of diabetes[57].

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[58].

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[59].

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[60].

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 *Sprague-Dawley* (SD) rats. Forty healthy male SD rats were induced to diabetes with a single intra-peritoneal 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 (ANOVA)[61].

Anoop *et al*.,(2016) evaluated the effect of *Ipomoea staphylina* leaves on Streptozotocin- Nicotinamide Induced Type-II Diabetes in *Wistar* Rats**.** The aim of the present study was to evaluate the antidiabetic activity of Ipomoea staphylina (IS) leaves in streptozotocin (STZ)-nicotinamide induced type-II diabetic in rats. Oral administration of ethanolic extract of IS leaves and its fractions at the doses of 100 mg/kg and 200 mg/kg was studied in glucose-loaded and STZ-nicotinamide induced diabetic rats. The IS extract and its fractions significantly reduced the blood glucose level in glucose-loaded rats. After treatment with IS extract and its fractions (100 and 200 mg/kg) for 21 days there was a significant decrease in blood glucose, total cholesterol, triglycerides, LDL-C, VLDL-C, plasma enzymes (SGOT, SGPT and ALP), serum urea, creatinine and significant increase in body weight and total protein levels was observed in treated diabetic rats. The activities of antioxidant enzymes SOD, CAT and GPx were also increased in diabetic mice after the treatment with IS extract and its fractions. Histological analysis showed improvement in the cellular architecture of pancreas, liver and kidney[62].

Sushma *et al*.,(2016) investigated the antidiabetic activity of methanolic extract of *Nepeta hindostana* herb in streptozotocin induced diabetes in rats. The present work was designed to evaluate the antidiabetic effect of *Nepeta hindostana* methanolic extract in streptozotocin-induced diabetes in rats. *Wistar* rats were divided into different groups and glibenclamide (2.5 mg/kg), and *Nepeta hindostana* methanolic extract (100, 200 and 400 mg/kg) treatments were given orally, for 28 d. *Nepeta hindostana* methanolic extract 200 and 400 mg/kg dose group have a significant change in weight as compared to diabetic control. All the extract treated groups showed a significant reduction in glucose level on 14th and 28th day as compared to diabetic control. The level of serum total cholesterol and triglyceride level were significantly reduced, and HDL level significantly increased in 400 mg/kg groups

after 28 d treatment. However, a significant increase in Hb concentration was observed in diabetic rats treated with 200 and 400 mg/kg, when compared to diabetic control. The microscopy of the pancreas showed both glibenclamide and 400mg/kg extract does appear to regulate diabetes at the cellular level resulting in restoration of near normal architecture pancreatic islets of langerhans and hepatocytes. It can be concluded that *Nepeta hindostana* methanolic extract exhibit significant antidiabetic activity against streptozotocin-induced diabetes model[63].

Dorin *et al*.,(2015) reported a review on the models to study *in vitro* antidiabetic activity of plants. Antidiabetic effect of plants and their active principles can be assessed *in vitro* using a variety of biological test systems. They play a major role in evaluation of antidiabetic properties as an initial screening tool prior to *in vivo* studies. The present review focuses on *in vitro* assays that are available to study potential antidiabetic activity of plant extracts and their active constituents. *In vitro* assay provides a basic platform for accusing these plant extracts and help us understand various mechanisms that would alleviate hyperglycaemia in diabetes. These assays provide information of various *in vitro* studies used in antidiabetic assessment, which can establish a mechanism for the antidiabetic activity of drug. Besides, intensive studies of the mechanism of action of the known drugs have provided further validation of several new molecular drug targets[33].

Agung *et al*.,(2015) reported that the antidiabetic and antioxidant activity of jackfruit (*Artocarpus heterophyllus*) extract. The present study was aimed to evaluate antidiabetic and antioxidant activity of aqueous extract of Jackfruit. The antidiabetic activity were determined by inhibition of haemoglobin glycation method. Phytochemical constituent like ascorbic acid, β-carotene and lycopene also determined. Antioxidant activity was measured by hydroxl radical and hydrogen peroxide scavenging activity, and chelating effect of ferrous iron. From the result of this study we can see the increasing of haemoglobin glycation concentration is followed by the increasing of jackfruit extracts concentration. The result of this study also showed that the extract of jackfruit has a phytochemical constituent with ascorbic acid is the highest, and followed by β-carotene and lycopene. Jackfruit also has antioxidant activity. The highest antioxidant activity is scavenging hydroxyl radical activity and followed by scavenging hydrogen peroxide and chelating of ferrous iron. The result of this study suggest that the jackfruit extract potential as an diabetic agent[64].

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[65].

Faizal *et al.*,(2014) studied the health benefits of *Moringa oleifera. Moringa oleifera* is a multi-purpose herbal plant used as human food and an alternative for medicinal purposes worldwide. It has been identified by researchers as a plant with numerous health benefits including nutritional and medicinal advantages. *Moringa oleifera* contains essential amino acids, carotenoids in leaves and components with nutraceutical properties, supporting the idea of using this plant as a nutritional supplement or constituent in food preparation. Some nutritional evaluation has been carried out in leaves and stems. An important factor that accounts for the medicinal uses of *Moringa oleifera* is its very wide range of vital antioxidants, antibiotic and nutrients includes vitamins and minerals. Almost all parts from moringa can be used as a source for nutrition with other useful values. For instance, it views the general nutrition content of the moringa up to several specific remedial properties including its anti-fibrotic, anti-inflammatory, anti-microbial, anti-hyperglycemic, antioxidant, anti-tumour and anti-cancer properties[66].

Tripathi *et.al*.,(2014) reported the comprehensive review on the different models used to induce diabetes. Diabetes mellitus is a group of heterogeneous metabolic disorders. Around 2.8 % population suffers from diabetes throughout the world. To reduce this data, many antidiabetic drugs are used and research is going on for more effective anti-diabetic drugs. For study on diabetes, many diabetic models, chemicals and diabetogenic hormones are used at research level. In this review give an overview of models used to induce diabetes, their chemical properties and mechanism of action. Conclusively many animal models are used to induce diabetes, which further help in the study of development and screening of new anti-diabetic drugs. Animal models for type 1 diabetes range from animals with spontaneously developing autoimmune diabetic to chemical ablation of the pancreatic beta cells and type 2 diabetes is studied in both obese and non-obese animal models with varying degrees of insulin resistance and beta cell failure. Large number of new genetically modified animals, chemical agents, surgical manipulations, viruses and diabetogenic hormones have been engineered for the study of diabetes[67].

Rashid *et al.*,(2014) reported a review on medicinal plants with antidiabetic activity**.** In the last few years, there has been an exponential growth in the field of herbal medicine and gaining popularity both in developing and developed countries because of their natural origin and less side effects. A comprehensive review was conducted to pile up information about medicinal plants used for the treatment of diabetes mellitus. It is a metabolic disorder of the endocrine system and affecting nearly 10% of the population all over the world also the number of those affected is increasing day by day. The profiles presented include information about the scientific and family name, plant parts and test model used, the degree of hypoglycemic activity, and the active chemical agents. The large number of plants described in this review (108 plant species belonging to 56 families) clearly demonstrated the importance of herbal plants in the treatment of diabetes. The effects of these plants may delay the development of diabetic complications and correct the metabolic abnormalities. This work stimulates the researchers for further research on the potential use of medicinal plants having antidiabetic potential[68].

Sampath *et al*.,(2014) investigated the *in vitro* antidiabetic, antioxidant and anti-inflammatory activity of *clitoria ternatea* L. Phytochemicals of leaves and flowers were analysed by using standard methods. *In vitro* antioxidant studies were carried out for the

ethanolic extract of the *Clitoria ternatea* leaves and flowers using various free radical models such a DPPH, reducing power assay and hydrogen peroxide scavenging assay. *In vitro* antidiabetic assay such as non-enzymatic glycosylation of haemoglobin assay, Glucose uptake in Yeast cells and Inhibition of salivary-amylase enzyme were carried in ethanolic extract. Preliminary phytochemical screening of ethanolic extract of the *Clitoria ternatea* revealed the presence of various bioactive components like alkaloids, flavonoids, steroids, glycosides, phenol, saponin, terpenoids and tannin in both leaves and flowers. Anthroquinone is absent in both the parts studied. The *in vitro* antidiabetic potential of plant extract was confirmed through non enzymatic glycation, glucose uptake by yeast cells and amylase inhibition methods. The result of the present study concluded that the ethanolic extracts of *Clitoria ternaea L.* leaves and flowers possess significant antidiabetic, antioxidant and anti-inflammatory activity. The potential pharmacological activity of *Clitoria ternaea L.* leaves and flowers might be due to the presence of phytochemicals[69].

Pinal *et al.*,(2014) reported the phytochemical analysis and antifungal activity of *Moringa oleifera*. The aim of the present study was to carried out phytochemical analysis of aqueous and ethanolic extract of *Moringa oleifera* and to find out antifungal property. The leaf extracts was used for plant component analysis and for determination of antifungal activity. *Saccharomyces cerevisiae, Candida albicans, Candida tropicalis* strain were used for experimental purpose. Well diffusion method was used to assess the antifungal effect of the extracts on micro-organisms. The phytochemical screening indicated the presence of flavonoids, tannins, steroid, alkaloid, saponins etc, in the both extracts. Antifungal activity of ethanolic and aqueous extract of *Moringa oleifera* leaf was highly active against *Saccharomyces cerevisiae* and active against *Candida tropicalis* and not showing activity against *Candida albicans*. The present study conclusively demonstrates that *Moringa oleifera* is a good source of various phytochemicals like alkaloids, flavonoids, carbohydrates, glycosides, saponins, tannins, terpenoids. The antifungal activity *Moringa oleifera* was clearly shown by the present study against various fungi like *Saccharomyces cerevisiae*, *Candida albicans* and *Candida tropicalis*. All these preliminary reports warrant an in depth analysis of the usefulness of *Moringa oleifera* as miracle drug against various ailments[70].

Asok *et al.*,(2013) reported the nephroprotective activity of ethanolic leaf extract of *Moringa pterygosperma* on paracetamol induced nephrotoxic rats. *Moringa pterygosperma*

was found to be an effective herbal medicine in the animal models of renal failure. From our pharmacological studies we demonstrated the renal protectant activity of *Moringa pterygosperma*, its toxic effects and undesired properties are none or minimal. The use of Moringa for treating further kidney and its associated diseases may be performed on the basis of our pharmacological investigations. The rat species are widely used for most of the pharmacological studies and can be compared with human diseased models. The efficacy of Moringa for curing or alleviating chronic renal failure (CRF) may be a light for developing a potential herbal medicine for the future. So, it needs further detailed pharmacological and clinical investigations to prove it as an effective therapeutic agent for renal failure in humans. The study carried out by using five group of rats. Furosemide was taken as standard drug. The parameters estimated are RBC content, haemoglobin content, urea, creatinine level. The extract showed nephro-protective activity by significantly reducing the levels of blood urea, serum creatinine, increasing the red blood cell count and haemoglobin content[71].

Uma Makheswari *et al*,.(2012) reported a database on antidiabetic indigenous plants of tamil nadhu, India. Diabetes mellitus is a chronic disease that requires long-term medical attention. Since ancient times, plants have been an exemplary source of medicine for Diabetes. Phytomedicine, in addition to their traditional values, also act as novel lead compounds for drug development. Hence the world is now moving towards the herbal medicine or phytomedicines that tend to cure diseases without any toxic side effects. The indigenous knowledge of plants used for the treatment of diabetics was collected through questionnaire and personal interviews. A total of 46 plants used to treat diabetes have been documented. The investigation revealed that, leaf materials (37%) followed by seeds (16%) and fruits (14%) were mostly used for the treatment of Diabetes. Anti-diabetic medicinal plants used by Tamil People have been listed along with plant parts used and its active chemical constituents[72].

Chandrashekar *et al*.,(2012) evaluated the *in-vitro* antidiabetic activity of stem bark of *Bauhinia purpurea* Linn. The objective of this work was to evaluate the antidiabetic activity of petroleum ether and aqueous extract of stem bark of *Bauhinia purpurea* L. The samples were studied for their effect on inhibition of glycosylation of haemoglobin, glucose transport across yeast cells and α- Amylase inhibition. Inhibition of glycosylation of haemoglobin and α-Amylase inhibition was in a dose dependent manner and glucose

transport differs with the sample and glucose concentration. From the results of the study, it is inferred that, *B. purpurea* stem bark possesses antidiabetic activity. However, these effects need to be confirmed using *in vivo* models and clinical trials for its effective utilization as therapeutic agents[73].

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[74].

Rajeeb *et al.*,(2012) reported a review on drumstick Tree (*Moringa pterygosperma* Gaertn): multiuse tree with higher economical values. The nature has provided a complete storehouse of remedies to cure all ailments of mankind. Since the dawn of civilization, in addition to food crops, man cultivated herbs for his medicinal needs. The knowledge of drugs has accumulated over thousands of years as a result of man's inquisitive nature, so that today we possess many effective means of ensuring health-care. *Moringa pterygosperma* Gaertn grown and used in many countries around the world is a multiuse tree with medicinal, nutritional and socio-economic values. In Senegal and Benin, *Moringa pterygosperma* Gaertn is dispensed as powder at health facilities to treat moderate malnutrition in children. It established the medicinal uses of *Moringa pterygosperma* Gaertn by local communities. The plant kingdom represent a rich storehouse of traditional medicines, folk medicines and organic compound that may lead to development of novel agent for various treatment. *Moringa pterygosperma* Gaertn commonly known by regional name such as horse radish tree, sajiwan, kelor murungai kaai, saijhan and sajna, is a natural as well as cultivated variety of the genus Moringa belonging to the family Moringaceae[75].

Gupta *et al.*,(2011) reported the antidiabetic efficacy of *Mangifera indica* seed kernels in rats: A comparative study with glibenclamide. The present study was conducted to examine the hypoglycemic potency of seed kernels of *Mangifera indica* ethanol extract (MIEtE) in streptozotocin diabetic rats. Remarkable abnormalities were observed in serum and tissue parameters in hyperglycemic rats after streptozotocin administration. Administration of MIEtE, 300 mg/kg b.w./day for 14 and 21 days resulted in their normalization. Data were in parallel analyzed with a standard drug, glibenclamide, to compare plant drug efficacy. Streptozotocin administration causes reduction in the number of β-cells and induces hyperglycemia. In our study, MIEtE was observed to decrease serum glucose level and increase serum insulin concentrationin treated rats. The possible mechanism by which MIEtE exerts its hypoglycemic action may be through potentiating the plasma insulin effect by increasing either pancreatic secretion of insulin from regenerated[76].

Lobo *et al.*,(2009) determined the *In vitro* Antioxidant Activity of *Moringa pterygosperma* (Gaertn) leaves. The human body produces reactive oxygen species (ROS) as a result of normal metabolic process. These ROS are capable of oxidizing biomolecules that can damage DNA, cells and contribute to chronic disease. In treatment of these diseases, antioxidant therapy is gained an utmost importance. The important role of dietary antioxidants maintaining the integrity of the living organisms is gaining ever increasing recognition. The oxidative stress induced due to ROS can be attenuated or perhaps reversed by diets containing vegetables that have an ability to scavenge reactive oxygen species. *Moringa pterygosperma* (Gaertn) leaves has been used in Indian cooking and herbal remedies. Its possible mechanism of action was examined in terms of antioxidant availability. AEMP has shown higher antioxidant activity as compared to EEMP in DPPH radical scavenging assay with IC50 values 3649.63±1.81 in AEMP and 3048.78±1.23 in EEMP respectively. Like antioxidant activity the reducing power and FRAP values of AEMP are better as compared to EEMP. The amounts of total phenolic and flavonoid content were also determined. In conclusion, the studies reveal that *Moringa pterygosperma* Gaertn. can scavenge radicals and reduce iron complex may explain the possible mechanism by which it exhibits beneficial effects[77].

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[53].

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[34].

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[78].

AIM AND OBJECTIVE OF THE STUDY

AIM:

The aim of the present work was to evaluate the antidiabetic activity of *Moringa pterygosperma* Gaertn against streptozotocin and nicotinamide - induced type 2 diabetes in rats

OBJECTIVE OF THE STUDY

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 defence system.

According to WHO, it is estimated that 3% of the world's population have diabetes and the prevalence is expected to double by the year 2025 to 6.3%. There will be a 42% increase from 51 to 72 million in the developed countries and 170% increase from 84 to 228 million in the developing countries. Thus by the year 2025 over 75% of all people with diabetes will be in the developing countries as compared to 62% in 1995. The reason behind this projected increase in prevalence rate are due to westernization and their associated life style changes, increase in life expectancy at birth, physical inactivity, obesity and possibly a genetic predisposition. India has today become the diabetic capital of the world with over 20million diabetics and this number is set to increase to 57 million by 2025. This astronomic increase in the prevalence of diabetes has made this disease a major public health challenge for India.

Eventhough there are many allopathic antidiabetic 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 effective and safe for the treatment of diabetes. Based on the WHO recommendation hypoglycaemic agents of plant origin used in traditional practice are approved for the treatment of diabetes. Herbal formulations are

frequently considered to be less toxic and more effective and also free from side effects than synthetic ones.

Moringa pterygosperma plant is one of the traditional herbal medicine reported to possess anti-inflammatory, local anaesthetic[54], nephroprotective activity[55], hepatoprotective effect[56], cardiovascular effect[57]. The objective of this study is to investigate the leaves of *Moringa pterygosperma* for its *in vitro* and *in vivo* antidiabetic activity.

PLAN OF WORK

STEP 1

Review of literature.

STEP 2

Collection and authentication of leaves of *Moringa pterygosperma*.

STEP 3

Aqueous and ethylacetate extraction of the *Moringa pterygosperma* leaves.

STEP 4

In vitro inhibition of α-amylase and non enzymatic glycosylation of haemoglobin.

STEP 5

In vivo screening of the *Moringa pterygosperma* extract against streptozotocin and nicotinamide - induced antidiabetic activity in rats.

STEP 6

Histopathological studies of pancreas.

STEP 7

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

MATERIALS AND METHODS

Chemicals and reagents used

Ethylacetate, starch, sodium potassium tartrate solution, 3,5-Dinitrosalicylic acid, Glucose, gentamycin, alpha tocopherol, 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. Total cholesterol, triglycerides, HDL, LDL, alanine transaminase, aspartate transaminase, alkaline phosphatase, serum urea, creatinine and uric acid kits were procured from agape diagnostic Ltd, Kerala.

Instruments used

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

PLANT MATERIAL

Collection and authentification

The leaves of *Moringa pterygosperma* 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/5/23/2018/Tech/1215 dated 23-07-2018.

Preparation of the extract

The leaves were rinsed under running tap and air dried at room temperature with adequate ventilation and pulverized with a blender. The pulverized samples were extracted with ethyl acetate and distilled water, 160 g of leaves is extracted using soxhlet apparatus by adding 650ml ethyl acetate into it and 50 g of leaves is taken and add water and shake every 30 min for 12hours. Both extracts are filtered using a muslin cloth. The filtrate was evaporated using a rotary evaporator and concentrated further using a water bath. The extract

was collected, weighed and stored in a sterile air tight container and kept in the refrigerator until required for use.[79,80]

Phytochemical screening

Chemical tests were carried out using the extract of *Moringa pterygosperma* for the presence of phytochemical constituents[81,82]

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 colouration

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 H_2SO_4 was added. A yellow colouration indicates the presence of flavonoids. The yellow coloration disappeared on standing
- Few drops of 1% $AlCl_3$ 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.

***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 colouring 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 coloured 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 540nm[64,83].

$$\text{Inhibition activity\%} = \text{Abs(control)} - \text{Abs(extract)} \div \text{Abs(control)} \times 100$$

Non enzymatic glycosylation of haemoglobin (HbA_1C)

Glycosylated haemoglobin is a blood test to determine level of glycemic control. Blood glucose binds to haemoglobin through a process called glycosylation. The higher the blood sugar the more glucose binds to haemoglobin. HbA_1C is the product of the glycosylation of glucose with the N-terminal residue of the B-chain of haemoglobin.

Procedure

Blood from healthy volunteers is placed into a bottle containing anticoagulant. Then blood is mixed with sodium chloride and one volume of carbon tetrachloride for the

preparation of hemosylate. The resulting hemosylate may contain debris, which is removed by centrifugation at higher rpm for 15minutes at room temperature.

Glucose (2%), haemoglobin(0.06%) and gentamycin(0.02%) solutions were prepared in phosphate buffer 0.01M, pH 7.4. 1ml each of above solution was mixed. Different concentration of plant extract was prepared. About 1ml of each concentration was added to above mixture. Mixture was incubated in dark at room temperature for 72hrs. The degree of glycosylation of haemoglobin was measured colorimetrically at 520nm. Alpha tocopherol (trolex) was used as a standard drug for assay[78].

$$\%\text{inhibition} = \text{Abs(control)} - \text{Abs(extract)} \div \text{Abs(control)} \times 100$$

EXPERIMENTAL ANIMALS

Female *Wistar* rats weighing 180-200 g were used for acute toxicity studies. Male *Wistar* rats weighing 150-200 g were used for streptozotocin and nicotinamide - induced diabetes activity. The animals were kept in polypropylene cages under ambient temperature (22 ± 3 ^{O}C) with 12 h light/dark cycle. Animals were provided with standard pellet feed and drinking water *ad libitum.* All animals procedures were performed in accordance with the recommendation for the proper care and use of laboratory animals.

Acute toxicity study

Acute oral toxicity testing was carried out in accordance with the OECD guidelines 420 Acute Oral Toxicity – Fixed Dose Procedure method (OECD, 2002).

Procedure

The acute toxicity study was done by two steps – sighting study and main study. The purpose of the sighting study is to allow selection of the appropriate starting dose for the main study. Healthy adult female (generally slightly more sensitive than male) *wistar* rats weighing between 180-200 g body weight was procured and kept in cages under ambient temperature (22 ± 3 ^{O}C) with 12 hr light/dark cycle. The animals were randomly selected, marked and kept in their cages for 7 days prior to dosing for acclimatization to laboratory conditions. The animals were fasted over night and were provided with water *ad libitum*. The test compounds were suspended in 0.5% CMC. Totally 18 animals were used for this study. Sighting study was conducted for a compound at dose levels 5, 50, 300 and 2000 mg/kg body weight. All animals survived without any toxic manifestations during the sighting study and

the same dose was selected for the main study. The main study was conducted for a compound at dose of 2000 mg/kg body weight using 5 animals. After the administration of the compounds, food was withheld for further 3-4 hours.

Animals were observed individually at least once during the first 30 min after dosing, periodically during the first 24 h (with special attention during the first 4 h) and daily thereafter for a period of 14 days. Once daily cage side observations included changes in skin and fur, eyes and mucous membrane (nasal), and also respiratory rate, circulatory (heart rate and blood pressure), autonomic (salivation, lacrimation, perspiration, piloerection, urinary incontinence and defecation) and central nervous system (drowsiness, gait, tremors and convulsions) changes.

Experimental induction of diabetes

Diabetes was induced by intraperitoneal injection of streptozotocin (55 mg/kg) dissolved in 0.1 M cold sodium citrate buffer (pH 4.5) in overnight fasting rats. 15min before giving the streptozotocin giving nicotinamide 120 mg/kg. The control rats received vehicle alone, and animals were allowed to drink 5% glucose solution overnight to overcome the drug-induced hypoglycemia. After 1 week time, the rats with moderate diabetes having glycosuria and hyperglycemia (blood glucose range of above 250 mg/dl) were considered as diabetic rats and used for the experiment[62].

Experimental design

The rats were divided into 6 groups, consisting 6 animals in group 1 and 6 and 8 animals in other groups [62].

Group 1: Control rats, received saline 10 ml/kg

Group 2: Diabetic control rats, received Streptozotocin 55 mg/kg+Nicotinamide 120mg/kg i.p

Group 3: Diabetic rats received 2.5 mg/kg, Glibenclamide p. o.

Group 4: Diabetic rats, received 100 mg/kg, *Moringa pterygosperma* p. o.

Group 5: Diabetic rats, received *Moringa pterygosperma* 200 mg/kg, p. o.

Group 6: Non diabetic rats administered *Moringa pterygosperma* 200 mg/kg

Glibenclamide (2.5 mg/kg) used as standard drug. All the test drugs were administered orally and treatment was continued for 28 days.

Sample collection

Blood samples were collected from tip of rat tail and blood glucose levels were estimated using glucocheck electronic glucometer weekly basis (0, 7, 14, 21and 28days) and body weight measured all days.

Estimation

On 29th day of experiment blood was collected from the retro-orbital plexus using ketamine/xylazine anaesthesia using capillary tubes in fresh vials containing EDTA and serum was separated. Serum analyzed for as alanine transaminase, aspartate transaminase, alkaline phosphatase, total cholesterol, triglycerides, high density lipoprotein, low density lipoprotein, VLDL, urea, creatinine, uric acid using standard commercial diagnostic kits. And the all the values were tabulated. Rats were sacrificed by cervical dislocation under ketamine/xylazine anaesthesia on day 29 and tissues such as liver, kidney and pancreas were removed and used for the preparation of homogenates.

Tissue processing of liver and pancreas

The tissue was removed and washed with ice-cold saline to remove as much as blood possible. Liver, kidney and pancreas were homogenated (5%w/v) in cold potassium phosphate buffer (50Mm, Ph 7.4) using a Remi homogenizer. The unbroken cell and cell debris were removed by centrifugation at 3000rpm for 10min. The obtained supernatant was used for the estimation of total protein, malondialdehyde, superoxide dismutase, catalase, glutathione reductase, glutathione peroxidise and reduced glutathione.

Estimation of biochemical parameters

Determination of serum glutamic pyruvic transaminase/alanine transaminase (SGPT/ALT) activity

Serum SGPT activity was determined according to the method of Thefeld *et al* (1994)

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.

L-Alanine α-Ketoglutarate $\xrightarrow{ALT}$ L-Glutamate + Pyruvate LDH

Pyruvate + NADH + H^+ $\xrightarrow{MDH}$ Lactate + NAD^+

Procedure

The working reagent was prepared by mixing 4 volume of Reagent 1 [Tris buffer (110 mmol/L, pH 7.5), L-Alanine (660mmol/L), LDH (1500 U/L)] with 1 volume of Reagent 2 [α-Ketoglutarate (16mmol/L, NADH (0.24mmol/L)] provided in the kit. About 100µL of serum was added to 1000µL of the working reagent. Mixed well and incubated for 1 minute at 37 ^{O}C. The change in absorbance was measured per minute for 3 minutes at 340 nm and the SGPT activity was expressed in U/L[84].

Determination of serum glutamic oxaloacetic transaminase/aspartate transaminase (SGOT/AST) activity

Serum SGOT activity was determined according to the method of Thefeld *et al.*,(1994)

Principle

SGOT catalyzes the transfer of amino group between L-Aspartate and α-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 decrease in absorbance which is proportional to the SGOT activity in the sample.

L-Aspartate + α-Ketoglutarate $\xrightarrow{AST}$ Oxaloacetate + L-Glutamate

Oxaloacetate + NADH + H^+ $\xrightarrow{MDH}$ Malate + NAD^+

Procedure

The working reagent was prepared by mixing 4 volume of Reagent 1 [Tris buffer (88 mmol/L, pH 7.8), L-Aspartate (260mmol/L), LDH (1500 U/L), MDH (900 U/L)] with 1 volume of Reagent 2 [α- Ketoglutarate (12mmol/L, NADH (0.24mmol/L)] provided in the kit. About 100µL of serum was added to 1000µL of the working reagent. Mixed well and incubated for 1 minute at 37 ^{O}C. The change in absorbance was measured per minute for 3 minutes at 340 nm and the SGOT activity was expressed in U/L[84].

Determination of serum alkaline phosphatase (ALP) activity

Serum ALP activity was determined according to the method of Rosalki *et al*.,(1993)

Principle

In the presence of magnesium, p-Nitrophenyl phosphate is hydrolyzed by phosphatases to form phosphate and p-Nitrophenol. The release of this coloured p-Nitrophenol is proportional to the ALP activity and can be measured photometrically at 403 nm(85).

p-Nitrophenyl phosphate + H_2O $\xrightarrow{ALP}$ phosphate + p-Nitrophenol.

Procedure

The working reagent was prepared by mixing 4 volume of Reagent1 [Diethanolamine buffer (125mmol/L, pH 10.2), Magnesium Chloride (0.625mmol/L)] with 1 volume of Reagent 2 [p-Nitrophenyl phosphate (50mmol/L)] provided in the kit. About 20µL of serum was added to 1000µL of the working reagent. Mixed well and incubated for 1 minute at 37^{O}C. The change in absorbance was measured per minute for 3 minutes at 405 nm and the ALP activity was expressed in U/L[84].

Determination of triglycerides

Serum TG was determined according to the method of Schettler and Nussel.,(1975).

Principle

Enzymatic colorimetric determination of triglycerides according to the following reactions.

Triglyceride + H_2O $\xrightarrow{\text{Lipoprotein lipase}}$ Glycerol + Fatty acids

Glycerol + ATP $\xrightarrow{\text{Glycerol kinase}}$ Glycerol-3- phosphate + ADP

Glycerol-3-PO_4 + $2O_2$ $\xrightarrow{\text{Glycerol-3- phosphate oxidase}}$ Dihydroxy acetone phosphate+ H_2O_2

$2H_2O_2$ + 4- Aminoantipyrine + p- Chlorophenol $\xrightarrow{\text{Peroxidase}}$ Violet quinone + $4H_2O$

Procedure

About10 µL of serum was added to 1000 µL of working reagent [Pipes buffer, pH 7.0- 50 mmol/L, p-Chlorophenol- 5.3 mmol/L, Potassium ferrocyanate- 10 mmol/L, Magnesium salt- 17 mmol/L, 4-Aminoantipyrine- 0.9 mmol/L, ATP- 3.15 mmol/L, Lipoprotein lipase >1800 U/L, Glycerol kinase > 450 U/L, Glycerol-3- phosphate oxidase > 3500 U/L, Peroxidase > 450U/L] provided in the kit. About 10 µL of the triglyceride standard (200 mg/dL) was also added to 1000 µL of the working reagent taken in another tube. Mixed well and incubated for 5 minute at 37 ^{O}C. Absorbance of both the sample and the standard was measured at 630 nm against reagent blank. The triglycerides concentration was expressed in mg/dL[86].

Determination of total cholesterol

Serum TC was determined according to the method of Zlatkis *et al*.(1953).

Principle

Enzymatic colorimetric determination of total cholesterol according to the following reactions.

Cholesterol ester + H_2O $\xrightarrow{\text{Cholesterol esterase}}$ Cholesterol + Fatty acids

Cholesterol + O_2 $\xrightarrow{\text{Cholesterol Oxidase}}$ 4- Cholesten- 3- one + H_2O_2

$2H_2O_2$ + Phenol + 4- Aminoantipyrene $\xrightarrow{\text{Peroxidase}}$ Red quinone + $4H_2O$

Procedure

About 10µL of serum was added to 1000 µL of cholesterol reagent [Pipes buffer- (pH 6.7)50 mmol/L,Phenol-24 mmol/L,Sodiumcholate-0.5 mmol/L,4-aminoantipyrene-0.5 mmol/L, Cholesterol esterase >180 U/L, Cholesterol oxidase > 20 U/L, Peroxidase > 1000 U/L] provided in the kit. 10 µL of the cholesterol standard (200 mg/dl) was also added to

1000 µL of the cholesterol reagent taken in another tube. Mixed well and incubated for 5 minute at 37 ^{O}C. Absorbance of both the sample and the standard was measured at 630 nm against reagent blank. The total cholesterol concentration was expressed in mg/dL[87].

Determination of high density lipoprotein (HDL)

Serum HDL level was determined according to the method of Assmann.,(1979).

Principle

The reaction between cholesterol other than HDL and the enzyme for cholesterol assay is suppressed by the electrostatic interaction between polyanions and cationic substances. Hydrogen peroxide is formed by the free cholesterol in HDL by cholesterol oxidase. Oxidative condensation of EMSE and 4-Aminoantipyrin is caused by hydrogen peroxide in the presence of peroxidise, and the absorbance of the resulting red purple quinone is measured to obtain the cholesterol value in HDL.

Other lipoproteins than HDL $\xrightarrow{\text{Polyanions}}$ Suppress reaction with enzyme

HDL (cholesterol esters) + H_2O $\xrightarrow{\text{Cholesterol esterase}}$ HDL (free cholesterol) + Free fatty acidss

HDL (free cholesterol) + O_2 + H^+ $\xrightarrow{\text{Cholesterol oxidase}}$ Cholestenone + H_2O_2

$2H_2O_2$ + 4-Aminoantipyrin + EMSE + H_2 + O $\xrightarrow{\text{Peroxidase}}$ Violet quinone + $5H_2O$

Procedure

About 5 µL of serum and 5 µL of calibrator was taken in separate tubes and was mixed with 450 µL of reagent 1 [N-Ethyl-N-(3-methylphenyl)-N'-succinylethyenediamine]. The tubes were incubated for 5 minutes at 37°C. 150mµL of reagent 2 [Cholesterol oxidase, 4-Aminoantipyrin] was then added. Mixed well and the mixture was again incubated for 5 minute at 37 ^{O}C. Absorbance of both the calibrator and the sample was measured at 578 and 630 nm against reagent blank. The HDL-C concentration was expressed in mg/dL[87]..

Determination of low density lipoprotein (LDL)

Principle

This assay method uses a surfactant for selectively solubilising LDL alone in the cholesterol assay system that employs cholesterol esterase and cholesterol oxidase. It passes the ester cholesterol and free cholesterol contained in the LDL to the cholesterol reaction system to determine LDL cholesterol. The enzyme reactions to other, non-LDL lipoproteins (HDL, VLDL and chilomicrons) are inhibited by the surfactant and by the sugar compounds. These lipoproteins are therefore not passed to the cholesterol reaction system and consequently remain in the reaction liquid as lipoproteins.

Procedure

About 5µL of serum and 5µL of calibrator was taken in separate tubes and was mixed with 450µl of reagent 1 [HSDA-1 mmol/L, good's buffer]. The tubes were incubated for 5 minutes at 37°C. 150 µL of reagent 2 [Cholesterol esterase- 2.0 U/L, Cholesterol oxidase- 1.0 mmol/L, 4-Aminoantipyrin- 2.5 mmol/L and good's buffer] was then added. Mixed well and the mixture was again incubated for 5 minute at 37 ^{O}C. Absorbance of both the calibrator and the sample was measured at 578 and 630 nm against reagent blank. The LDL-C concentration was expressed in mg/dL[89].

Determination of very low density protein (VLDL)

VLDL fractions were estimated by Friedwald *et al.,* (1972) formula which states

VLDL = Triglyceride/5 and the VLDL-C concentration was expressed in mg/Dl.

Determination of urea

Serum urea was determined by using DAM method

Principle

Urea react with hot acidic Diacetylmonoxime in presence of thiosemicarbazide and produces a rose purple colored complex which is measured colorimetrically

Procedure

Dilute 1 ml of urea reagent to 5 ml with purified water. From that taken 2.5 ml of reagent and was mixed with 0.01ml of serum and 0.25 ml reagent 2 (Diacetylmonoxime DAM). Mix well and keep the tubes in the boiling water exactly for 10 minutes. Cool immediately under running water for 5minutes, mix by inversion and measure the absorbance at 535mm within 10 minutes[90].

Urea in mg/dl=absorbance of test/absorbance×30

Determination of creatinine

Serum creatinine was measured by using alkaline picrate methods

Principle

Creatinine in a protein free solution reacts with alkaline picrate and produces a red colored complex. which is measured colorimetrically.

Procedure

About 0.5ml of serum was mixed with 0.5ml purified water and 3.0ml of reagent 1(picric acid). Keep in a boiling water bath for 1 minutes. Cool immediately under running tap water and centrifuge and filter. Into that added working standard 0.5 ml and 1.5ml reagent 1 (picric acid) and 0.5 ml reagent 2 (sodium hydroxide). Mixed well and allow standing at room temperature exactly for 20minutes. Measure optical density at 520 nm[91]

Creatinine in mg/100ml=O.D test-O.D blank/O.D std-O.D blank ×3.0

Determination of uric acid

Uric acid level was measured using uricase/POD method

Principle

Uricase converts uric acid to Allenton and hydrogen peroxides. The hydrogen peroxide formed further react with a phenolic compound and aminoantipyrine by the catalytic action of peroxidise and form a red colored quinoneimine dye complex. Intensity of the color complex is directly proportional to the amount of uric acid present in sample

Procedure

About 0.5 ml of reagent buffer, 0.5ml enzyme reagent and 0.02 ml sample was mixed well and incubated for 15min. Measure the absorbance of the standard and test sample against the blank at 520nm within 30 min[92].

Estimation of tissue homogenate

Total protein

The amount of total protein in the tissue homogenate was estimated by the method of Lowry (1951) using bovine serum albumin as the standard.

Principle

Protein + Cu^{2+} ⟶ Cu-protein complex

Procedure

To 0.1mL of tissue homogenate, 4.0mL of alkaline copper solution was added and allowed to stand for 10 min. Then, 0.4 ml of phenol reagent was added very rapidly, mixed quickly and incubated in room temperature for 30 min for colour development. Reading was taken against blank prepared with distilled water at 610 nm in UV-visible spectrophotometer. The protein content was calculated from standard curve prepared with bovine serum albumin and results were expressed as µg/mg wet tissue[93].

Estimation of malondialdehyde (MDA)

The level of lipid peroxidation in serum was measured as malondialdehyde (MDA) according to the method of Niehaus and Samuelson, (1986).

Principle

- The lipid radicals readily react with molecular oxygen to produce peroxyl radicals which initiate lipid peroxidation
- The chief secondary product is MDA.
- MDA+ thiobarbituric acid to form a chromogenic adduct which is a pink colored complex

Procedure

About 0.1 mL of the tissue homogenate was combined with 2 mL of TCA-TBA-HCl reagent (1:1:1) (15% trichloro acetic acid (TCA) and 0.375% thiobarbituric acid (TBA) in 0.25 N HCl) and placed in water bath for 15 min, cooled and centrifuged at 100xg for 10 min. The precipitate was removed after cooling by centrifugation at 1000xg for 10 min. The absorbance of clear supernatant was measured against a reference blank at 535 nm. The levels of MDA were calculated using extinction coefficient calculation. The values are expressed as nmoles of MDA formed/min/mg protein[94].

Assay of catalase (CAT):

The assay of CAT was done by the method of Sinha,(1972)

Principle

Decomposition of H_2O_2 to form H_2O and O_2

$$2H_2O_2 \xrightarrow{\text{catalase}} 2H_2O+O_2$$

Procedure

The reaction mixture contained 1.0mL of 0.01 M phosphate buffer pH 7 and 0.1 mL of tissue homogenate and was incubated at 37^{O}C for 15 min. The reaction was started by the addition of 0.4mL of H_2O_2. The reaction is stopped by the addition of 2.0mL dichromate acetic acid reagent (5% potassium dichromate and glacial acetic acid are mixed in 1:3 ratio). The absorbance was measured at 620 nm. CAT activity was expressed as the amount of enzyme using the decomposition of μmoles H_2O_2/min/mg protein[95].

Assay of glutathione peroxidase (GPx)

GPx activity was measured by the procedure given by Paglia and Valentine,(1967)

Principle

Glutathione peroxidase degrades H_2O_2 in presence of glutathione there by depletion of GSH occurs.GSH remaining is measured using DTNB which gives a coloured complex

Procedure

About 0.2 mL of the heart homogenate was mixed with 0.2mL of 0.4M Tris-buffer pH 7.0, 0.1mL of 10mM sodium azide, 0.1 mL of 0.042 % H_2O_2 and 0.2 mL of 200 mM glutathione and was incubated at 37 ^{O}C for 10 min. The reaction was stopped by the addition of 0.1mL 10% trichloroacetic acid and the absorbance was measured at 340 nm. GPx activity was expressed as nmoles/min/mg protein[96].

Assay of superoxide dismutase (SOD)

The activity of SOD was determined according to the method of Kakkar,(1984)

Principle

- The ability of superoxide dismutase to inhibit auto oxidation of adrenaline is the basis of the SOD assay.
- Superoxide radicals which react with 2-(4-iodophenyl)-3-(4-nitrophenol)-5-phenyltetrazoliumchloride (INT) to form a red formazan dye.

Procedure

To 150µL of heart homogenate, 1.8mL of carbonate buffer (30 mM, pH 10.2), 0.7mL of distilled water and 400 µL of epinephrine (45 mM) were added and mixed well. The inhibition of autocatalyzed adrenochrome formation in the presence of heart tissue homogenate was measured at 480 nm using a spectrophotometer. Autooxidation of epinephrine to adrenochrome was performed in a control tube without the homogenate. SOD activity was expressed as nmoles/min/mg protein[97].

Assay of glutathione reductase (GSSH)

The activity of GSSH in the tissue was determined by the method of Racker,(1955)

Principle

ß-NADPH+GSSG $\xrightarrow{\text{Glutathione reductase}}$ ß-NADP+2GSH

Procedure

The reaction mixture contained 2.1mL of 0.25mM potassium phosphate buffer pH 7.6, 0.1mL of 0.001M NADPH, 0.2mL of 0.0165M oxidized glutathione and 0.1mL of

bovine serum albumin (10 mg/mL). The reaction was started by the addition of 0.02mL of tissue homogenate with mixing and the decrease in absorbance at 340 nm was measured for 3 min against a blank. GSSH activity was expressed as nmoles/min/mg protein[98].

Assay of reduced glutathione (GSH)

The activity of GSH was determined by Ellman's method

Principle

Ellman reagent (DTNB), which reacts with sulfydryl compounds to give a relatively stable yellow color

Procedure

About 1.0mL of tissue homogenate was treated with 0.5 mL of Ellman's reagent (19.8 mg of 5,5'- Dithiobis-(2-Nitro benzoic acid) [DTNB] in 100 mL of 0.1 % sodium citrate) and 3.0mL of phosphate buffer(0.2 M,pH8).The absorbance was read at 412 nm using a spectrophotometer. GSH activity was expressed as nmoles/min/mg protein[98].

HISTOPATHOLOGICAL STUDIES

At the end of study, the liver and pancreas is excised and washed with ice cold saline. The tissue was fixed in 10% buffered neutral formalin solution. After fixation tissues were embedded in paraffin-wax and five micrometer thick sections were cut and stained with hematoxylin and eosin. The slides were observed under light microscope, photomicrograph was taken and examined the histopathological changes.

STATISTICAL ANALYSIS

Statistical analysis was performed using one way ANOVA followed by Dunnett's test. Values are expressed as mean ±SEM and $p<0.05$ were considered statistically significant.

RESULTS

PHYTOCHEMICAL SCREENING

Table 7 Phytochemical screening of aqueous (AEMP) and ethylacetate extract of *Moringa pterygosperma* (EAEMP)

Chemical constituent	*Tests*	*AEMP*	*EAEMP*
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 constituents

-? Absence of constituents

Phytochemical screening of aqueous extract of *Moringa pterygosperma* revealed the presence of glycosides, triterpenoids, alkaloids, and tannins, saponins. The ethyl acetate extract of *Moringa pterygosperma* revealed the presence of alkaloids, tannins, saponins.(**Table 7)**

IN VITRO METHODS

The *in vitro* antidiabetic activity was investigated through the inhibition of α-amylase and non-enzymatic glycosylation of haemoglobin.

Evaluation of *in vitro* α-amylase inhibitory activity using *Moringa pterygosperma* leaves

The *Moringa ptrygosperma* leaves extract (MPLE) showed significant α-amylase inhibitory activity at varying concentrations (0.5, 1, 2, 4, 8, 16µg/ml). There was a dose dependent increase in the percentage inhibition for all the concentrations. The aqueous extract of *Moringa ptrygosperma* (AEMPL) at a concentration of 0.5µg/ml showed a percentage inhibition of 20.60, for 16µg/ml it became 80.01µg/ml **(Table 8).** The IC_{50} value was found to be 1.4µg/ml. In the case of ethylacetate extract of *Moringa ptrygosperma* (EAEMPL) at a concentration of 0.5µg/ml showed a percentage inhibition of 32.56, for 16µg/ml it was 77.19µg/ml .The IC_{50} value was 2.9µg/ml.

Acarbose was used as a standard drug for the determination of α-amylase inhibitory activity. The concentration of acarbose varied from 0.5 to 16µg/ml. Acarbose at concentration 0.5µg/ml exhibited a percentage inhibition of 38.22 and for 16µg/ml it was found to be 89.86. A graded increase in percentage of inhibition was observed for the increase in the concentration of acarbose. The IC_{50} value of acarbose was found to be 1µg/ml. All determinations were done in triplicate and the mean value were determined.

Table 8: α-amylase inhibitory activity of different concentration of aqueous and ethylacetate extract of *Moringa ptrygosperma*

Concentration (µg/ml)	Percentage inhibition (Mean±S.E.M)		
	AEMP	EAEMP	STANDARD acarbose
0.5	20.60±9.33	32.56±2.18	38.22±2.11
1	33.06±0.45	42.21±1.64	50.51±3.55
2	54.81±0.42	48.33±0.44	56.003±1.84
4	62.79±3.27	54.103±1.63	70.003±0.51
8	71.13±1.16	66.14±3.06	84.08±1.93
16	80.01±0.07	77.19±0.62	89.86±1.03
IC_{50}	**1.4±0.54**	**2.9±0.13**	**1±0.34**

In vitro non-enzymatic glycosylation of haemoglobin

The AEMP showed higher inhibition of glycosylation, which shown in **table 9**. The results revealed that AEMP showed 11.82 percent of inhibition for 0.5µg/ml and for 32µg/ml it was found to be 71.92. IC_{50} value was found to be 12.50µg/ml. In the case of EAEMP at a concentration of 0.5µg/ml showed a percentage inhibition of 6.643, for 32µg/ml it become 58.05. The IC_{50} value was found to be 24.2.

α-Tocopherol was used as the standard drug for the determination of haemoglobin glycosylation inhibitory effect. The concentration of α-tocopherol varies from 0.5 to 32µg/ml. The dose of 0.5µg/ml produced an inhibitory percentage of 39.63 and the same at 32 µg/ml produced 84.94%. A dose dependent increase in the percentage of inhibition could be observed for all the concentrations used. The IC_{50} value of α- tocopherol was found to be 3.2µg/ml.

Table 9: Effect of aqueous and ethylacetate extract of *Moringa pterygosperma* on Haemoglobin glycosylation

Concentration (µg/ml)	Percentage inhibition (Mean±S.E.M)		
	AEMP	EAEMP	STANDARD α-tocopherol
0.5	11.82±2.63	6.643±1.85	32.96±3.67
1	12.45±1.45	8.93±3.88	40.63±4.76
2	13.28± 0.56	13.68±4.06	46.34±3.24
4	22.75±1.85	17.33± 3.83	64.68±4.52
8	38.32± 2.03	26.64± 2.91	75.74±5.30
16	54.64 ±2.65	41.72± 2.94	79.94±5.59
32	71.92±1.53	58.08±3.03	84.94±5.94
IC_{50}	**12.5±2.76**	**24.2±2.67**	**3.2±3.63**

Both *in-vitro* studies shows that the percentage inhibition is higher in aqueous extract compared to the ethyl acetate extract of *Moringa pterygosperma*. So aqueous extract of *Moringa pterygosperma* was used for the further studies.

IN-VIVO STUDIES

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

Among the two extracts tested the aqueous extract of *Moringa pterygosperma* was 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 18-200 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. Sighting study was conducted with a lower dose of 5mg/kg using 0.5% CMC. After administration, the animals were observed for occurrence of toxic effects. No toxic effect were observed and after sufficient interval of time (2-3days). The second, third and fourth rats were administered with 300 and 2000 mg/kg dose of *Moringa pterygosperma* and the rats were observed for signs of acute toxicity. 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 4^{th} 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 was no changes in normal behavioural pattern and no signs and symptoms of toxicity and mortality in rats. As per the OECD 420 guidelines *Moringa pterygosperma* can be included in the category 5 or unclassified category of globally harmonized classification system (GHS). Hence based on these results the *Moringa pterygosperma* were considered non-toxic and $1/10^{th}$ and $1/20^{th}$ dose were used for the biological evaluation (antidiabetic activity) and the studies were conducted at dose levels of 100 and 200 mg/kg body weight.

Table 10 : 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	Dyspnea	-	-	-	-	-	-	-	-	-	-	-	-
	Apnea	-	-	-	-	-	-	-	-	-	-	-	-
	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	-	-	-	-	-	-	-	-	-	-	-	-

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
Piloerection	Contraction of erectile tissue of hair	-	-	-	-	-	-	-	-	-	-	-	-
Gastro intestinal signs	Diarrhoea	-	-	-	-	-	-	-	-	-	-	-	-

Table 11: LIVE PHASE OBSERVATION

LIVE PHASE ANIMALS	OBSERVATIONS
1. Body weight every day	Normal
2. Food consumption daily	Normal
3. Water consumption daily	Normal
4. Home cage activity	Normal

Note: Acute toxicity study of test compound were performed and found to be non-toxic up to 2000 mg/kg dose

Table 12: MORTALITY RECORD FOR TEST COMPOUND IN ACUTE ORAL TOXICITY STUDY

Dose	Sighting study				Main study				
	5 mg/kg	50 mg/kg	300 mg/kg	2000 mg/kg	2000 mg/kg	2000 mg/kg	2000 mg/kg	2000 mg/kg	2000 mg/kg
No. of animals	1	2	3	4	5	6	7	8	9
Body weight (g)	180	185	180	190	180	185	180	200	200
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
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

	Sighting study				Main study				
Dose	5 mg/kg	50 mg/kg	300 mg/kg	2000 mg/kg	2000 mg/kg	2000 mg/kg	2000 mg/kg	2000 mg/kg	2000 mg/kg
No. of animals	1	2	3	4	5	6	7	8	9
Body weight (g)	180	185	180	190	180	185	180	200	200
Sex	Female	Female	Female	Female	Female	Female	Female	Female	Female
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	0/1	0/1	0/5				

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

In vivo* antidiabetic activity of *Moringa pterygosperma

The biological evaluation was carried out using 100mg/kg and 200mg/kg dose of *Moringa pterygosperma* aqueous leaf extract. **Table 13** show the body weight of control and experimental animals on 0, 7, 14, 21 and 28 days of treatment. There was significant reduction of body weight in diabetic control animals compared to test drug treated animals. On 7^{th} day significant reduction of body weight was observed in diabetic control animals (159.85±13.95) and drug treated animals (168±9.38, 170.14±13.32 and 173±8.3) when compared to control rats (193.14±2.47). Reduction in body weight indicates the induction of diabetes. However, there was no reduction in non diabetic extract treated group (199.83±4.44). The normal control (226.14±7.1) and drug treated rats gained significant weight (179.14±9.65, 188.85±12.40, and 193.28±5.090) ($P<0.01$) on 28 days of treatment. However, the increases in body weight in drug treated rats were significantly lower than non diabetic rats.ss

Table 14 shows the effect of aqueous extract of *Moringa pterygosperma* on blood glucose levels of diabetic rats. It was found that a significant ($P<0.01$) increase in blood glucose level was observed in STZ-nicotinamide induced diabetic rats (289.16±7.11) compared to normal control (70.14±9.6). After the daily treatment for 28 days showed significant ($p<0.01$) reduction in blood glucose levels with the doses of 100 mg/kg (160.83±11.65) and 200 mg/kg (153.66±23.87) *p.o* of aqueous extract of *Moringa pterygosperma* and 2.5 mg/kg, *p.o* of glibenclamide (156.66±6.31) as compared to diabetic control group. However a slight fall in blood glucose level was observed in non-diabetic AEMP treated group (59.30±6.31), showing the potency of test drug.

Table 15 shows the level of serum lipoproteins such as total cholesterol, triglycerides, LDL, HDL and VLDL. Serum total cholesterol, triglyceride, LDL, HDL and VLDL levels were significantly elevated in diabetic group when compared with control group animals. Administration of AEMP at dose 100 and 200mg/kg and standard drug glibenclamide results in a significant fall of these serum lipoproteins when compared to diabetic rats. Non diabetic MPLE treated group did not exhibit a significant change in the level of serum lipoproteins. HDL level was decreased in the diabetic group when compared to the non diabetic rats. After 28 days of AEMP and glibenclamide supplementation, there was a significant elevation in HDL level in serum.

Total cholesterol level was increased significantly P<0.01 in diabetic control (147.6±2.63) group compared with normal control (73.10±1.82). After treatment showed significant reduction (P<0.01) with 200 mg/kg (100.08±2.47) 100 mg/kg (125.89±2.13) of AEMP and glibenclamide (79.53±2.56) compared with diabetic control.

Level of triglyceride was also decreased in treatment group after 28 days treatment with AEMP 200 mg/kg (102.54± 2.26) 100 mg/kg (113.4± 2.26) and glibenclamide treated group (97.10±2.10) compared with negative control group (120.45±7.04).

LDL and VLDL level was significantly (P<0.01) increased in negative control group (96.63±2.98, 34.77±0.63) compared control group (21.40±1.28, 16.96±0.53). After 28 days of treatment with both doses of a AEMP significant decreases (P<0.01) in LDL and VLDL levels were observed compared to the negative control. HDL level in diabetic control group was decreased (16.2±1.0) significantly (P<0.05) compared with normal control (34.2±1.38). Treatment with 200 mg/kg (28.7±1.73) 100 mg/kg (22.37±1.7) of AEMP and glibenclamide (29.94±2.08) increaseD the HDL level compared with normal control (34.2±1.38).

The effect of *Moringa pterygosperma* on ALT, AST and ALP level in streptozotocin and nicotinamide-induced diabetes rats are shown in Table 16. In diabetic rats a significant increase in activities of ALT, AST and ALP was observed. After treatment with aqueous extract of *Moringa pterygosperma* (100 and 200 mg/kg, p.o.) and glibenclamide (2.5 mg/kg) the ALT, AST and ALP activities were significantly(P<0.01) reduced compared to diabetic rats .

The ALP level in the high (200 mg/kg) and low dose (100mg/kg) of AEMP (2334.81±4.34, 2800.15±9.54) was significantly decreased compared with diabetic control group (3507.43±7.84). Level of AST in diabetic control group was significantly (P<0.0.05) increased (3293.66±9.56) compared with the normal control group (1267.86±4.89). After 28 days of treatment with 200 mg/kg (2384.81±1403) and 100 mg/kg of AEMP(2634.04±8.61) and glibenclamide (2238.04±4.05) a significant (P<0.01) reduction of AST level was obtained compared with the negative control group. ALT level also significantly (P<0.01) reduces after treatment with high (2589.84±9.04) and low dose (2814.64±7.36) of AEMP and glibenclamide (2693.85±7.68). Whereas in extract alone (200 mg/kg) group the ALT level was similar to that of normal control group.

In diabetic rats a significant ($p<0.01$) increase in the levels of serum urea, creatinine and uric acid was observed when compared to normal rats. The urea level in diabetic control group (50.99±0.50) was significantly ($P<0.01$) increased compared with the normal control group (31.23±0.36) upon treatment with AEMP at both doses a significant decrease (37.18±0.23, 41.02±0.22, 34.12±0.20) in the urea level was observed compared with the negative control group. Diabetic rats treated with AEMP 100 mg/kg (1.34±0.03) and 200 mg/kg (0.85±0.02) showed significant ($P<0.01$) reduction in serum creatinine levels when compared with diabetic rats (2.06±0.01). Diabetic rats treated with AEMP, 100 mg/kg (9.98±0.43) and 200 mg/kg (8.75±0.42) also showed the significant reduction in the level of uric acid when compared with diabetic rat (15.36±0.18).(Table 17)

The level of protein after 28 days of treatment in liver, kidney and pancreas is given in Table 18,19 and 20. A decrease in protein level was found in diabetic control (121.37±5.60, 170.62±6.32, 129.12±7.37) rats compared with control rats (244.125±8.28, 344.5±3.96, 286.7±8.06). Administration of AEMP (100 and 200 mg/kg), AEMP treated non diabetic rats (247.25±9.45) and glibenclamide treated rats (247.25±9.35) restored the protein level significantly near to normal levels. The results was found to be statistically significant ($P<0.01$).

The effect of MPLE on MDA shown in (**Table-18, 19, 20)**. MDA level was found to be elevated in streptozotocin and nicotinamide-induced diabetic rat (44.92±3.09, 39.65±0.64, 51.10±0.37) compare to control rats (6.94±0.56, 10.96±0.20, 10.02±0.19) in liver, kidney, pancreas. This level was significantly ($P<0.01$) reduced in the diabetic rats treated with the AEMP at the dose of 100 mg/kg (, 16.84±0.23, 16.12±0.19) and 200 mg/kg (7.26±0.28, 10.40±0.33, 13.94±0.15, 17.06±0.16) and standard glibenclamide (8.08±0.20, 14.31±0.35, 16.89±0.46) treated groups.

A significant ($P<0.01$) decrease was observed in the activities of CAT (95.33±0.38, 109.75±1.06, 134.66±3.53) SOD (382.24±8.64, 275.17±5.34, 219.80±3.17) GSSH (1.21±0.38, 0.89±0.23, 1.1±0.42) GP_X (0.067±0.01, 0.041±0.008, 0.07±0.009) and GSH (0.46±0.19, 0.34±0.11, 0.51±0.08) in liver, kidney and pancreas homogenates of diabetic rats compared to control rats **(Table-18,19,20).**

Administration of AEMP high dose (188.55±4.25, 167.76±6.39, 187.52±5.81) and standard glibenclamide (188.55±4.25, 167.76±6.36, 187.52±5.81) significantly ($P<0.01$) increased the activity of CAT enzymes compared to the negative control group. Non diabetic

AEMP 200mg/kg (198.34±3.06, 196.05±7.94, 202.89±5.42) treated group also showed a significant increase in the level of CAT enzyme.

The level SOD was increased significantly (P<0.01) in AEMP high dose (526.35±6.9, 511.38±7.69, 506.16±4.63) low dose (512.11±5.54, 508.27±8.7, 490.25±5.48) and glibenclamide (587.34±7, 511.33±5.8, 501.17±5.7) treated groups compared with the negative control group. GSSH (3.26±0.52, 2.45±0.46, 4.09±0.12) and GP_X (0.85±0.12, 0.95±0.23, 0.86±0.11) level was also increases with the treatment of AEMP compared with the negative control in liver, kidney, pancreas.

The non-enzymatic antioxidant, GSH level also decreased in diabetic control group compared with the negative control group. Treatment with high dose 200 mg/kg (2.47±0.23, 1.53±0.32, 2.02±0.67) low dose 100 mg/kg (2.26±0.57, 1.40±0.10, 1.86±0.60) of AEMP and glibenclamide (2.50±0.40, 1.44±0.23, 1.98±0.38) significantly (P<0.01) increases this enzymes level compared with the negative control group in liver, kidney, pancreas (**(Table-18,19,20).**

Table 13 - Effect of aqueous extract of *Moringa pterygosperma* leaves on the body weight in streptozotocin and nicotinamide-induced type 2 diabetes mellitus rats

GROUPS	DRUG TREATMENT	AVERAGE BODY WEIGHT (b.w/kg)				
		0^{th} DAY	7^{th} DAY	14^{th} DAY	21^{st} DAY	28^{th} DAY
I	0.5% w/v CMC (1ml/ 200g b.w.)	181.71±4.49	193.14±2.47	209.28±2.28	217.714±1.49	226.14±7.1
II	Diabetic control	164.14±5.30$^{\#\#}$	159.85±3.95$^{\#\#}$	154.28±2.29$^{\#\#}$	146.85±11.40$^{\#\#}$	139.71±9.94$^{\#\#}$
III	Diabetic + AEMP (100mg/kg)	165.85±6.89**	168±9.38*	172.42±9.19**	174.71±8.95**	179.14±9.65**
IV	Diabetic + AEMP (200mg/kg)	164.28±8.71**	169.14±3.32**	175.57$^{\pm 3.32}$**	182±8.91**	188.85±12.40**
VI	Diabetic + Glibenclamide (2.5mg/kg)	167.42±4.72**	173±8.3**	179.71±4.88**	185.85±4.84**	193.28±5.0**
V	AEMP (200mg/kg)	184.16±5.03^{ns}	199.83±4.44^{ns}	200.83±4.708^{ns}	208.5±4.68^{ns}	221.5±4.231^{ns}

One-way ANOVA followed by Dunnett's test. All the values are Mean± S.E.M., $^{\#\#}$P<0.01 when negative control compared with normal control group., **P<0.01,*P<0.05 when treatment groups compared with negative control groups, ^{ns}P>0.05 extract alone treated group compared with normal control

Group	Body weight	
	Initial	Final (%change)
0.5% w/v CMC (1ml/ 200g b.w.)	175.32±3.2	226.14±7.1** (↑22.61)
Diabetic control	159.56±2.1	139.71±9.94ns (↓-13.80)
Diabetic + AEMP (100mg/kg)	160.43±6.4	179.14±9.65ns(↑11.32)
Diabetic + AEMP (200mg/kg)	158.53±7.7	188.85±12.40*(↑16.3)
Diabetic + Glibenclamide (2.5mg/kg)	161.63±8.3	193.28±5.0*(↑16.70)
AEMP (200mg/kg)	174.46±9.2	221.5**±4.231(↑21.44)

Students paired *t* test. All the values are Mean± S.E.M., Values in parentheses are the percent increase (↑) or decrease (↓) from their corresponding initial readings. *denotes P<0.05 when compared to initial readings. nsP>0.05 extract alone treated group compared with normal control

Table 14 - Effect of aqueous extract of *Moringa pterygosperma* leaves 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	21st DAY	28th DAY
I	0.5% w/v CMC (1ml/ 200g b.w.)	73.5±9.77	72.83±12.01	72.13±6.8	71.56±8.5	70.14±9.6
II	Diabetic control (STZ-55mg/kg NIC 120mg/kg)	251.5±20.60##	266.83±21.73##	271.5±18.41##	280.16±12.05##	289.16±7.11##
III	Diabetic + AEMP (100mg/kg)	240.16±19.03**	230.16±16.94**	190.66±11.32**	174.33±9.54**	160.83±11.65**
IV	Diabetic+ AEMP (200mg/kg)	255.83±14.0**	239.66±22.15*	171.57±15.15**	162.66±3.61**	153.66±23.87**
VI	Diabetic + glibenclamide (2.5mg/kg)	244.33±13.47**	228.33±10.82**	61.83±2.04**	161.16±7.96**	156.66±6. 31**
V	AEMP (200mg/kg)	73.16±4.40**	73±2.36**	72.83±2.04**	71.5±1.36**	70.30±6.31**

One-way ANOVA followed by Dunnett's test. All the values are Mean±S.E.M., ##P<0.01 when negative control compared with normal control group., **P<0.01, *P<0.05 when treatment groups compared with negative control groups, nsP>0.05 extract alone treated group compared with normal control.

Table 15 - Effect of aqueous extract of *Moringa pterygosperma* leaves on the lipid profile level in streptozotocin and nicotinamide-induced type 2 diabetes mellitus rats

GROUPS	DRUG TREATMENT	LIPID PROFILE (mg/dL)				
		TC	TG	HDL	LDL	VLDL
I	0.5% w/v CMC (1ml/ 200g b.w.)	73.10± 1.82	45.82±7.15	34.2±1.38	21.40±1.28	9.16± 0.53
II	Diabetic control (STZ-55mg/kg NIC-120mg/kg)	147.6± 2.63$^{\#\#}$	120.45±7.04$^{\#\#}$	16.2±1.0$^{\#}$	96.63±2.98$^{\#\#}$	24.09± 0.63$^{\#\#}$
III	Diabetic + AEMP (100mg/kg)	125.89± 2.13**	101.4± 2.26**	22.37±1.7*	75.00±2.08**	20.29± 0.45**
IV	Diabetic + AEMP (200mg/kg)	100.08± 2.47**	97.54± 2.26 **	28.7±1.73**	48.65±3.27**	19.50± 0.45**
VI	Diabetic + glibenclamide (2.5mg/kg)	87.42± 2.23 **	97.10±2.10*	29.94±2.08**	38.05±1.50**	19.42± 0.39**
V	AEMP (200mg/kg)	79.53±2. 56^{ns}	41.39±4. 08^{ns}	38.56±2.06^{ns}	23.46±2.47^{ns}	8.27±0. 21^{ns}

One-way ANOVA followed by Dunnett's test. All the values are Mean±S.E.M., $^{\#}$P<0.05, $^{\#\#}$P<0.01 when negative control compared with normal control group., **P<0.01, *P<0.05 when treatment groups compared with negative control groups. ^{ns}P>0.05 extract alone treated group compared with normal control.

Table 16 - Effect of aqueous extract of *Moringa pterygosperma* leaves on the liver function test in streptozotocin and nicotinamide-induced type 2 diabetes mellitus rats

GROUPS	DRUG TREATMENT	LIVER FUNCTION TEST		
		ALP (U/L)	AST(U/L)	ALT (U/L)
I	0.5% w/v CMC (1ml/ 200g b.w.)	1914.47±9.89	1267.86±4.89	1103.03±11.44
II	Diabetic control (STZ-55mg/kg NIC-120mg/kg)	3507.43±7.84$^{\#\#}$	3293.66±9.56$^{\#\#}$	3311.46±11.38$^{\#}$
III	Diabetic + AEMP (100mg/kg)	2800.15±9.54**	2634.04±8.61**	2814.64±7.36**
IV	Diabetic + AEMP (200mg/kg)	2334.81±4.34**	2334.81±14.03**	2589.84±9.04**
VI	Diabetic+ glibenclamide (2.5mg/kg)	2231.36±5.78**	2238.04±4.05**	2693.85±7.68**
V	AEMP (200mg/kg)	1910.66±14.09**	1259.51±7.8*	1094.42±10.82**

One-way ANOVA followed by Dunnett's test. All the values are Mean±S.E.M., $^{\#}$P<0.05, $^{\#\#}$ P<0.01 when negative control compared with normal control group., **P<0.01, *P<0.05 when treatment groups compared with negative control groups. ^{ns}P>0.05 extract alone treated group compared with normal control.

Table 17 - Effect of *Moringa pterygosperma* leaves on the serum urea, Creatinine, uric acid level in streptozotocin and nicotinamide-induced type 2 diabetes mellitus rats

GROUPS	DRUG TREATMENT	KIDNEY FUNCTION TEST		
		UREA (mg/dL)	CREATININE(mg/dL)	URIC ACID (mg/dL)
I	0.5% w/v CMC (1ml/ 200g b.w.)	31.23±0.36	0.65±0.01	6.51±0.36
II	Diabetic control (STZ-55mg/kg NIC-120mg/kg)	50.99±0.50$^{\#\#}$	2.06±0.01$^{\#\#}$	15.36±0.18$^{\#\#}$
III	Diabetic + AEMP (100mg/kg)	41.02±0.22**	1.34±0.03**	9.98±0.43**
IV	Diabetic + AEMP (200mg/kg)	37.18±0.23**	0.85±0.02*	8.75±0.42**
VI	Diabetic+ glibenclamide (2.5mg/kg)	34.12±0.20**	0.84±0.02**	8.31±0.45**
V	AEMP (200mg/kg)	29.07±0.14*	0.54±0.01**	6.32±0.35*

One-way ANOVA followed by Dunnett's test. All the values are Mean±S.E.M., $^{\#\#}$P<0.01 when negative control compared with normal control group., **P<0.01, *P<0.05 when treatment groups compared with negative control groups. ^{ns}P>0.05 extract alone treated group compared with normal control

Table 18 – Effect of *Moringa pterygosperma* leaves on the serum urea, Creatinine, uric acid level 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)	GP_X (µmoles/min/ mg protein)	GSH (µmoles/min/ mg protein)
0.5% w/v CMC (1ml/ 200g b.w.)	244.125±8.28	6.94±0.56	686.03± 6.92	209.16±4.63	5.17±0.82	1.21±0.04	3.15±0.53
Diabetic control (STZ-55mg/kg NIC-120mg/kg)	121.37±5.60$^{\#\#}$	44.92±3.09$^{\#\#}$	382.24±8.64$^{\#\#}$	95.33±2.58$^{\#\#}$	1.21±0.38$^{\#\#}$	0.067±0.01$^{\#\#}$	0.46±0.19$^{\#\#}$
Diabetic + AEMP (100mg/kg)	226.25±9.98**	10.405±0.33**	512.11±5.54**	170.44±5.98**	3.11±0.48**	0.84±0.10**	2.26±0.57*
Diabetic + AEMP (200mg/kg)	229.62±7.94**	7.26±0. 28**	526.35±6.9**	188.55±4.25**	3.26±0.52**	0.85±0.12**	2.47±0.23*
Diabetic + glibenclamide (2.5mg/kg)	230.25±9.881**	8.08±0.20**	587.34±7**	191.15±3.54**	4.17±0.69*	0.85±0.13**	2.50±0.40**
AEMP (200mg/kg)	239.37±7.68**	6.89±0.11**	673.56±11.62**	198.34±3.06**	5.14±0.87*	1.03±0.12*	2.65±0.47**

One-way ANOVA followed by Dunnett's test. All the values are Mean±S.E.M., $^{\#\#}$P<0.01when negative control compared with normal control group.,**P<0.01,*P<0.05 when treatment groups compared with negative control groups.^{ns}P>0.05 extract alone group compared with control.

Table 19 - Total protein, MDA and antioxidant parameters of *Moringa pterygosperma* using kidney homogenate

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)	GP_X (µmoles/min/ mg protein)	GSH (µmoles/min/ mg protein)
0.5% w/v CMC (1ml/ 200g b.w.)	344.5±3.96	10.96±0. 20	587.98± 9.81	209.76±4.33	3.84±0.66	1.47±0. 44	1.91±0.44
Diabetic control (STZ-55mg/kg NIC 120mg/kg)	170.62±6.32$^{\#\#}$	39.65±0.64$^{\#\#}$	275.17±5.34$^{\#\#}$	109.75±1.06$^{\#\#}$	0.89±0.23$^{\#\#}$	0.041±0.008$^{\#}$	0.34±0.11$^{\#\#}$
Diabetic + AEMP (100mg/kg)	316.25±3.24**	16.84±0.23**	508.27±8. 7**	162.99±4.94**	2.37±0.45**	0.88±0.25**	1.40±0.10**
Diabetic + AEMP (200mg/kg)	333.75±7.20**	13.94±0.15**	511.38±7.68**	167.76±6.39**	2.45±0.46**	0.95±0.20**	1.53±0.32*
Diabetic + glibenclamide (2.5mg/kg)	331.75±6.29**	14.31±0.35**	511.33±5. 8**	180.05±6.46**	2.74±0.70**	0.86±0.23**	1.44±0.23**
AEMP (200mg/kg)	334.75±5.57**	9.30±0.4 2**	573.11±6.23*	196.05±7.94**	3.03±0.10*	1.43±0.10*	1.93±0.09**

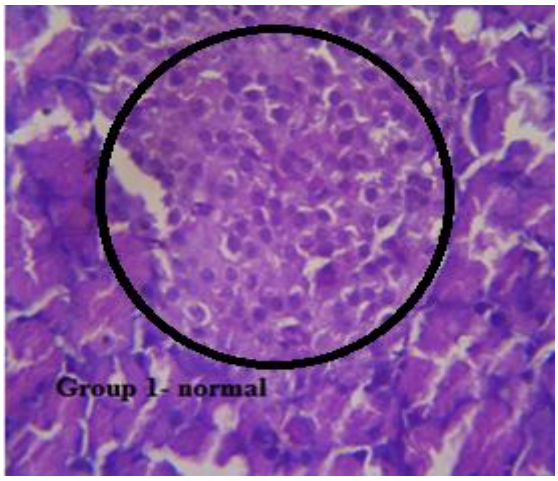
One-way ANOVA followed by Dunnett's test. All the values are Mean±S.E.M., $^{\#\#}$P<0.01, $^{\#}$P<0.05 when negative control compared with normal control group., **P<0.01, *P<0.05 when treatment groups compared with negative control groups. ^{ns}P>0.05 extract alone treated group compared with normal control.

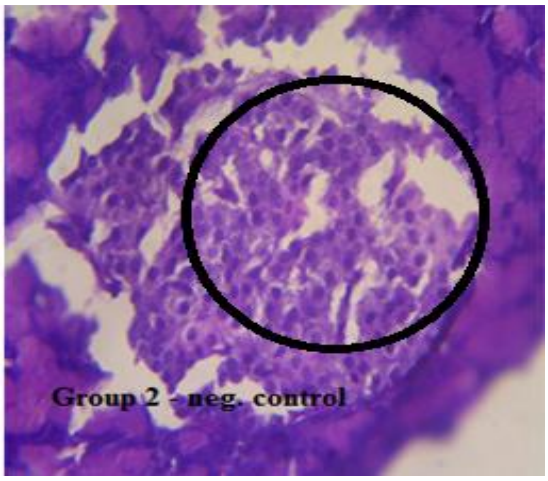
Table 20 - Total protein, MDA and antioxidant parameters of *Moringa pterygosperma* using pancreas homogenate

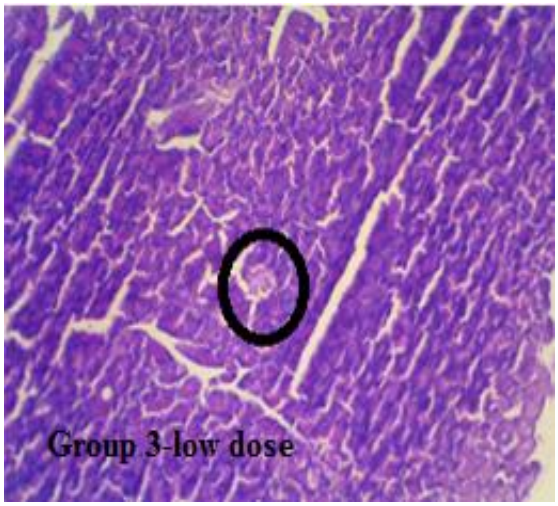
DRUG TREATMENT	Total protein (mmoles/min/ tissue)	MDA (nmoles/min/mg protein)	SOD (nmoles/min/ mg protein)	CAT (µmoles/min/ mg protein)	GSSH (µmoles/min/ mg protein)	GP_X (µmoles/min/ mg protein)	GSH (µmoles/min/ mg protein)
0.5% w/v CMC (1ml/ 200g b.w.)	286.7±8.06	10.02±0.19	570.1± 5.23	223.39±3.71	5.40±0.69	1.64±0.27	2.86±0.58
Diabetic control (STZ-55mg/kg NIC 120mg/kg)	129.12±7.37$^{\#\#}$	51.10±0.37$^{\#\#}$	219.80±3.17$^{\#}$	134.66±3.53$^{\#\#}$	1.1±0.4 2$^{\#\#}$	0.07±0.009$^{\#\#}$	0.51±0.08$^{\#\#}$
Diabetic+ AEMP (100mg/kg)	227±5.50**	16.12±0.19**	490.25±5.48**	181.29±7.92**	3.84±0.28**	0.85±0.23**	1.86±0.60**
Diabetic+AEMP (200mg/kg)	247.37±6.25**	17.06±0.16**	506.16±4.63**	187.52±5.81**	4.09±0.12*	0.86±0.11**	2.02±0.67**
Diabetic+ glibenclamide (2.5mg/kg)	247.25±9.35**	16.89±0.46**	501.17±5.7**	189.21±5.98**	4.21±0.33**	0.98±0.23**	1.98±0.32**
AEMP (200mg/kg)	277.25±9.45**	12.22±0.25**	562.09±3.61**	219.89±5.42**	5.35±0.27*	1.52±0.56**	2.56±0.10*

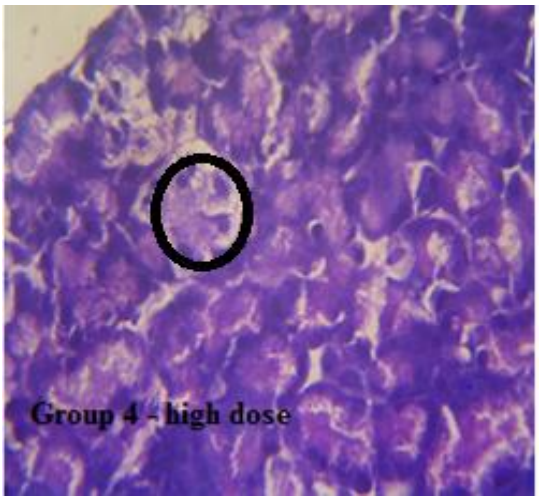
One-way ANOVA followed by Dunnett's test. All the values are Mean±S.E.M., $^{\#\#}$P<0.01, $^{\#}$P<0.05 when negative control compared with normal control group., **P<0.01, *P<0.05 when treatment groups compared with negative control groups. ^{ns}P>0.05 extract alone treated group compared with normal control

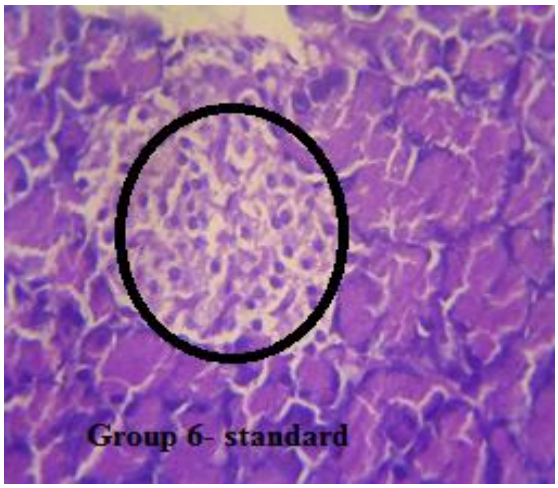
Histopathology of pancreas

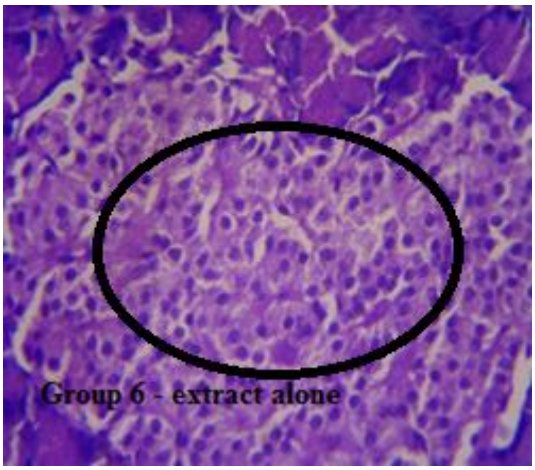












Group 1 (Normal control) : Section from pancreas shows normal acini. Islets are normal in number and morphology. Blood vessels show congestion. There is no inflammation or cytoplasmic vacuolation seen in the section studied.

Group 2 (Negative control) : Section from pancreas shows cytoplasmic vacuolation. Islets are small in size and reduced in number

Group 3 (MPLE-100mg/kg) : Section from pancreas shows normal pancreatic acini. Islets are small in size and reduced in number. There is a focal mild cytoplasmic vacuolation.

Group 4 (MPLE-200mg/kg) : Section from pancreas shows normal pancreatic acini. Islets are normal in number with few showing small in size. There is no evidence of inflammation and cytoplasmic vacuolation.

Group 5 (Glibenclamide): Section from pancreas shows normal acini. Islets are normal in number with mild decrease in size. There is no evidence of destruction / cytoplasmic vacuolation.

Group 6 (Extract alone) : Section from pancreas shows normal islets. Acini shows normal morphology. There is no evidence of destruction/ cytoplasmic vacuolation.

The results of histopathology of pancreas show the normal control group having normal islet architecture and normal in number (Group 1). The negative control group (Group 2) shows cytoplasmic vacuolation and islets are small in size, partially destructed and reduced in number compared to normal control group. Treatment with 200mg/kg of AELP (200 mg/kg) and glibenclamide (2.5 mg/kg) has shown partial to near normal reversal of changes induced by streptozotocin-nicotinamide injections suggesting regeneration and hypertrophy of beta islet cells in the AELP and glibenclamide-treated groups.

DISCUSSION AND CONCLUSION

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 *in-vitro* antidiabetic activity of the both ethylacetate and aqueous extracts have been evaluated by measuring its α-amylase inhibitory activity and non-enzymatic glycosylation of haemoglobin.

α-amylase is an enzyme which converts starch to oligosaccharide. Inhibition of this enzyme can retard glucose absorption, and thereby can produce hypoglycaemic action.Glycosylated haemoglobin is a blood test to determine level of glycemic control. Blood glucose binds to haemoglobin through a process called glycosylation. The higher the blood sugar the more glucose binds to haemoglobin can be determined by using non-enzymatic glycosylation of haemoglobin test.

The results of both *in-vitro* study with alpha amylase and non-enzymatic glycosylation of haemoglobin exhibited potential inhibitory activity for the aqueous extract of plant *Moringa pterygosperma* and it was compared with standard acarbose and α-tocopherol .

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 hypoglycaemic activity of aqueous extracts of MPLE carried out on STZ and nicotinamide induced type 2 diabetic rats.

In streptozotocin and nicotinamide-induced type 2 diabetic mellitus, oral administration of aqueous extract of AEMP for 28 days showed a significant reduction in body weight in diabetic rats is due to excessive break down of tissue protein. Treatment with AEMP or gibenclamide improved body weight significantly inducing prevention of muscle wasting due to hyperglycaemic condition.

The percentage difference in body weight is large in control group compared with negative control group. In treatment group also increases the body weight compared with the diabetic control group.

The hypoglyamic activity of MPLE was compared with glibenclamide,a standard second generation hypoglycemic drug. Acute administration of sulfonyl urea

increases insulin release from the pancreas, Sulfonyl ureas such as gibenclamide have used for many years to treat diabetes, to stimulate insulin secretion from ß-cells principally by inhibiting ATP-sensitive K^+ (KATP) channels in the plasma membrane. Further, it is known that sulfonylureas have a direct effect on ß-cell exocytosis and that effect is mediated by a mechanism that does not involve direct activation of protein kinase-C, which place a major role in controlling the ß-cell potential. The inhibition of ATP sensitive channels leads to membrane depolarization, activating Ca channels, increased calcium influx, a rise in cytosolic (Ca^{2+}) and there by insulin release. Oral administration of MPLE and glibenclamide to the STZ and nicotinamide-induced diabetic rats decreased the blood glucose levels.

The extent of complications of diabetes appears to correlate with elevated blood glucose concentrations and it is widely thought that excessive glucose is the major cause of tissue injury. Indeed, several mechanism exists by which glucose and other sugars can damage tissues which include the enzyme glucokinase which converts glucose to glucose-6-phosphate.

Increase in concentration of total cholesterol, triglycerides, LDL and VLDL and decreased HDL is observed in diabetes untreated rats. Hyperlipidemia is a recognized consequence of diabetes mellitus. Administration of AEMP and glibenclamide normalized serum lipids, secondary to the diabetes state. Diabetes induced hyperlipidaemia is attributable of excess mobilization of fat from the adipose tissue due to the under utilization of glucose.

Increase in ALT, AST and ALP are the predictors of diabetes and are common sign of liver disease. In streptozotocin-nicotinamide induced type 2 diabetic, rats increase in serum SGOT, SGPT and ALP level may be due to the leakage of these enzymes from liver cytosol into blood stream as a result of the hepatotoxic effect of STZ. Treatment with aqueous extract of MPLE decreased the levels of ALT, AST and ALP in diabetic animals, which indicates that the extract tends to prevent liver damage in diabetes by maintaining integrity of plasma membrane, thereby suppressing the leakage of enzymes through membrane. Insulin deprivation in diabetic state causes a profound increase in protein catabolism.

In this study, fall in plasma total protein and rise in serum urea, creatinine and blood urea levels were observed in diabetic rats. Urea and creatinine in the serum are significant markers to detect the renal dysfunction, might be due to increased protein catabolism in the body. Accumulation of urea in experimental diabetes may due to the enhanced breakdown of both liver and plasma proteins. The decrease in serum urea and creatinine levels on treatment with extract indicated that the extract has prevented the progression of renal damage in diabetic rats.

Free radical have been implicated in the causation of several disorders, which includes diabetes and the agents that scavenge free radicals may have great potential in ameliorating these disease processes. Antioxidants play a important role in protecting the human body against damage ROS. Increased oxidative stress has been postulated in the diabetic state. Oxidative stress in diabetes co exists with a reduction in the antioxidant status, which can increase the deleterious effects of free radicals.

Generation of these 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 is 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 involoved in the direct elimination of ROS. SOD is an important defence enzyme which catalyses the dismutation of superoxide radical and CAT is a haemoprotein which catalyses the reduction of H_2O and protects the tissue from hydroxyl radicals. GP_X, a selenium containing enzyme present in significant concentration detoxifies H_2O_2 to H_2O through the oxidation of reduced glutathione. The reduced activity of SOD, CAT, GP_X, GSSH, GSH in the liver, kidney, pancreas during diabetes is a result of deleterious effects which results in the accumulation of superoxide anion radicals and H_2O_2. The activity of enzymatic and non enzymatic antioxidants are increased significantly in AEMP treated animals ($P<0.01$).

Marked increase in the concentration of MDA was observed in the liver, kidney, pancreas of diabetes rats. AEMP 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 hyperglycaemic state after the administration of AEMP which reduce the severity of oxidative and acuity of hyperglycaemia, a process that closely linked to glucose oxidation and formation of free radicals. Our results suggested that AEMP 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 *Moringa pterygosperma* can be successfully utilized for the management of diabetes due to their anti-hyperglycaemic action. Further studies on the nature of functional group involved and isolation of active constituents would enlighten the exact mechanism and thus help to rationalize their use in the treatment of diabetes more effectively.

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सं. भा.व.स./द.क्षे.के./No.: BSI/SRC/5/23/2018/Tech. /1215

दिनांक/Date: 23rd July 2018

सेवा में / To

Ms. Karthika P
II Year M. Pharm.
Department of Pharmacology
Sri Ramakrishna Institute of Paramedical Sciences
395, Sarojini Naidu Road
Siddhapudur, Coimbatore – 641 044

महोदया/Madam,

The plant specimen brought by you for authentication is identified as *Moringa pterygosperma* Gaertn. - MORINGACEAE. The identified specimen is returned herewith for preservation in their College/ Department/ Institution Herbarium.

धन्यवाद/Thanking you,

भवदीय/Yours faithfully,

23/07/2018

डॉ सी मुरुगन/Dr. C. Murugan
वैज्ञानिक 'डी' एवं कार्यालय अध्यक्ष /
Scientist 'D' & Head of Office

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COMMITTEE FOR THE PURPOSE OF CONTROL AND SUPERVISION OF EXPERIMENTS ON ANIMALS (CPCSEA)

INSTITUTIONAL ANIMAL ETHICS COMMITTEE (IAEC)

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

IAEC PROTOCOL APPROVAL CERTIFICATE

Principal Investigator	: **P. Karthika**	
Title of the Project	: Evaluation of antidiabetic activity of *Moringa pterygosperma* Gaertn. against streptozotocin and nicotinamide-induced type 2 diabetes in rats.	
Proposal Number	: COPSRIPMS/IAEC/PG/Pharmacology/003/2018-2019	
Approval date	: 19/09/2018	
Animals	: *Wistar* rats	
No. of animals sanctioned	: Male: 44	Female: 06
Expiry date (Termination of the Project)	: 18/09/2019	
Name of IAEC chairperson	: Dr. T. K. Ravi	
Name of CPCSEA Main Nominee	: Dr. G. Arihara Sivakumar	

Signature
IAEC Chairperson
CHAIRMAN
IAEC

Signature
CPCSEA Main Nominee
MAIN NOMINEE
CPCSEA