# EVALUATION OF *INVITRO* AND *INVIVO* ANTI DIABETIC ACTIVITY OF ETHANOLIC EXTRACT OF *Portulaca Quadrifida L.* ON STREPTOZOTOCIN INDUCED DIABETES IN RATS

*A dissertation submitted to*

**THE TAMILNADU DR.M.G.R MEDICAL UNIVERSITY**

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*In the partial fulfillment of the requirements*

*for the award of the degree of*

**MASTER OF PHARMACY**

**IN**

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**Submitted by**

**Reg No: 261226054**


**INSTITUTE OF PHARMACOLOGY**

**MADRAS MEDICAL COLLEGE**

**CHENNAI – 600 003.**

**APRIL – 2013-14**

# CERTIFICATE

This is to certify that the dissertation entitled **"EVALUATION OF *INVITRO AND INVIVO* ANTI-DIABETIC ACTIVITY OF WHOLE PLANT OF Portulaca quadrifida Linn. ON STREPTOZOTOCIN INDUCED DIABETES IN RATS"** submitted by **Registration No.261226054** in partial fulfillment of the requirements for the award of Degree of Master of Pharmacy in Pharmacology by the Tamilnadu Dr. M. G. R. Medical University, Chennai is a bonafide work done by her during the academic year 2013-2014.

The Dean,

Madras Medical College,

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The Director and HOD,

Institute of Pharmacology,

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**Mrs. M. Sakthi abirami, M.pharm.,**

Tutor in Pharmacy,

Institute of Pharmacology,

Madras Medical College,

Chennai - 600 003.

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## ABBREVIATIONS

| | |
|---|---|
| DM | Diabetes Mellitus |
| WHO | World Health Organisation |
| PQ | Portulaca quadrifida |
| GOD-POD | Glucose Oxidase-Peroxidase |
| HMP | Hexose Mono Phosphate |
| G6P | Glucose-6-phosphate |
| LDH | Lactate dehydrogenase |
| G6PD | Glucose-6-phosphate dehydrogenase |
| NADP | Nicotinamide adenine dinucleotide phosphate |
| GLP-1 | Glucagon-likepeptide-1 |
| CPCSEA | Committee for the Purpose of Control and Supervision on Experiment on Animals |
| GI | Gastro intestinal |
| SU | Sulfonyl Urea |
| PPAR | Peroxisome Proliferator Activated receptor |
| DPP-4 | Dipeptidyl Peptidase inhibitors |
| STZ | Streptozotocin |
| PPHG | Post-prandial hyperglycemia |
| ALT | Alanine Aminotransferase |

| | |
|---|---|
| AST | Aspartate Aminotransferase |
| FDA | Food and Drug Administration |
| Rtd | Retired |
| CSIR | Council for Scientific and Industrial Research |
| EDTA | Ethylene diamine tetra acetic acid |
| ATP | Adenine Tri Phosphate |
| NAD | Nicotinamide Adenine Dinucleotide |
| NADH | Reduced Nicotinamide Adenine Dinucletide |
| IAEC | Institutional Animal Ethical Committee |
| OECD | Organisation for Economic Co-operation and Development |
| RBC | Red Blood Corpuscles |
| SGOT | Serum Glutamate Oxaloacetic Transaminase |
| SAP | Serum Alkaline Phosphate |
| SGPT | Serum Glutamic Pyruvic Transaminase |
| HDL | High Density Lipoprotein |
| $H_2O_2$ | Hydrogn Peroxide |
| ALP | Alkaline Phosphatase |
| ACP | Acid Phosphatase |
| HK | Hexokinase |
| ANOVA | Analysis of Variance |
| SD | Standard Deviation |

| | |
|---|---|
| ROS | Reactive Oxygen Species |
| GD | Glucose Diffusion |
| p.o | Oral route |
| b.w | Body Weight |
| rpm | Revolutions per minute |
| mins | Minutes |
| Hrs | Hours |

# INTRODUCTION

India has an ancient heritage of traditional medicine. Traditional medicine is a very important part of health care. Most of the population in the developing countries still relies mainly on indigenous traditional medicines for satisfying their primary health care needs. Materia medica of India provides much information on the folklore practices and traditional aspects of therapeutically important natural product. Today we find in every country the folklore living in the nook and corner are habituated to use their plant resources. They have developed ethnic systems of medicines. Some of them have developed based on prolonged experience [(1).]

Traditional use of herbal medicine is the basis and integral part of various cultures, which was developed within an ethnic group before the developed and spread of modern science. Herbal drugs constitute a major part in all the traditional systems of medicine. These have made a great contribution in maintaining human health. A majority of the world's population still rely on herbal medicines to meet its health needs. The practice continues today because of its biomedical benefits and its place in culture beliefs in many part of world.

India, china and several other nations have an ancient tradition of herbal remedies. The written records in Ayurveda, the ancient system of medicine in India, contain more than 800 herbal remedies. The Charaka Samhita and Sushruta Samhita are two treasure troves containing knowledge of plant based drugs and are even today, held in the highest esteem the world over.

Inspite of all the advances in therapeutics, Diabetes still remain a major cause of morbidity and mortality in the world. Diabetes Mellitus is the most common metabolic disorder known to the ancient Indian physician some 3000 years ago, as can be seen, from the medicinal texts such as Charaka Samhita and Sushruta Samhita. They have discussed the

honey urine in detail. The most ancient medicinal systems of India provide information that madhumeha (diabetes) has long been treated with various herbs and herbomineral drugs. It is observed that some are used as anti-diabetic medicine by folklore. Notable among them are the use of fresh juice of Bael, Onion and Garlic.

However, the name Diabetes was given by the two Roman physician Celsius and Aretaeus in 1st A.D in 1921.Banting and Best solved the problem of Diabetes to a great extent by discovering Insulin as a therapeutic agent in Insulin Dependent Diabetes Mellitus. The first oral hypoglycemic agents suitable for clinical use were the sulfonylureas developed by Auguste Loubatieres in the year 1940.(2) Conventionally, insulin dependent diabetes mellitus is treated with exogenous insulin(3) and non-insulin-dependent diabetes mellitus with synthetic oral hypoglycemic agents like sulphonylureas and biguanides(4). However the hormonrifails as curative agent for complications of diabetes and synthetic oral drugs produce adverse health effects.(5) Therefore, different medicinal systems are using the active plant constituents, which discovered as natural hypoglycemic medicine came from the virtue of traditional knowledge.(6) Asia's large population and rapid economic development have made it an epicenter of the epidemic. Asian populations tend to develop diabetes at younger ages and lower BMI levels. Several factors contribute to accelerated diabetic epidemic in Asians, including the "normal-weight metabolically obese" phenotype, high prevalence of smoking and heavy alcohol use; high intake of refined carbohydrates (e.g. White rice) and dramatically decreased physical activity levels.

Estimates for the worldwide prevalence of diabetes have increased from around 60 million in 1980 to about 118 million in 1995 and are set to increase to 220 million by the year 2010(Amoes et al., 1997). According to the International Diabetes Federation (IDF), Diabetes affects at least 285 million people worldwide, and that number will be expected to reach 438 million by the year 2030.

# Introduction

More than 400 different plants and plant extracts have been described as reputedly beneficial for the diabetic patient. Most of these plants have been claimed to possess hypoglycemic properties but most claims are anecdotal and few have received adequate medical or scientific evaluation. Those that have been evaluated may be grouped into three categories:

1. Plants from which a reputedly hypoglycemic compound or partially characterized hypoglycemic fraction has been prepared.
2. Plants reported to exert a hypoglycemic effect, but the nature of the active principle is unestablished.
3. Plants that reputedly exert a hypoglycemic effect, but the scientific evidence is equivocal. These categories exclude the numerous traditional plants for which an independent scientific or medical has not been published.

*Portulaca quadrifida L.* is an important medicinal herb and this plant is a small, diffuse, annual and erect herb found throughout the tropical parts of India. It is said to be useful in asthma, cough, urinary discharges, inflammations and ulcers. A poultice of the plant is applied in abdominal complaints, erysipelas, and haemorrhoids. *Portulaca quadrifida L.* has been reported to possess antifungal activity against Aspergillus fumigates and Candida albicans. The effect of ethanolic extract of *Portulaca quadrifida L.* on central and peripheral nervous system were studied by using spontaneous motor activity, *in vivo* muscle relaxant activity (Grip strength) and anticonvulsant activity[11]. However, till date there has been no investigation supporting the anti-diabetic properties of this plant. Hence, this study has been taken with an aim to evaluate the anti-diabetic potential of ethanolic extract of whole plant of *Portulaca quadrifida L.* in *in-vitro* and *in-vivo* Streptozotocin model.

# AIM AND OBJECTIVES

1. Phytochemical evaluation of the whole plant of ethanolic extract of *Portulaca quadrifida L.*

2. To determine the glucose diffusion property of the prepared extract of the *Portulaca quadrifida L.* using dialysis tube and GOD-POD kit.

3. To evaluate the safety of the effective extract of *Portulaca quadrifida L.* by acute toxicity study in Wistar albino rats.

4. To investigate the *in-vivo* anti diabetic effect of active extract of *Portulaca quadrifida L.* in Streptozotocin induced diabetic Wistar albino rats.

# REVIEW OF LITERATURE

## DIABETES MELLITUS

The word “Diabetes” is derived from “Greek” word “Diabainein” which means “to pass through”. It is characterized by an excess of glucose in blood and urine, hunger, thirst and gradual loss of weight. Insulin is a hormone which regulates the carbohydrates and triglyceride metabolism through its action at several sites and facilitates the entry of glucose into the cell. Insulin also stimulates the synthesis of glucokinase and moderates the degree of gluconeogenesis. In the diabetic patient, there is an aberration in the functioning of insulin.

## WHO CLASSIFICATION OF DIABETES MELLITUS

### 1) TYPE 1 DIABETES MELLITUS[12]

The hallmark also known as Insulin Dependent Diabetes Mellitus (IDDM). It is not associated with obesity, and may be associated with acidosis or ketosis. Administration of insulin is essential in patients with type I diabetes. Type I diabetes is further subdivided into immune and idiopathic types.

The immune form is the most common form of type I diabetes. Although most patients are younger than 30 years of age at the time of diagnosis, the onset can occur at any age. When it occurs at infancy (due to congenital disorder) or in childhood, it is called Juvenile diabetes. Susceptibility appears to involve a multi factorial genetic linkage, but only 15-20% of patients have a positive family history.

**Causes**

i. Degeneration of β cells in the islets of Langerhans of pancreas.
ii. Destruction of β cells by viral infection.
iii. Congenital disorder of β cells.
iv. Destruction of β cells due to autoimmune disorder where there is development of antibodies against β cells.

**2) TYPE 2 DIABETES MELLITUS**

Type 2 diabetes is characterized by tissue resistance to the action of insulin combined with a relative deficiency in insulin secretion. It usually occurs after 40 years; hence it is called as maturity onset diabetes mellitus. It is also known as adult-onset diabetes or Non-Insulin Dependent Diabetes Mellitus (NIDDM).

The impaired insulin action affects fat metabolism, resulting in increased free fatty acid flux and triglyceride levels, and reciprocally low high-density lipoprotein (HDL) levels.

**Causes**

In this type of diabetes, the structure and function of β cells and the blood level of insulin are normal. The diabetes develops due to the absence or reduced number of insulin receptors in the cells of the body.

**The major causes for type 2 diabetes are:**

**Heredity disorders**

Heredity disorders which may be associated with DM or impaired glucose tolerance can be subdivided into syndromes such as maternally inherited diabetes, down, turner and

klinefelter syndromes, metabolic diseases such as cystic fibrosis and hemochromatosis. Patients with a heredity disorder run a higher risk of developing DM**.**

**Endocrine disorders**

It is very common 'in some of the disorders like Gigantism, Acromegaly, and Cushing's syndrome. The hyperglycemia in these conditions causes excess stimulation, in turn causes burning out and degeneration of β cells. The β cells exhaustion leads to permanent DM. This type of DM is called secondary diabetes.

**3) GESTATIONAL DIABETES (GDM)[(13)]**

It is defined as any abnormality in glucose levels noted for the first time during pregnancy and resolves after delivery. Gestational diabetes mellitus develops during the second or third trimester of pregnancy in about 2% of pregnancies and is treated by diet. During pregnancy, the placenta and placental hormones create an insulin resistance that is most pronounced in the last trimester. However, insulin injections may be required. Women who have GDM are at higher risk for developing Type 2 diabetes mellitus within 5-10 years.

**4) MATURITY ONSET DIABETES IN YOUNG (MODY)**

MODY is defined as hyperglycemia diagnosed before the age of 25 years and treatable for more than 5 years without insulin. Patients with MODY are found to have mutation in the glucokinase (Hexokinase IV) gene on chromosome 7p, and the primary cause of their NIDDM is due to reduction in glucose stimulated insulin secretion. This disease was shown to be linked genetically to ADA (adenosine deaminase) locus on chromosome 20q.

**5) GENETICS[14]**

Susceptibility to both IDDM and NIDDM is determined to a substantial extent by genetic factors. These probably interact with an environmental trigger to induce expression of the disease state. The major contribution comes from a gene or genes within the HLA region, on the short arm of chromosome 6, at least one of which lies close to or within the DQ sub region.

There is a stronger inheritance pattern for type 2 diabetes. Those with first-degree relatives with type 2 have a much higher risk of developing type 2 diabetes.

## SIGNS AND SYMPTOMS OF DM[15]

These are mainly due to hyperglycemia with decreased utilization of glucose by cells; as a result, there is an extracellular glucose excess and intracellular glucose deficiency, a situation called starvation in the midst of plenty.

1. Hyperglycemia (raised blood glucose), it predisposes to infection like boils and urinary tract infection.
2. Glycosuria (presence of glucose in the urine)
3. Polyuria (excessive urine production)
4. Dehydration
5. Polydipsia (increased thirst) is a result of the dehydration that results from the osmotic diuresis.
6. Polyphagia (excessive eating)- low glucose utilization by glucostat cells of ventromedial nucleus in hypothalamus(satiety center) results in no inhibition of lateral nucleus in hypothalamus(feeding center) which eventually produces increased hunger.
7. Loss of weight.

8. Ketonuria.
9. Poor resistance to infections due to protein depletion.
10. Hyperlipedemia (abnormally high serum lipid levels). Insulin deficiency decreases LDL receptor availability, which decreases serum cholesterol clearance. This decreased clearance produces hypercholesterolemia, or high blood cholesterol.
11. Electrolyte depletion.

## DIAGNOSIS OF DIABETES[16]

Diabetes can be confirmed by estimating the blood glucose level. This may be performed using the **Glucometer and Blood glucose test strips** with small drop of blood from finger**.**

**Test for blood glucose levels are:**

**a) Fasting plasma glucose (FPG) test**

It is also known as fasting blood sugar test. It measures blood glucose in a person who has not eaten anything for at least 8 hours.

| **Plasma glucose result (mg/dL)** | **Diagnosis** |
|---|---|
| 99 or below | Normal |
| 100 – 125 | Pre-diabetes (impaired fasting glucose) |
| 126 or above | Diabetes |

**b) Postprandial blood glucose test**

It is a test that determines the amount of glucose in the blood 2 hours after a meal.

| **Plasma glucose result (mg/dL)** | **Diagnosis** |
|---|---|
| <140 mg/dL | Normal |
| 140-200 mg/dL | Pre-diabetes (impaired glucose tolerance) |
| >200 mg/dL | Diabetes |

**c) Oral glucose tolerance test (OGTT)**

This test is done to assess the glucose level of a person, 2 hours after the administration of 75 gms of glucose dissolved in water with overnight fasting for atleast 8 hours.

| **2-hour plasma glucose result (mg/dL)** | **Diagnosis** |
|---|---|
| 139 or below | Normal |
| 140-199 | Pre-diabetes (impaired glucose tolerance) |
| 200 or above | Diabetes |

**d) Glycosylated haemoglobin test**

A test that measures the amount of haemoglobin bound to glucose. It indicates the amount of sugar in a person's blood during the past 2-4 months. It is used to monitor effectiveness of diabetes treatment.

| Plasma glucose result (mg/dL) | Diagnosis |
|---|---|
| 4% - 6% | Normal |
| More than 6% | Diabetes |

**e) Random plasma glucose test**

It is also known as casual plasma glucose test, which measures blood glucose level randomly. Symptoms of diabetes + random plasma glucose concentration ≥ 200 mg/dL (11.1mmol/l) is the diagnostic criteria for diabetes mellitus.

## COMPLICATIONS OF DM[17]

***(i)Diabetic ketoacidosis*** is a serious consequence of poorly controlled IDDM. It is characterized by elevated blood glucose level, ketonemia, increased serum osmolarity and elevated stress hormone levels (the counter regulatory hormones to insulin – cortisol, GH, glucagon, epinephrine). These elevated hormones aggravate the metabolic disorder. The patients have acidosis and decreased vascular volume.

(ii)***Nonketotic Hyperosmolar Coma*** (NHC) can occur with either IDDM or NIDDM. People with NHC have extremely high serum hyperosmolarity and glucose.

(iii)***Insulin shock*** is produced due to the administration of excessive amounts of insulin which can cause confusion, convulsions, loss of consciousness and even death.

## LONG TERM SEQUELAE OF DM

Long-term problems associated with DM include Neuropathies, Nephropathies, Microangiopathies, Macroangiopathies, and Retinopathies.

***(i)Neuropathies:*** Peripheral nerve damage (neuropathy) can occur as a result of metabolic or osmotic damage to neurons or Schwann cells. Diabetic patients can exhibit sensory loss, paraesthesia and even pain as a result of the neurologic damage. Neuronal transmission is slowed.

***(ii)Nephropathies:*** Diabetes is a common cause of renal failure (nephropathy). The glomerular capillary basement membrane thickens, which is thought to produce glomerulosclerosis and subsequent renal insufficiency.

***(iii)Microangiopathies:*** microscopic changes occur in the microcirculation with prominent thickening of capillary basement membranes.

***(iv)Macroangiopathies:*** Atherosclerosis develops in diabetic patients at an accelerated rate. Diabetic patients are more likely to have coronary artery disease and myocardial infarction than are non-diabetic individuals.

***(v)Retinopathies:*** Retinal abnormalities develop in diabetic patients and are a major cause of blindness in the United States. The retinal changes are characterized by aneurysms, increased capillary permeability, small retinal hemorrhages and excessive micro vascular proliferation.

***(vi)Nonretinal Visual Problems:*** As blood glucose and blood osmolarity rise, the volume of the lens rise, distorting vision and the diabetic patients commonly have cataracts.

## CHEMICAL AGENTS CAPABLE OF INDUCING DIABETES[18]

- Alloxan
- Streptozotocin

## ALLOXAN INDUCED DIABETES

Alloxan, a cyclic urea analogue, was the first agent producing permanent diabetes in animals. It is a highly reactive molecule readily reduced to diuleric acid, then auto-oxidized back to alloxan resulting in the production of free radicals. These free radicals damage the DNA of β-cells and cause cell death. Second mechanism proposed for alloxan is its ability to react with protein SH groups, especially the membrane proteins like glucokinase on the β-cells, finally resulting in cell necrosis.

### *Drawbacks*

- High mortality in rats.
- Causes ketosis due to free fatty acid generation.
- Diabetes induced is reversible.
- Some species like guinea pigs are resistant to its diabetogenic action.

## STREPTOZOTOCIN INDUCED DIABETES

STZ [2-deoxy-2-(3-methyl-3-nitrosourea) 1-D-glucopyranose] is a broad-spectrum antibiotic, which is produced from *Streptomyces achromogens.* STZ causes β-cell damage by process of methylation, free radical generation and Nitric oxide production.

### *Advantages*

STZ has almost completely replaced alloxan for inducing diabetes because of:

- Greater selectivity towards β-cells
- Lower mortality rate and
- Longer or irreversible diabetes induction.

***Disadvantage***

Guinea pigs and rabbits are resistant to its diabetogenic action.

## HORMONE-INDUCED DIABETES MELLITUS

Dexamethasone, a long acting glucocorticoid, is used to produce NIDDM at a dose of 2-5mg/kg i.p. twice daily over a number of days in rats.

## INSULIN ANTIBODIES-INDUCED DIABETES

Giving bovine insulin along with CFA to guinea pigs produces anti-insulin antibodies. Intravenous injection of 0.25-1.0 ml guinea pig anti-insulin serum to rats induces a dose dependent increase in blood glucose levels up to 300%. This unique effect to guinea pig anti-insulin serum is due to neutralization of endogenous insulin by the insulin antibodies. It persists as long as the antibodies are capable of reacting with insulin remaining in the circulation. Slow i.v. infusion or i.p. injection prolongs the effect for more than a few hours. However, large doses and prolonged administration are accompanied by ketonemia, ketonuria, glycosuria and acidosis and are fatal to animals. After lower doses, the diabetic syndrome is reversible after a few hours.

## VIRAL AGENTS-INDUCED DIABETES

Viruses are thought to be one of the etiologic agents for IDDM. Viruses may produce diabetes mellitus by:

- Infecting and destroying of β-cells in pancreas,
- A less infecting or cytologic variant producing a comparable damage by eliciting immune auto reactivity to the β-cells,
- Viruses producing systemic effect, not directly affecting the β-cells.

Various human viruses used for inducing diabetes include RNA picornoviruses, Coxsackie-B4 (CB4), encephalomylocarditis (EMC-D and M variants), Mengo-2T, as well as two other double stranded RNA viruses, reovirus and lymphocytic choriomeningitis virus (LMCV, Armstrong variant).

## SURGICALLY INDUCED DIABETES

Surgical removal of all or part of the pancreas can induce Diabetes Mellitus. In partial pancreactectomy more than 90% of the organ must be removed to produce diabetes. Depending on the amount of intact pancreatic cells, diabetes may range in duration from a few days to several months. Total removal of the pancreas results in an insulin-dependent form of diabetes, and insulin therapy is required to maintain experimental animals. The portion of the pancreas usually left intact following a subtotal pancreatic resection is typically the anterior lobe or a portion thereof.

***Disadvantages***

- Surgical removal of pancreas results in loss of α- and δ-cells in addition to β-cells. This causes loss of counter-regulatory hormones, glucagon and somatostatin.
- There is a loss of the pancreatic enzymes necessary for proper digestion; therefore, the diet must be supplemented with pancreatic enzymes.

## MANAGEMENT OF DIABETES MELLITUS[19]

The goals of treatment for diabetes are to reduce and control blood glucose levels, relieve the symptoms of the disease and prevent complications. Intensive treatment and careful control of blood glucose levels can reduce the risk of complications of diabetes.[20]

## I. NON PHARMACOLOGICAL INTERVENTIONS:

The major environmental factors that increase the risk of type 2 diabetes, presumably in the setting of genetic risk are nutrition and sedentary lifestyle with consequent over weight and obesity. Medical nutrition therapy (i.e., diet) and exercise are important aspects of non-pharmacologic treatment for diabetes. Weight loss is a vital part of treatment for type 2 diabetes because it can help improve the sensitivity of cells to insulin and the uptake of glucose by cells.

A goal of medical nutrition therapy is to attain and maintain blood glucose levels in the normal range or as close to normal as possible. Patients with type 2 diabetes typically have dyslipidemia and another goal for these patients is to improve the lipid profile.

## II. PHARMACOLOGICAL INTERVENTIONS:

### A) Injectable anti-diabetic agents[21]

### 1) INSULIN:

All patients with type 1 diabetes require insulin injections. Patients with type 2 disease who have multiple symptoms of hyperglycemia are pregnant or have ketosis also should use insulin injections. Currently, insulin used for treatment is derived from beef and pork pancreas as well as recombinant (human) DNA technology.

In the 1980s, the human insulin was produced by recombinant DNA technology in *Escherichia coli.* The side effects of intensive treatment include serious hypoglycemia, local reactions (swelling, erythema and stinging), allergy and edema. Newer insulin delivery devices have been made to improve ease and accuracy of insulin administration. These are insulin syringes, pen devices, inhaled insulin, insulin pumps, and implantable pumps [20].

| Type | Zinc content | Action ( hours) | | |
|---|---|---|---|---|
| | | Onset | peak | Duration |
| **Rapid:** | | | | |
| a) Regular soluble | 0.01- 0.04 | 0.5-0.7 | 1.5-4 | 5-8 |
| b) Lispro | 0.02 | 0.25 | 0.5-1.5 | 2-5 |
| **Intermediate:** | | | | |
| a) NPH | 0.016 | 1-2 | 6-12 | 18-24 |
| b) Lente | 0.2-0.25 | 1-2 | 6-12 | 18-24 |
| **Slow:** | | | | |
| a) Ultralene | 0.2-0.25 | 4-6 | 16-18 | 20-36 |
| b) Protamine zinc | 0.2-0.25 | 4-6 | 14-20 | 24-36 |
| c) Glargine | 0.03 | 2-5 | 5-24 | 18-24 |

**2) GLUCAGON LIKE PEPTIDE- 1 (GLP- 1) AGONIST:**

The glucagon like peptide –1 (GLP-1) is an important incretin that is released from the gut in response to oral glucose. It is difficult to use clinically because of rapid degradation by the enzyme dipeptidyl peptidase-4 and it is injected subcutaneously twice daily one hour before meals acts for 6-10 hours. Nausea is an important side effect.

**3) AMYLIN AGONISTS:**

This synthetic amylin (a polypeptide produced by pancreatic β-cells which reduces glucagon secretion from α-cells and delays gastric emptying) analogue attenuates postprandial hyperglycemia when injected subcutaneously just before meal and exerts a centrally mediated anorectic action.

**B) Oral hypoglycemic drugs:**

Oral hypoglycemic drugs include sulfonyl ureas, biguanides, meglitinide /phenylalanine analogues, thiazolidinediones, α-glucosidase inhibitors, dipeptidyl peptidase inhibitors and incretin mimetics.

**1) SULFONYL UREAS: [e.g. Glibenclamide, Glimepiride, Glipizide, etc.]**

Sulfonyl ureas act primarily by increasing insulin secretion from pancreatic β-cells and cause hypoglycemia. Side effects include hypoglycemia, weight gain, allergic rashes and bone marrow damage.

**2) BIGUANIDES: [e.g. Metformin]**

Metformin reduces blood glucose concentrations by increasing glucose uptake in the peripheral muscles and decreasing the amount of glucose produced and released in the liver i.e., suppress hepatic gluconeogenesis. Side effects include lactic acidosis, vitamin $B_{12}$ deficiency and gastrointestinal disturbances.

**3) MEGLITINIDE / D-PHENYLALANINE ANALOGUES:**

**[e.g.Repaglinide, Nateglinide]**

The glinides stimulates insulin secretion, although they bind to a different site within the sulfonylurea receptor. They have a shorter circulating half-life than the sulfonylurea must be administered more frequently.

**4) THIAZOLIDINEDIONES:**

Thiazolidinediones are insulin sensitizers that increase insulin sensitivity and action in liver, muscle and fatty tissues to endogenous and exogenous insulin by binding and activating

the nuclear receptor peroxisome proliferator – activated receptor γ (PPAR-γ). Side effects include weight gain, fluid retention and GI disturbances.

**5) α-GLUCOSIDASE INHIBITORS :( e.g.Acarbose, Miglitol, Voglibose)**

These drugs inhibit the action of intestinal disaccharidases enzymes that break down carbohydrates. These oral anti-diabetic agents delay glucose absorption. Side effects include flatulence, abdominal bloating and diarrhea.

**6) DIPEPTIDYL PEPTIDASE INHIBITORS :( e.g.Sitagliptin)**

DPP-4 inhibitors increase insulin secretion and decrease glucagon level. It does not affect weight and does not induce hypoglycemia. Side effects include Gastro intestinal disturbances, headache, peripheral edema, and severe pain in upper stomach spreading to back.

**7) INCRETIN MIMETICS :( e.g.Liraglutide)**

These drugs increase insulin secretion, suppress glucagon secretion and slows gastric emptying. Side effects include Gastro intestinal disturbances, headache, dizziness, asthenia, hypoglycemia, increase sweating and injection site reaction.

## ALTERNATIVE MEDICINES FOR DIABETES[22]

Insulin therapy is the only satisfactory approach in diabetes mellitus, even though it has several drawbacks like insulin resistance, anorexia, brain atrophy and fatty liver in chronic treatment. There are several oral hypoglycemic agents used therapeutically but certain adverse effects and weak effectiveness of them has led to the search for more effective agents. Therefore, herbal drugs are gradually gaining popularity in the treatment of

diabetes mellitus. The major qualities of herbal medicine seem to be their supposed efficacy, low incidence of serious adverse effects and low cost.

## MOST STUDIED AND COMMONLY USED ANTI DIABETIC MEDICINAL PLANTS

**Kumari et al., 1995,[23]**

*Allium cepa* (Onion) is an essential plant cultivated throughout India, belongs to the family Liliaceae. Various parts such as seedling, callus, bulb etc., are known to possess anti-diabetic activity. Investigations reveal the presence of sulfur containing amino acid, which when administered orally to alloxan-induced diabetic rats (200 mg/kg for 45 days) significantly controlled blood glucose and lipids in serum and tissues and normalized the activity of liver hexokinase, glucose-6-phosphatase and HMG-CoA reductase. The effect was in accordance with that of glibenclamide and insulin.

**Zacharias et al., 1980.[24]**

*Allium sativum* (Garlic) belongs to the family of Alliaceae. Aqueous extract of garlic increased hepatic glycogen, and free amino acid content when given orally to sucrose fed rabbits (10 ml/kg/day). Garlic is known to decrease fasting blood sugar, triglyceride level in serum liver and aorta and protein levels when compared with sucrose controls.

**Faiyaz Ahmed and Asna Urooj, et al., 2008[25].**

The present investigation evaluated the antihyperglycemic activity of the bark powder and aqueous extract of *Ficus glomerata* (Moraceae) in streptozotocin induced diabetic rats. Oral administration of bark powder (FGB) and aqueous extract (FGAE) at 500 mg/kg caused 21% and 52% reduction in fasting blood glucose, respectively and also

decreased glycosuria significantly. Histology of pancreas suggested normalization of islets of Langerhans and β-cells with respect to their number and cellular architecture. The results suggest that, the bark of *Ficus glomerata* has significant anti-hyperglycemic activity in experimental animals and has potential to be used as an adjunct in the management of diabetes mellitus.

**M.R.M.Rafiullah and A.W.Siddiqui et al., 2006.[26]**

The effect of aqueous extract of *Syzygium cumini.Linn*, *Gymnema sylvestre* (Retz) Schult and *Portulaca olearacea.Linn* were investigated in fasting normal and streptozotocin (STZ) induced diabetic rats. The effects of extract on oral glucose tolerance in normal fasting rats were also studied. The aqueous extracts of *S.cumini* (200 mg/kg) and *G. sylvestre* (200 mg/kg) decrease the blood glucose in normal rats significantly at 2 and 4 hour of extracts administration ($p < 0.05$, $p < 0.01$). The *S. cumini* and *G.sylvestre* extracts decrease the increase of glucose levels significantly ($p < 0.05$) at 90 and 180 minutes after the glucose load in glucose tolerance test. In STZ induced diabetic animals, the aqueous extracts of *S.cumini* and *G.sylvestre* decrease the blood glucose significantly ($p < 0.05$) at 4 hour. The aqueous extract of *P.olearacea* did not show any hypoglycemic activity.

**Rajasekaran et al., 2005.[27]**

*Aloe barbabensis*, commonly called as Aloe vera is a medicinal herb; belongs to the family Liliaceae; Leaf gel, Leaf pulp and dried sap are known to possess anti-diabetic and anti-oxidant activity oral administration of ethanoloic extract at a concentration of 300 mg/kg body weight for 21 days, proved more effective in controlling oxidative stress found in diabetes.

**Gomes et al., 1995, Anderson and Polansky, 2002.[30]**

*Camellia sinensis* belongs to the family Theaceae, commonly known as Tea. The blood glucose level lowering activity has been extensively investigated. Antihyperglycemic activity of hot water extract of green tea in STZ induced diabetic rats were studied by Gomes *et al.*(1995), these findings have been supported by Anderson and Polansky(2002). Tea polyphenols possess antioxidant capacity; have also been reported to inhibit α-amylase.

## AVAILABLE STUDIES ON *Portulaca quadrifida Linn.*

**Syed kamil M *et al.,* 2010.[31]**

The effect of ethonalic extract of *Portulaca quadrifida Linn.*were studied in mice using various models. The effect of ethonalic extract of *Portulaca quadrifida Linn.*on central and peripheral nerves system were studied by using spontaneous motor activity, anti-nociceptive activity, *in vivo* muscle relaxants activity (grip strength) and anti-convulsant activity. The extract (400 and 800 mg/kg i.p) showed a significant reduction in spontaneous motor activity, antinociceptive activity and also showed reduction in time to recover from the electrically induced convulsion. The effect of extract on grip strength was found non-significant. Results from the present study indicate that *Portulaca quadrifida Linn.* has significant effect on central nerves system but not on peripheral nerves system.

**Patil AG, Patil DA *et al.,* 2012[32]**

The herbal products today symbolize safety in contrast to synthetic drugs that are regarded unsafe to the human and environment. *Portulaca quadrifida Linn.* a traditional medicinal plant, valued for its benefits in the management of urinary and inflammatory disorders. The decoction of plant acts as anthelmintic and used in the treatment of stomach complaints and gonorrhea. The powder microscopy showed the presence of tannins, mucilage, steroidal compounds and carbohydrates.

**Syed kamil mulla and Paramjyothi swami, 2012[33]**

*Portulaca quadrifida Linn.* (Portulacaceae) is traditionally used for the treatment against various ailments in tropical and sub-tropical parts of India without any scientific knowledge. Polyphenols, present in different fruits and vegetables, have retained attention in

recent years. In the present study the effects of ethanol and polyphenol extracts of *Portulaca quadrifida Linn.* on the proliferation of human colon cancer HT-29 and a normal L-6 cell lines were investigated by MTT assay and Trypan blue dye exclusion assay followed by DNA fragmentation assay. The results confirm that the ethanolic and polyphenolic extracts exhibited fragmentation of DNA in HT-29 cell lines and are found less effective against normal L-6 cell lines indicating the cancer specific effect of *Portulaca quadrifida Linn.*

## PLANT PROFILE[29]

**Name** : Portulaca quadrifida L.

**Synonym** : Portulaca anceps A.Rich

Portulaca geniculate Royle

## TAXONOMY

**Kingdom** : Plantae

**Phylum** : Magnoliophyta

**Class** : Magnoliatae

**Order** : Caryophyllales

**Genus** : Portulaca

**Family** : Portulacaceae

**Vernacular names** :

| | | |
|---|---|---|
| English | - | chickenweed |
| Tamil | - | Siru pasalai, Tharai pasalai |
| Sanskrit | - | Paciri, Paviri |
| Telugu | - | Goddu paveli |
| Malayalam | - | Neelakeera |
| Others | - | Lunki buti, Pulikeerai |

**Habitat** : Arenosol, subtropical, tropical, prostrate herb, upto 20cm.

**Distribution** : Pan tropical, Common in tropical India and Africa, U.S. Virgin Islands and Asia.

## *Portulaca Quadrifida L.* – FLOWER

## WHOLE PLANT

## Description

**The plant:**

A small diffused succulent, annual herb/forb. Stem is rooting at the nodes. The diameter of stem was found to be 0.1 cm**.**

**Leaves:**

- Leaves are opposite, fleshy, ovate, acute apex, entire margin; stipulate with a ring of silvery hairs.
- The length of leaf is about 0.9 cm, and breadth is about 0.3 cm.

**Flowers:**

- Flowers are small, Dicot, terminal, and solitary, surrounded by a 4-leaved involucre and copious white hair.
- Petals are four and yellow in colour.

**Capsule:**

Capsule dehisces horizontally, and contains minutely tubercled seeds.

**Chemical composition:**

Alkaloids, glycosides, mucilage,

Tannins, proteins, flavonoids,

Saponins, carbohydrates, triterpenoids

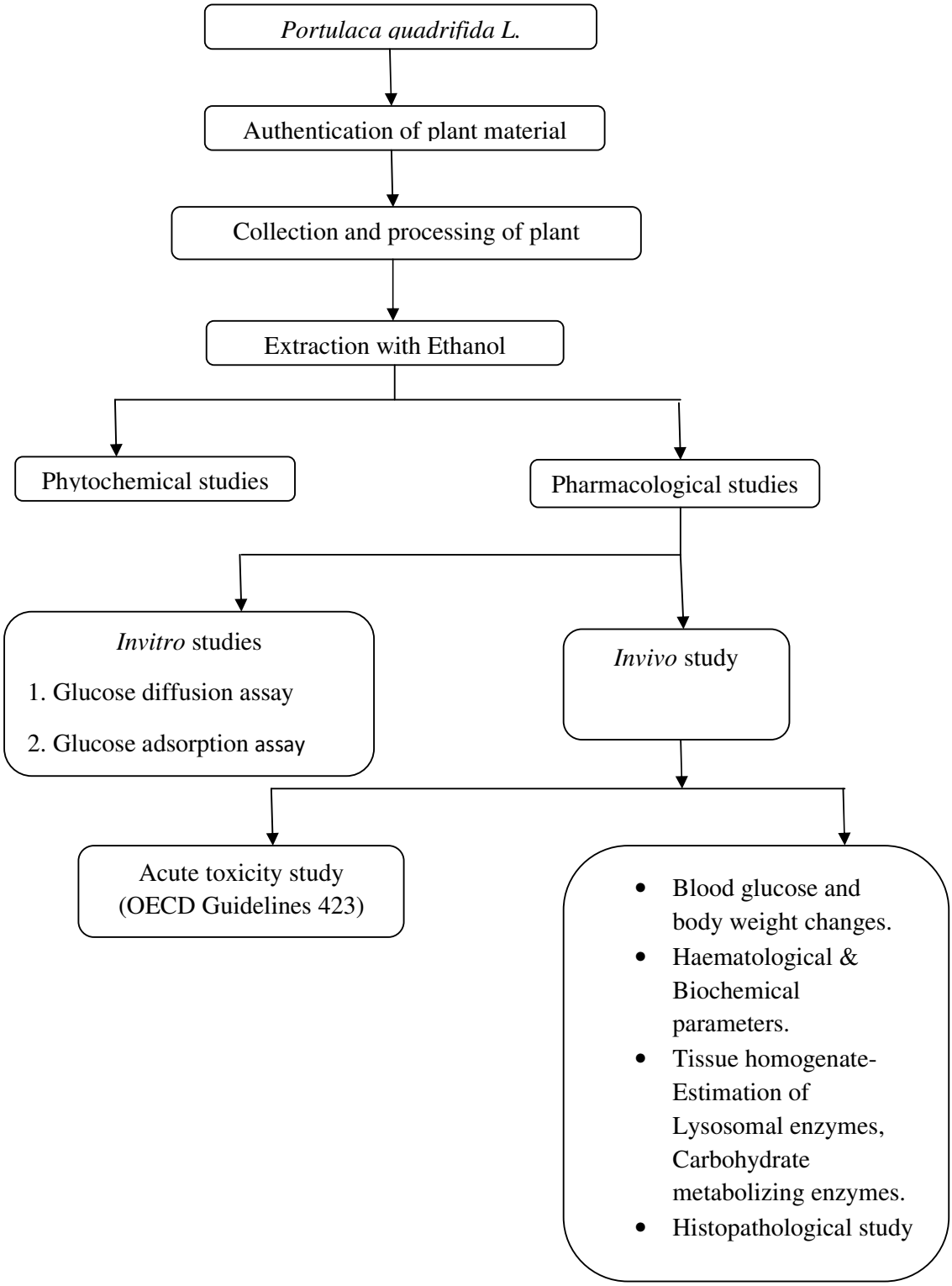
**Parts used:**

Whole plant.

**Properties and Use:**

- *Portulaca quadrifida L.* is used as a laxative.
- It cures fevers, asthma, cough and urinary disorders.
- Good for eye diseases, skin diseases and ulcers.
- Leaves are used as a local application to swellings.
- *Portulaca quadrifida L.* has been reported to possess antifungal activity against Aspergillus fumigates and Candida albicans.[31]
- It has a significant effect on central nervous system.[30]
- It has anticonvulsant activity and anticancer activity.[32]

**STUDY DESIGN**



# MATERIALS AND METHODS

## PLANT COLLECTION AND IDENTIFICATION

Dried entire plant of *Portulaca quadrifida L.* was collected from the forest around Komaneri, Tuticorin district, Tamilnadu (INDIA), in the month of July. It was authenticated by Prof. V. Chelladurai, Research Officer- Botany (Scientist - C) (Rtd), Central Council for Research in Ayurveda & Siddha, Govt. of India.

## PREPARATION OF PLANT EXTRACT

The powdered plant material (50 g) was extracted by hot continuous soxhlet extraction method. The plant material was extracted with ethanol (99.9% v/v) (500 ml) for 2 days in a percolator.

It is a process of continuous extraction method in which the solvent can be circulated through the extractor for several times. The vapours from the solvent are taken to the condenser and the condensed liquid is returned to the extract for continuous extraction. The apparatus consist of body of extractor (thimble) attached with side siphon tube, lower end attached with distillation flask and the mouth of extractor is fixed to the condenser by the standard joints.

**Procedure:**

- Weighed about 50g of dried powdered plant and transferred into thimble for packing.
- While packing, the content was wetted with ethanol (99.9% v/v) and then poured until the siphon tube was filled.
- A piece of porcelain was added into the round bottom flask to avoid bumping effect.

solution to second portion and the gelatin salt reagent to third portion. Precipitation with the latter reagent or with both the gelatin salt reagent was indicative of the presence of tannins. Precipitation of salt solution indicated a false-positive test. Positive tests were further confirmed by the addition of a few drops of dilute ferric chloride (1% $FeCl_3$) to test extracts, which gave black or green colouration.

The extract was mixed with lead acetate solution and observed for white precipitate.

**4. Test for saponins:**

- ***Foam test:*** A small amount of extract was extracted with petroleum ether. To the insoluble residue left after extraction, a few ml of water was added and shaken vigorously for 15 minutes and was observed for the formation of honeycomb froth that persisted for at least 30 minutes.

**5. Test for terpenoids:**

- ***Noller's test:*** The extract was warmed with tin and thionyl chloride. Pink colouration indicates the presence of terpenoids.

**6. Test for glycosides:**

- ***Borntrager's test:*** A small amount of extract was hydrolyzed with hydrochloric acid for few hours on a water bath and the hydrolysate was extracted with benzene. The benzene layer was treated with dilute solution and was observed for formation of reddish pink colour.

- ***Legal test:*** the extract was dissolved in pyridine and was made alkaline with few drops of 10% sodium hydroxide and then freshly prepared sodium nitroprusside was added and observed for the formation of blue colour.

**7. Test for phytosterols:**

- ***Libermann – Burchard test:*** 1 mg of the extract was dissolved in few drops of chloroform, 3 ml of acetic anhydride and 3 ml of glacial acetic acid. Warmed, cooled under tap water and drops of concentrated sulphuric acid was added along the side of the test tube, formation of bluish green colour indicates the presence of steroids.

## DRUGS AND CHEMICAL

Sodium Chloride, D- glucose, Ethanol, Sodium citrate tribasic-dehydrate, Citric acid monohydrate, Streptozotocin, Glibenclamide.

## *II) IN VITRO* ANTIDIABETIC EVALUATION

## PHYSICAL METHOD

Ability of the plant materials to retard the movement of glucose from the intestine into the blood was evaluated by physical methods *in vitro,* because the absorbed glucose from the intestine will cause the rise in postprandial hyperglycemia (PPHG) which complicates the diabetic condition associated with risk factors. The following are the convenient models for assessing the materials which affect the absorption of glucose *in vitro.*

**A. GLUCOSE DIFFUSION ASSAY[(33)]:**

Plant extracts were mixed with glucose and placed in the sealed dialysis membrane and kept, and in the orbit shaker bath at 37º C, at 150rpm, and the movement of glucose across the membrane into the external solution was measured at periodic intervals using commercial GOD-POD kit.

**REQUIREMENTS:**

- Dialysis membrane
- 0.15 M Sodium chloride solution
- D- (+) – Glucose - (25Mm in sodium chloride solution)
- Orbit shaker
- GOD – POD kit

**PROCEDURE:**

Dialysis membrane containing 2 ml of 25mM glucose solution was mixed with 1ml of different concentrations of plant extracts (100, 250,500,1000 and 1500µg/ml) and was placed in the centrifuge tube containing 45ml 0.15M NaCl and then kept in orbit shaker bath at 37ºC,at 150rpm. The movement of glucose into the external solution was monitored at 0, 1, 2, 3, 4, 5 hours using GOD-POD kit. Glucose concentration in the external solution was expected as mg/dl/hr.

**B.GLUCOSE ADSORPTION ASSAY[34]**

**Principle**

Glucose binding ability of the plant materials was determined by the method of Ou *et al*., 2001. Plant extract samples were incubated for 6 hours with various concentrations of glucose and the free glucose concentration at the end of the incubation period was determined using GOD-POD kit.

Sample (1%w/v) + Glucose solution (conc. In mM) —incubated at 37°C for 6 hours→ Centrifuged

↓

Glucose in supernatant was measured

**Requirements**

- Glucose solution (D-(+)-Glucose in various concentrations in distilled water)
- GOD-POD kit
- Orbit shaker bath

**Procedure**

Plant extracts (1%) were mixed with 25ml of glucose solution of increasing concentration (5, 10, 25, 50 and 100 mM) and incubated in the orbit shaker bath at room temperature for 6 hours, centrifuged at 4000 rpm for 20 minutes and the glucose content in the supernatant was determined by using the GOD-POD kit. Glucose bound was calculated using the formula:

$$\textbf{Glucose bound (nm)} = \frac{\mathbf{G_0 - G_6}}{\textbf{Weight of the sample (mg)}} \textbf{ x Vol. of the solution (ml)}$$

Where, $G_0$ – glucose concentration at '0' hours expressed as mg/dL

$G_6$ – glucose concentration at '6' hours expressed as mg/dL

## III) *IN VIVO* ANTI DIABETIC STUDIES

**Experimental Animals:**

The present study was conducted after obtaining approval from the Institutional Animal Ethics Committee and this protocol met the requirements of national guidelines of CPCSEA (PROPOSAL NO: IAEC 13/243/CPCSEA). The Wistar albino rats (150-200 gm) used for this study were procured from Animal house, Madras Medical College, Chennai, India.

**Quarantine and Acclimatization:**

Quarantine is the separation of newly received animals from those already in the facility until the health and possibly the microbial status of the newly received animals have been determined. The newly procured Wistar albino rats were quarantined for the period of one week to minimize the chance of introduction of pathogens into established animals and allowed to develop the psychological, physiological and nutritional stabilization before their use.

**Housing:**

The animals were housed in well ventilated animal house which was maintained at a constant temperature and relative humidity of 55 to 60%. The animals were housed in spacious polypropylene cages and paddy husk was utilized as bedding material.

**Diet and water:**

The animals were maintained on standard pellet diet and purified water. The animals were provided with food and water ad libitum except during fasting. The bed material was changed twice a week.

**Animal identification:**

All animal cages used in the study had a proper identification i.e., labels. Each animal in the cage was marked either on head or body or tail with picric acid for their appropriate identification

**A) TOXICITY STUDIES:**

Acute toxicity was designed as per the OECD guidelines (423).[37]

**ACUTE TOXICITY STUDY:**

**Principles and purpose:**

The main purpose of acute toxicity is to evaluate the degree of toxicity in a quantitative and qualitative manner with the purpose of comparing it with other drug substances (e.g. other drug candidates for the same indication).

**Experimental Animals:**

Three healthy adult Wistar albino rats weighing between 150-250g were selected for the study. For all the three animals food, but not water was withheld overnight prior to dosing.

**Selection of dose levels and administration of doses:**

Being a traditional herbal medicine, the mortality was unlikely at the highest starting dose level (2000mg/kg body weight). Hence a limit test at one dose level of 2000mg/kg body weight was conducted in all the three animals.

**B) *INVIVO* ANTIDIABETIC EVALUATION:**

The antidiabetic activity of *Portulaca quadrifida Linn* was evaluated in diabetic Wistar rats. Diabetes was induced by intra-peritoneal injection of Streptozotocin 50mg/kg body weight. The antidiabetic effect of plant extract was compared with standard drug Glibenclamide.

**(i) Induction of diabetes in rats:**

After a week of acclimatization, the rats were subjected to overnight fasting. Diabetes was induced by intraperitoneal injection of Streptozotocin, freshly dissolved in citrate buffer pH 4.5. The animals were allowed to drink water 5% glucose solution overnight to overcome the drug induced hypoglycemia due to massive release of insulin from β-cells. After 3 days, blood glucose levels were measured and the animals with a blood concentration of more than 250 mg/dl were considered as diabetic and taken for the experiment. Administration of the plant extract was started on the $4^{th}$ day after streptozotocin injection and this was considered as the $1^{st}$ day of treatment, which was continued for 21 days.

**(ii)Experimental design:**

The fasting glucose and body weight of all animals were recorded at the beginning of the study. The blood glucose was checked by one – touch glucometer throughout the study, in the experiments, 24 rats were divided into 4 groups of six rats each.

GROUP 1: Normal control rats, received distilled water.

GROUP 2: STZ induced diabetic rats received distilled water and served as diabetic control for 21 days.

GROUP 3: STZ induced diabetic rats received standard drug Glibenclamide (5mg/kg BW p.o) for 21 days.

GROUP 4: STZ induced diabetic rats received the plant extract (200mg/kg BW p.o) for 21 days.

GROUP 5: STZ induced diabetic rats received the plant extract (400mg/kg BW p.o) for 21 days

For all rats, body weight was measured before the induction of diabetes (-4$^{th}$ day) and on 7, 14 and 21$^{st}$ day of the treatment. Blood glucose level was measured on 1, 7, 14 and 21$^{st}$ days throughout the study period by tail tip cutting method. At the end of the experiment on 21$^{st}$ day, sufficient blood was collected by retro-orbital bleeding from all the animals under anaesthesia for estimation of haematological and biochemical parameters.

**(iii) Biochemical parameters**

The blood samples were centrifuged at 3000rpm for 5 minutes using REMI (412 LAG) cooling centrifuge. The serum was kept at -80°C until analyzed. Levels of serum glutamate oxaloacetic transaminase (SGOT), serum glutamate pyruvic transaminase (SGPT), serum alkaline phosphatase (ALP), serum creatinine, urea, total cholesterol, triglycerides (TGL), HDL and total protein were determined with an automatic analytical instrument (Hitachi 911, Japan).

**A.ESTIMATION OF TOTAL PROTEIN (Biuret Method)[38]**

The serum content of the soluble proteins, those circulating in extracellular and intracellular fluids, has been used as a marker to aid in clinical diagnosis. Serum total protein including albumin is mainly involved in the maintenance of normal water distribution between tissues and the blood and responsible for maintaining the osmotic pressure of plasma and is used to transport many substances including macromolecules.

**Principle**

In the biuret reaction, a chelate is formed between the $Cu^{2+}$ ion and the peptide bonds of the proteins in alkaline solutions to form a violet coloured complex whose absorbance is

measured photometrically. The intensity of the colour produced is proportional to the concentration of the protein in the sample.

**$Cu^{2+}$ + Serum protein ⟶ Copper-protein complex**

**Reagents**

- Biuret reagent
- 1ml of protein standard
- Serum sample

**Procedure**

1ml of biuret reagent which is stored under 2-8°C and 10µl of serum sample/standard are mixed well and then it is incubated for 5 mins. The intensity of the colour is measured at 540 nm.

**Calculation**

$$\text{Total protein in gm/dL} = \frac{\text{Absorbance of test}}{\text{Absorbance of standard}} \times 5.5$$

**B.ESTIMATION OF LIPID PROFILE[39]**

**Principle**

The cholesterol esters and cholesterol present in the sample are acted upon by Cholesterol Esterase to release Cholesterol and Fatty acids. The Cholesterol is oxidized by Cholsterol Oxidase to yield 4-Cholesten 3-one and Hydrogen peroxide as by product. Hydrogen peroxide together with 4-aminoantipyrin and phenolic compound in the presence of peroxidase gives the coloured complex. The intensity of the colour is proportional to the total cholesterol in the sample and is measured at 550 nm or with Green filter.

**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-aminoantipyrine** $\xrightarrow{\text{peroxidase}}$ **Red quinone + $4H_2O$**

**Requirements**

- Enzyme reagent
- HDL ppt reagent

**Procedure**

1ml of enzyme reagent and 10µl of test/standard were mixed well and incubated at 37°C for 5mins. The absorbance of test and standard were measured at 505 nm or using Green filter.

**Calculation**

$$\text{Cholesterol conc. mg/dl} = \frac{\text{Absorbance of test}}{\text{Absorbance of standard}} \times \text{Conc. of Standard (200)}$$

**HDL Cholesterol[40]:**

**Procedure**

Step 1:

200µl of serum and 300 µl of HDL ppt reagent were mixed well and allowed to for 10mins. Then the mixture was centrifuged at 3000rpm for 10mins and the supernatant was separated.

Step 2:

1ml of enzyme reagent and 100 µl of supernatant from step A were mixed together and incubated for 5mins at 37°C. The absorbance is read at 505 nm.

**Calculation**

$$\text{HDL Cholesterol conc. mg/dl} = \frac{\text{Absorbance of test}}{\text{Absorbance of standard}} \times \text{Conc. of Standard (50)}$$

**TRIGLYCERIDE**[41]

In human nutrition, triglyceride is the most prevalent glycerol esters encountered. They constitute 95% of tissue storage fat are the predominant form of glycerol ester found in plasma. The investigation of triglyceride is part of the overall evaluation of lipids disorders.

**Principle**

$$\text{Triglyceride} + H_2O \xrightarrow{\text{LPL}} \text{Glycerol} + \text{free fatty acid}$$

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

$$\text{Glycerol-3-phosphate} + O_2 \xrightarrow{\text{GPO}} \text{DAP} + H_2O_2$$

$$H_2O_2 + \text{4AAP} + \text{3, 5-DHBS} \xrightarrow{\text{Peroxidase}} \text{Quinoeimine Dye} + 2H_2O_2$$

**Requirements**

- Pipes buffer pH 7.0
- 4-AAP 0.4mmol/l
- Magnesium 2mmol/l
- ATP 2mmol/l
- GK
- POD
- LPL
- GPO
- Surfactants

**Procedure**

1ml of reagent mixed with 10 µl of sample and incubated for 5min at 37°C.Then the absorbance is measured at 505 nm.

**Calculation**

Triglyceride mg/dl = $A_x/A_s$ x Concentration of Standard

Where, $A_x$ – absorbance of sample, $A_s$- absorbance of standard

**C. ESTIMATION OF UREA[41]**

Urea is the end product of the protein metabolism. It is synthesized in liver from the ammonia produced by the catabolism of ammonia acids. It is transported by the blood to the kidneys from where it is excreted. Increased levels are bound in renal diseases, urinary obstructions, shock, congestive heart failure and burns. Decreased levels are found in liver failure and pregnancy.

**Principle:**

Urea is an acidic medium condenses with Diacetyl monoxime at 100°c to form a red coloured complex. Intensity of the colour formed is directly proportional to the amount of urea present in the sample.

$$Urea + H_2O \xrightarrow{Urease} 2NH_3 + CO_2$$

Urea + Diacetyl monoxime ----------→ Red Coloured Complex

| **Contents:** | **25 ml** | **75 ml** |
|---|---|---|
| $L_1$: Urea Reagent | 75 ml | 150 ml |
| $L_2$: Acid Reagent | 75 ml | 150 ml |
| L3: DAM Reagent | 75 ml | 150 ml |

S: Urea Standard (40 mg/dl) 5 ml 5 ml

**Procedure**

| Addition sequence | Blank (B) ml | Standard (S) ml | Test (T) ml |
|---|---|---|---|
| Urea reagent ($L_1$) | 1.0 | 1.0 | 1.0 |
| Acid reagent ($L_2$) | 1.0 | 1.0 | 1.0 |
| DAM reagent | 1.0 | 1.0 | 1.0 |
| Distilled water | 0.01 | - | - |
| Urea Standard (S) | - | 0.01 | - |
| Sample | - | - | 0.01 |

Pipette into clean dry test tubes labeled as Blank (B), Standard (S) and Test (T).

Mix well and keep the test tubes in boiling water ($100^{o}$c) for 10min. Cool under running tap water and measure the absorbance of the standard (Abs. S), and Test sample (Abs. T) against the blank.

**Calculation**

$$\text{Urea in mg/ dl} = \frac{\text{Abs. T}}{\text{Abs. S}} \times 40$$

## D. ESTIMATION OF CREATININE[41]

Creatinine is a waste product formed from creatine phosphate, a high energy storage compound. It is removed from plasma by glomerular filtration and then excreted in urine. Creatinine is a useful indicator of renal function.

Elevated levels of creatinine are associated with abnormal renal function as it relates to glomerular filtration.

Serum creatinine levels are used in combination with Urea/BUN to differentiate between pre-renal and renal causes of azotemia (condition of increased BUN level)

**Principle**

Creatinine reacts with picric acid in alkaline environment to form an orange-red colour complex. Developing of this orange-red colour may be followed photometrically at 500-520 nm.

Creatinine + Picric acid $\xrightarrow{NaOH}$ orange-red formation

**Requirements**

- Picric acid 11mmol/l
- NaOH 0.3mmol/l
- Creatinine standard

**Procedure**

1ml of reagent and 100µl of sample/standard are mixed and incubated for 60seconds at 37°C. Then the absorbance ($A_1$) measured at 505 nm. After exactly 60second absorbance ($A_2$) was measured.

**Calculation**

Creatinine mg/dl = $A_2 - A_1$(sample)/ $A_2 - A_1$(standard) x concentration of standard

## E.ESTIMATION OF ASPARTATE AMINOTRANSFERASE (AST/ SGOT)[42]

Aspartate transaminase (AST) also referred to serum glutamate oxaloacetate transferase (SGOT) is an enzyme involved in amino acid metabolism. AST is widely distributed in liver, RBCs, heart, pancreas and kidney. Low level of SGOT in blood is observed in severe liver disease, myocardial infarction, heart failure, kidney disease and lung disease.

**Principle**

**α-Ketoglutarate + L-Aspartate** $\xrightarrow{\text{SGOT}}$ **L-Glutamate + Oxaloacetate**

**Oxaloacetate + NADH+ $H^+$** $\xrightarrow{\text{MDH}}$ **L-Malate + $NAD^+$**

The rate of NADH consumption is measured photometrically and is directly proportional to the SGOT concentration in the sample.

**Reagents**

- L-Aspartate >200mmol/l
- Malate dehydrogenase > 200mmol/l
- α-Ketoglutarate >35mmol/l
- NADH >1.05mmol/l

**Procedure**

800µl of L-Aspartate & Malate dehydrogenase and 200µl of α-Ketoglutarate are mixed together and incubated at 37°C for 2 minutes and 100µl of sample is added. The change in absorbance is measured at 340 nm.

**Calculation**

**AST = ΔAbs/ min x Factor (1746)**

## F.ESTIMATION OF ALANINE AMINOTRANSFERASE (ALT/ SGPT)[43]

Alanine aminotransferase / serum glutamate pyruvate transferase is an enzyme involved in amino acid metabolism.

**Principle**

**α-Ketoglutarate + L-Alanine** $\xrightarrow{\text{SGPT}}$ **L-Glutamate + Pyruvate**

**Pyruvate + NADH+ $H^+$** $\xrightarrow{\text{LDH}}$ **L-Lactate + $NAD^+$**

The rate of NADH consumption is measured photometrically and is directly proportional to the SGPT concentration in the sample.

**Reagents**

- L-Alanine >200mmol/l
- Lactate dehydrogenase > 1500mmol/l
- α-Ketoglutarate >35mmol/l
- NADH >1.05mmol/l
- Tris buffer 80mmol/l pH 7.5

**Procedure**

800µl of L-Alanine & Lactate dehydrogenase and 200µl of α-Ketoglutarate are mixed together and incubated at 37°C for 2 minutes and 100µl of sample is added. The change in absorbance is measured at 340 nm.

**Calculation**

**ALT = ΔAbs / min x Factor (1746)**

## (iv) Estimation in tissue homogenates

### A) PREPARATION OF TISSUE HOMOGENATE

At the end of 21st day, all the experimental animals were sacrificed and liver, pancreas and kidney were removed, free from adhering tissues and washed with ice-cold normal saline solution (0.9%). About 0.3g tissue was homogenized in 3ml of 0.01 M Tris-HCl with the help of Teflon-homogenizer. The homogenate was centrifuged at 10,000 rpm for 20mins at 4°C. The supernatant obtained was used for estimation of lysosomal enzymes and some carbohydrate metabolizing enzymes

### B) CARBOHYDRATE METABOLIZING ENZYMES ESTIMATION

### 1) HEXOKINASE ESTIMATION[44]

**Principle**

Hexokinase (HK) activity was determined by the method of Brandstrup et al., (1957). The assay is based upon the reduction of $NAD^+$ through a coupled reaction with glucose-6-phosphate dehydrogenase and is determined spectrophotometrically by measuring the increase in absorbance at 340nm.

**D-Glucose + ATP** $\xrightarrow{\text{hexokinase}}$ **Glucose-6-phosphate + ADP**

**Glucose-6-phosphate** $\xrightarrow{2P}$ **Gluconate-6-phosphate + $NADH^+H^+$**

One unit of activity reduces one micromole of $NAD^+$/min at 30°C and pH 8.0 under the specified conditions.

**Reagents**

- 0.05M Tris-Hcl buffer, pH8.0 with 13.3mM $MgCl_2$
- 0.67M Glucose in above Tris-$MgCl_2$ buffer
- 16.5mM Adenosine 5'Triphosphate in above Tris-$MgCl_2$ buffer
- 6.8mM NAD in above Tris-$MgCl_2$ buffer

- Glucose -6-phosphate dehydrogenase (G6PD)

**Procedure**

The assay mixture containing 2.28 ml of Tris-$MgCl_2$ buffer, 0.5 ml of 0.67M Glucose, 0.1 ml of 16.5mM ATP, 6.8mM NAD and 0.01 ml of G6PD was mixed well. To the above mixture 0.1ml of tissue homogenate was added and the increase in absorbance was measured at 340nm for 5minutes.

**2) ESTIMATION OF GLUCOSE-6-PHOSPHATE DEHYDROGENASE (G6PD)[45]**

**Principle**

G6PD activity was estimated by the method of Anderson & Nordlie *et al.,* (1968). This G6PD is rather unique in that it possesses dual coenzyme specificity. When assayed under conditions that are optimal for the particular coenzyme, the ratio of observed catalytic activity is NAD/NADP = 1.8. The reaction velocity is determined by measuring the increase in absorbance at 340nm resulting from the reduction of NAD or NADP.

**Reagents**

- 0.055M Tris-HCl buffer pH 7.8 containing 0.0033 M Magnesium Chloride
- 0.06M Nicotinamide Adenine Dinucleotide (NAD)
- 0.1M Glucose-6-phosphate
- G6PD enzyme

**Procedure**

The assay mixture containing 2.7 ml of 0.055M Tris-HCl buffer, pH7.8 with 0.0033M $MgCl_2$, 0.1ml of 0.06M NAD and 0.1 ml of Glucose-6-phosphate. 0.1 ml of tissue homogenate was added to the above assay mixture and the change in absorbance was measured at 340nm for 5mins

### 3) LACTATE DEHYDROGENASE (LDH) ASSAY[46]

**Principle**

The assay was performed according to King's *et al.,* (1965) method, when the enzyme is supplied with Pyruvate and $NADH^+$, the LDH catalyzed reaction starts to produce lactate. At certain time point the reaction is terminated by the addition of 2,4-Dinitrophenylhydrazine, which reacts with lactate at acidic pH. After alkalization (addition of NaOH) the resulting hydrazine derivate gives a yellowish-orange colour suitable for quantification by means of spectrophotometry at the wavelength of 440 nm.

**Requirements**

- 0.1M phosphate buffer (pH 7.5)
- NADH(6.6mM)
- Sodium pyruvate (30mM)
- Dinitro phenyl hydrazine

**Procedure**

0.1ml of tissue homogenate was added with 2.7 ml buffer, 0.1ml NADH, and 0.1ml sodium pyruvate. The mixture was heated for 15 mins at 37°C. Then 0.5ml of dinitro phenyl hydrazine was added and incubated at room temperature for 15 mins. The reaction was stopped by addition of 5ml 0.1N NaOH. The developed colour was measured at 440nm.

## C.LYSOSOMAL ENZYMES ESTIMATION IN TISSUE PREPARATIONS

### 1) ASSAY OF ALKALINE PHOSPHATASE (ALP)[47]

**Principle**

When the enzyme incubated with p-Nitro phenyl phosphate and Tris buffer (pH 9.6), in alkaline condition inorganic phosphate and p-Nitro phenol are formed by the catalytic

action of alkaline phosphatase. Amount of p-nitro phenol liberated by the enzyme is measured at 420 nm.

**2-amino-methyl-1-propanol + p-Nitro phenyl phosphate + $H_2O$**

↓

**4-Nitro phenol + 2-amino-2-methyl-1-propanol phosphate**

**Requirements**

- P-nitro phenyl phosphate (10 mM)
- Tris-HCl pH 9.6 (80mM)
- NaOH (0.1N)

**Procedure**

1ml of p-nitro phenyl phosphate and 1.5 ml of buffer were added with 100µl of homogenate. The mixture was incubated at 37°C for 30mins. Then the reaction was stopped by addition of 0.1 N NaOH. The absorbance of liberated p-nitro phenol was measured at 420nm.

**Calculation**

ALP U/I = ΔA/ 2764

**2) ASSAY OF ACID PHOSPHATASE (ACP)[48]**

**(α-Naphthylphosphate Kinetic method)**

**Principle**

In acidic condition, the incubation of α-Naphthylphosphate with ACP will liberate α-Naphthol and inorganic phosphate due to catalytic action of ACP. The α–Naphthol is coupled with Fast Red to form a diazo dye complex. The rate of formation of this complex is measured as an increase in absorbance which is proportional to the ACP activity in the sample.

**α –Naphtholphosphate + $H_2O$** $\xrightarrow{\text{ACP}}$ **α –Naphthol + Phosphate**

**α –Naphthol + Fast Red TR Salt ⟶ Diazo dye complex**

**Requirements**

- α –Naphthol phosphate (4.5mM)
- acetate buffer (pH5.0)
- NaOH (0.2N)

**Procedure**

1ml of α –Naphthol and 1.9 ml of buffer were added with 100µl of homogenate. The mixture was incubated at 37°C for 30mins. The absorbance of liberated α–Naphthol was measured at 420 nm.

**Calculation**

ACP activity in U/L = ΔA/min x 750

## HISTOPATHOLOGICAL STUDIES:

At the end of 21st day, all the animals were sacrificed to collect the pancreas and liver. The organs were rinsed in ice cold 0.9% saline and were fixed in 10% formalin embedded in paraffin and cut into 5 µm thick sections in a microtome. Sections were mounted on glass slides using standard techniques. After staining with Hematoxylin-Eosin, the sections were examined under 100X magnification and photographed under a light microscope equipped for photography (Olympus CK 40).

## STATISTICAL ANALYSIS:

All the parameters were analysed by ANAVO followed by Dunnet's test. The results were expressed as Mean ± SD. The statistical analysis was performed by using Graph and Prism, version 6.0 software.

## PRELIMINARY PHYTOCHEMICAL ANALYSIS

| TEST | RESULTS |
|---|---|
| **Test For Flavonoids** | |
| a) Shinado's test | Present |
| b) Sodium hydroxide test | Present |
| **Test For Tannins** | |
| With lead acetate | Present |
| **Test For Saponins** | |
| Foam test | Present |
| **Test For Tepenoids** | |
| With Tin and thionyl chloride | Present |
| **Test For Glycosides** | |
| a) Libermann-burchard's test | Present |
| b) Legal's test | Present |
| c) Borntrager's test | Present |
| **Test For Phytosterols** | |
| Libermann test | Present |
| **Test For Mucilage** | |
| Swelling test | Present |
| **Test For Protein** | |
| a) Biuret test | Present |
| b) Million's test | Present |
| **Test For Carbohydrates** | |
| Molish's test | Present |
| **Test For Alkaloids** | |
| a) Drangendroff's test | Present |
| b) Mayer's test | Present |
| c) Hager's test | Present |
| d) Wagner's test | Present |

**Table shows the preliminary phytochemical analysis of ethanolic extract of *Portulaca quadrifida L*.**

# TOXICITY STUDIES

## ACUTE TOXICITY STUDY:

The crude ethanolic extract of *Portulaca quadrifida L.* was subjected to acute toxicity study.

| S.NO. | PARAMETERS | RESULT |
|---|---|---|
| 1. | Toxic signs | Absent |
| 2. | Pre-terminal deaths | Nil |
| 3. | Body weight | No specific change |
| 4. | Cage side observation | Normal |
| 5. | Motor activity | Normal |
| 6. | Tremors | Absent |
| 7. | Convulsions | Absent |
| 8. | Straub reaction | Absent |
| 9. | Pilo erection | Absent |
| 10. | Righting reflex | Present |
| 11. | Lacrimation and salivation | Normal |
| 12. | Unusual vocalization | Absent |
| 13. | Sedation | Absent |
| 14. | Body temperature | Normal |
| 15. | Analgesia | Absent |
| 16. | Ptosis | Absent |
| 17. | Diarrhea | Absent |
| 18. | Skin colour | Normal |
| 19. | Respiration | Normal |
| 20. | Scratching | Absent |
| 21. | Grooming | Absent |
| 22. | Aggressiveness and restlessness | Absent |

# Results

Animals were observed for behavioral signs of toxicity like motor activity, tremor, ect., and no significant toxic signs were observed during 14 days. The results of the acute toxicological studies revealed that the administration of ethanolic extract of *Portulaca quadrifida L*. by oral route upto 2000mg/kg body weight did not produce any mortality and it was tolerated.

**Table 2 GLUCOSE ADSORPTION ASSAY**

| S.No. | Concentration of the Glucose Solution (mM) | Glucose bound (nm) |
|---|---|---|
| 1 | 5 | 8.6±0.88 |
| 2 | 10 | 23.5±1.31 |
| 3 | 25 | 39.5±0.71 |
| 4 | 50 | 53.73±0.87 |
| 5 | 100 | 26.77±o.74 |

**Table 2 shows the glucose adsorption capacity of PQ extract at 1% concentration, values expressed as mean glucose bound (nm) with SD.**

Glucose solution in a concentration of 50mM showed maximum glucose adsorption of 53 nm, at 100mM concentration the value was reduced to 27 nm.

**Figure 1 GLUCOSE ADSORPTION ASSAY**

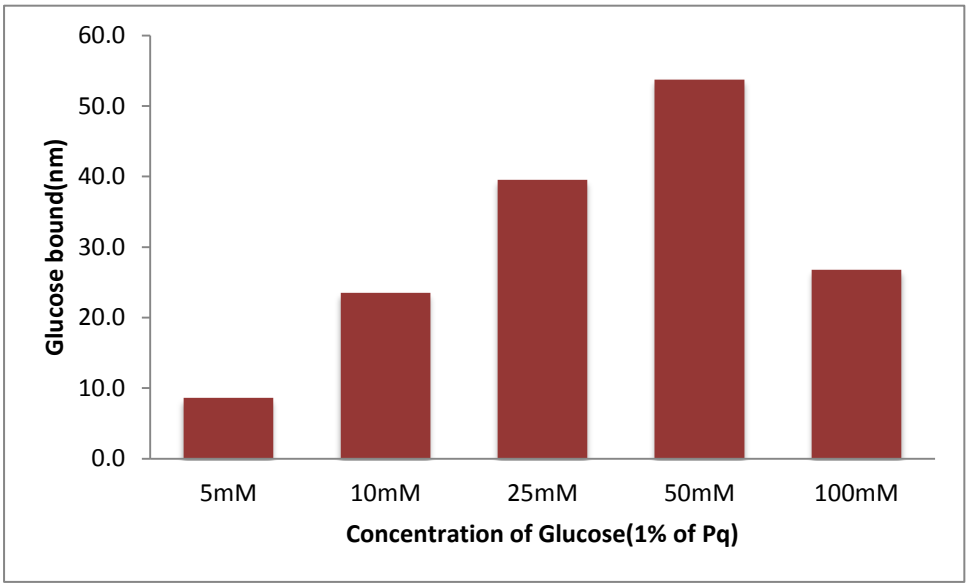



**Figure 1 shows the graphical representation of mean glucose adsorption**

**Table 3 GLUCOSE DIFFUSION ASSAY**

| Concentration (µg/ml) | Glucose concentration (mg/ml) | | | | | |
|---|---|---|---|---|---|---|
| | 0 hour | 1st hour | 2nd hour | 3rd hour | 4th hour | 5th hour |
| 100 | 2.48±0.08 | 33.69±1.44 | 44.44±1.69 | 43.54±0.49 | 47.37±1.13 | 66.83±2.23 |
| 250 | 2.79±0.02 | 32.02±1.75 | 41.9±2.52 | 45.07±1.26 | 45.96±2.07 | 54.76±0.81 |
| 500 | 2.41±0.01 | 27.93±1.34 | 38.2±1.03 | 42.1±1.12 | 443.2±2.81 | 50.63±1.52 |
| 1000 | 2.54±0.04 | 25.63±0.93 | 30.7±0.70 | 34.9±0.91 | 39.37±0.61 | 44.83±2.21 |
| 1500 | 2.43±0.17 | 23.63±0.82 | 25.63±0.52 | 27.1±1.39 | 34.1±4.06 | 33.37±1.44 |
| Control (0 µg/ml) | 3.16±0.34 | 35.37±1.15 | 47.3±0.4 | 53.57±0.49 | 58.87±1.59 | 73.57±2.16 |

**Table 3 shows the mean glucose intensity in the external solution of various concentrations of PQ at different time intervals. Glucose values are expressed as Mean ± SD**

PQ extract decreased the concentration of glucose into the external solution by retarding its diffusion through membrane. After 5 hrs the control showed 73.57 mg/dl of glucose in the external solution whereas the PQ extract at 1500µg/ml showed only 33.3 mg/dl of glucose. PQ extract possesses a dose dependent effect in glucose diffusion and it showed highest activity at concentration of 1500µg/ml.

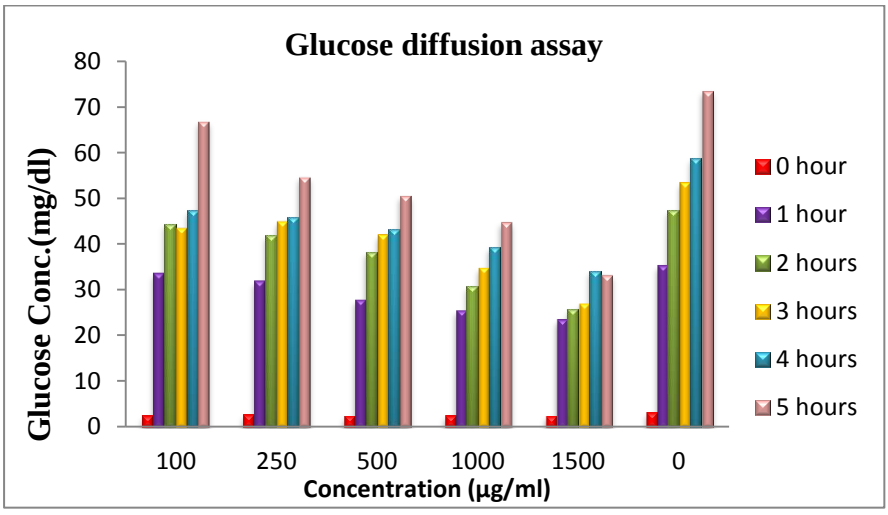



**Figure 2 is the graphical representation of Table**

## *INVIVO* ANTIDIABETIC ACTIVITY

**Table 4: Effect of ethanolic extract of *Portulaca quadrifida L.* on Body Weight in Streptozotocin induced diabetic rats.**

| GROUP | TREATMENT | AVERAGE BODY WEIGHT IN DAYS(gm) | | | |
|---|---|---|---|---|---|
| | | 1st Day | 7th Day | 14th Day | 21st Day |
| 1 | Normal control | 147.9±2.9 | 149.63±2.3 | 154.5±2.4 | 162.85±2.9 |
| 2 | Diabetic control STZ (150mg/kg) | 150.65±1.0 | 144.21±2.9 | 138.05±2.8 | 131.3±2.0 |
| 3 | Glibenclamide (5mg/kg) | 152.06 | 147.9±2.3 | 144.18±1.2 | 139.05±1.07 |
| 4 | *Portulaca quadrifida* (200mg/kg) | 151.7±1.49 | 144.5±1.32 | 141.75±1.9 | 136.5±3.15 |
| 5 | *Portulaca quadrifida* (400mg/kg) | 150.42±2.14 | 145.95±1.9 | 143.33±1.8 | 140.8±0.9 |

**Data were expressed as mean ±SD, n=6**

Table shows the mean body weight during the study period of all groups. The mean body weight showed a decrease in GP2, GP3, GP2 & GP5 at 21st day on comparing with 1st day.

GP2 (diabetic rats) showed a significant reduction in body weight $P<0.05$.when compared within the groups.

**Figure 3 EFFECT ON BODY WEIGHT**

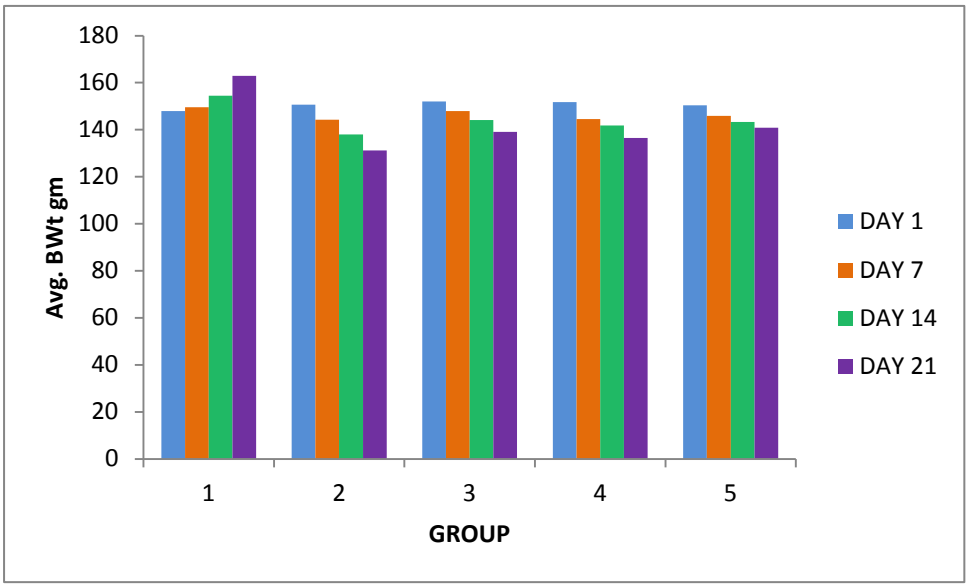



**Figure 3 shows the average body weight of STZ induced diabetic Wistar rats after 3 weeks of treatment with ethanolic extract of *Portulaca quadrifida L.***

**Table 5: Effect of ethanolic extract of *Portulaca quadrifida L* on Whole blood Glucose (WBG) in Streptozotocin induced diabetic rats.**

| GROUP | TREATMENT | Whole Blood Glucose (mg/dL) | | | |
|---|---|---|---|---|---|
| | | 1st Day | 7th Day | 14th Day | 21st Day |
| 1 | Normal control | 79.3±3.02 | 75.07±0.67 | 80.05±0.80 | 92.32±0.44 |
| 2 | Diabetic control STZ (150mg/kg) | 395.5±1.46 | 411.05±1.52 | 419.02±1.41 | 423.3±1.3 |
| 3 | Glibenclamide (5mg/kg) | 346.62±2.04 | 275.97±1.42 | 214.58±0.69 | 188.1±1.19 |
| 4 | *Portulaca quadrifida* (200mg/kg) | 355.22±7.53 | 281.81±1.01 | 218.48±1.44 | 190.93±1.06 |
| 5 | *Portulaca quadrifida* (400mg/kg) | 411.4±4.97 | 383.72±1.15 | 252.03±1.52 | 185.23±9.58 |

**Data were expressed as mean ±SD, n=6**

**Within group analysis:**

The mean blood glucose showed a significant reduction in both GP3, GP4& GP5 on comparing 21st day with 1st day

**Between group analysis:**

When compared with GP2, the mean body glucose level was lower in GP3, GP4 & GP3 at day 21. On comparing GP1 with GP2, GP3, GP4 & GP5, a statistical significant ($p<0.05$) difference was observed in GP2. On comparing GP2 with GP3, GP4 & GP5, a statistically significant ($p<0.05$) difference was seen in both the groups;

**Figure 4 MEAN BLOOD GLUCOSE**

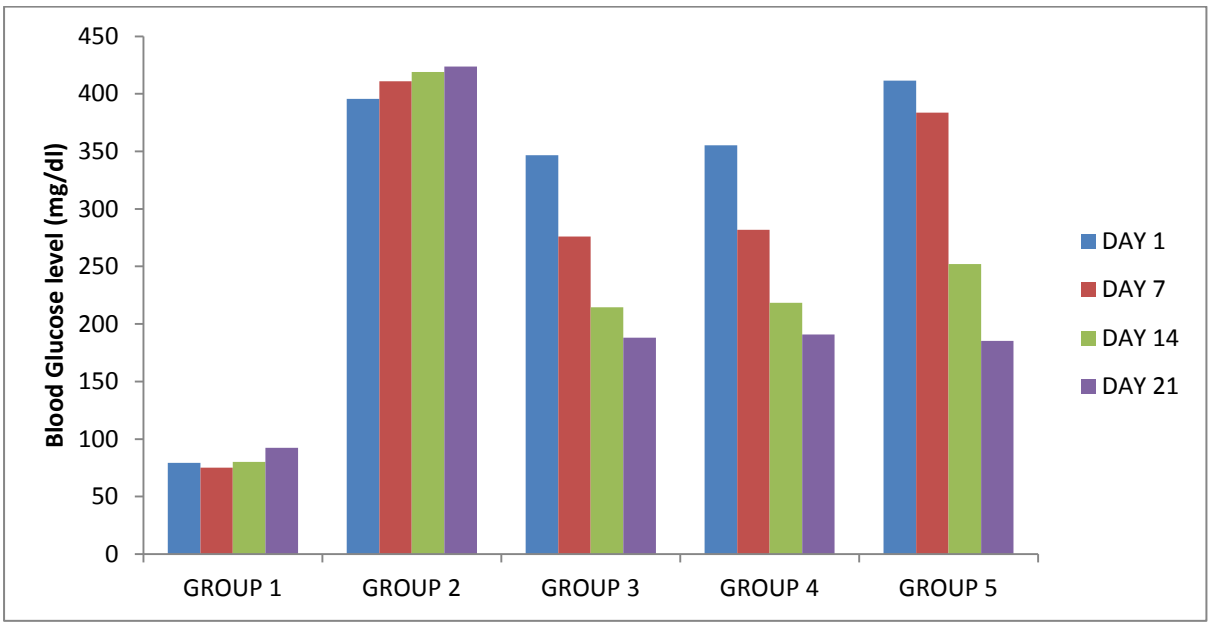



**Figure 4 is the graphical representation of mean blood glucose level.**

**Table 6** **LIPID PROFILE**

| Group | Treatment | Cholesterol | Triglycerides | HDL |
|---|---|---|---|---|
| 1 | Normal control | 109.2± 0.65 | 106.7± 0.63 | 16.6± 0.9 |
| 2 | Diabetic control STZ(150mg/kg) | 155.9± 3.5 | 252.2± 0.9 | 25.12± 0.49 |
| 3 | Glibenclamide (5mg/kg) | 130.63± 1.06 | 164.7± 0.6 | 16.6± 0.7 |
| 4 | Pq (200mg/kg) | 139.7± 0.6 | 172.9± 2.14 | 18.3± 0.57 |
| 5 | Pq (400mg/kg) | 133.4± 1.9 | 158.43±0.49 | 21.06± 1.8 |

**The value are expressed as mean± SD**

The mean cholesterol, triglycerides levels were higher in **GP2**, than the groups with **GP3, GP4** & **GP5.**

**GP2** Vs **GP3, GP4** & **GP5-** A significant ($p < 0.05$) decrease in Triglycerides Cholesterol level was observed

**Figure 5** **LIPID PROFILE**

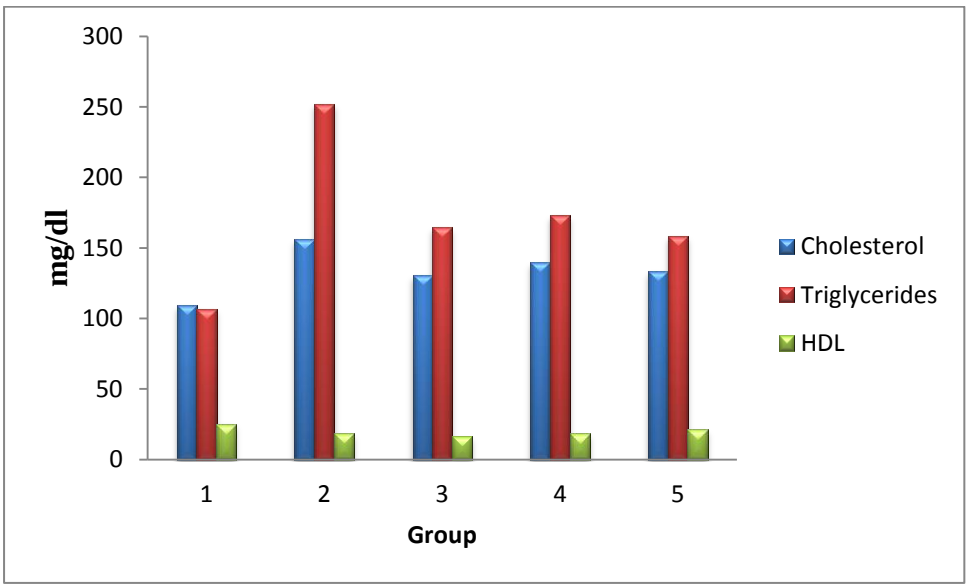



**Figure 5 is the graphical representation of Lipid profile level.**

**Table 7 ASSAY OF ALP (U/ml)**

| Group | Treatment | ALP (U/ml) |
|---|---|---|
| 1 | Normal control | 0.9±0.47 |
| 2 | Diabetic control (STZ 5mg/kg) | 7.1±0.21 |
| 3 | Glibenclamide (5mg/kg) | 1.7±0.21 |
| 4 | Pq (200mg/kg) | 1.4±0.25 |
| 5 | Pq (400mg/ml) | 1.3±0.23 |

The values are expressed as mean ± SD, n=6

In **GP2** the mean ALP was higher than GP1, GP3 & GP4.

GP1 Vs GP2, GP3, GP4 &GP5- A significant ($p < 0.05$) increase in ALP level was observed in GP2, GP3, GP4 & GP5.

**Figure 6 ALKALINE PHOSPHATE**

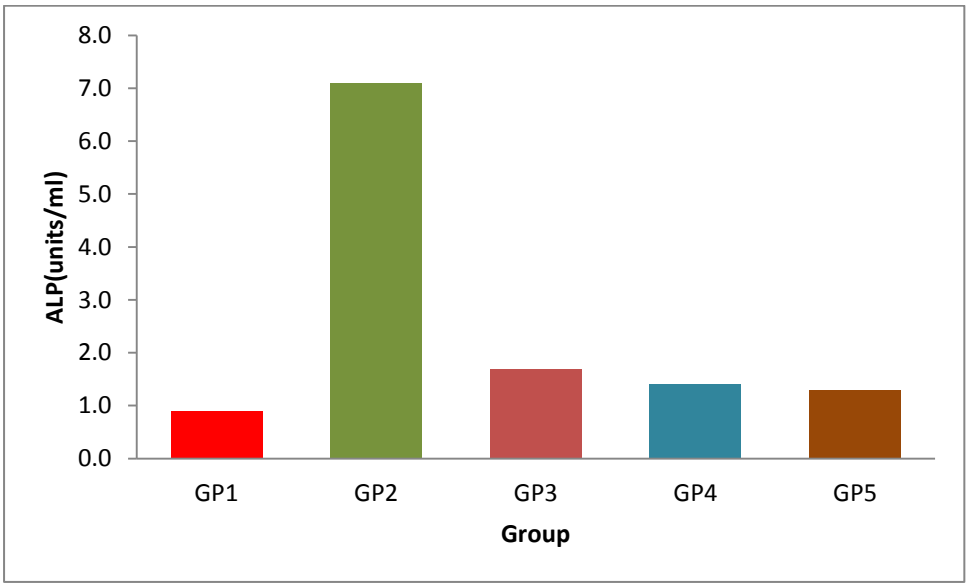



**Figure 6 is the graphical representation of ALP levels**

**Table 8 ASSAY OF ACP (U/ml)**

| Group | Treatment | ACP (U/ml) |
|---|---|---|
| 1 | Normal control | 0.6±0.2 |
| 2 | Diabetic control (STZ 5mg/kg) | 1.8±0.08 |
| 3 | Glibenclamide (5mg/kg) | 0.7±0.12 |
| 4 | Pq (200mg/kg) | 0.6±0.15 |
| 5 | Pq (400mg/ml) | 0.56±0.20 |

The values are expressed as mean ± SD, n=6

In GP2 the mean ACP level was higher than GP1, GP3, GP4 & GP5.

GP1 Vs GP2, GP3, GP4 & GP5 - A significant ($p<0.05$) decrease in ACP level was seen in GP3, GP4 & GP5.

**Figure 7 ACID PHOSPHATASE**

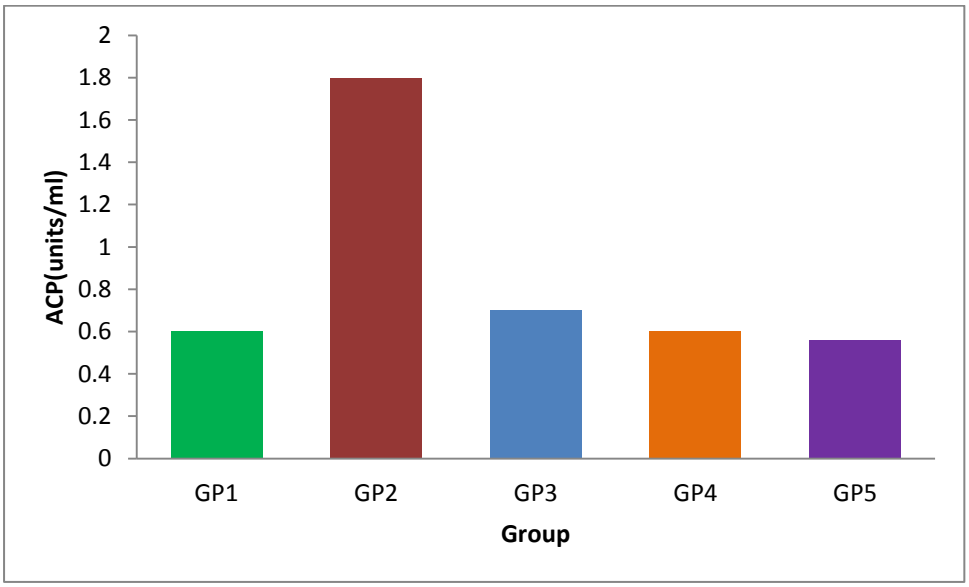



**Figure 7 is the graphical representation of ACP level**

**Table 9 ESTIMATION OF HEXOKINASE (U/ml) ACTIVITY**

| Group | Treatment | Hexokinase(U/ml) |
|---|---|---|
| 1 | Normal control | 2.5±0.08 |
| 2 | Diabetic control (STZ 5mg/kg) | 1.2±0.22 |
| 3 | Glibenclamide (5mg/kg) | 2.3±0.09 |
| 4 | Pq (200mg/kg) | 2.4±0.12 |
| 5 | Pq (400mg/ml) | 2.56±0.17 |

The values are expressed as mean ± SD, n=6

In GP2 the mean hexokinase level was lower than GP1, GP3, GP4 & GP5.

GP1 Vs GP2, GP3, GP4 & GP5- A significant ($p<0.05$) decrease in the HK level was observed in GP2

**Figure 8 HEXOKINASE**

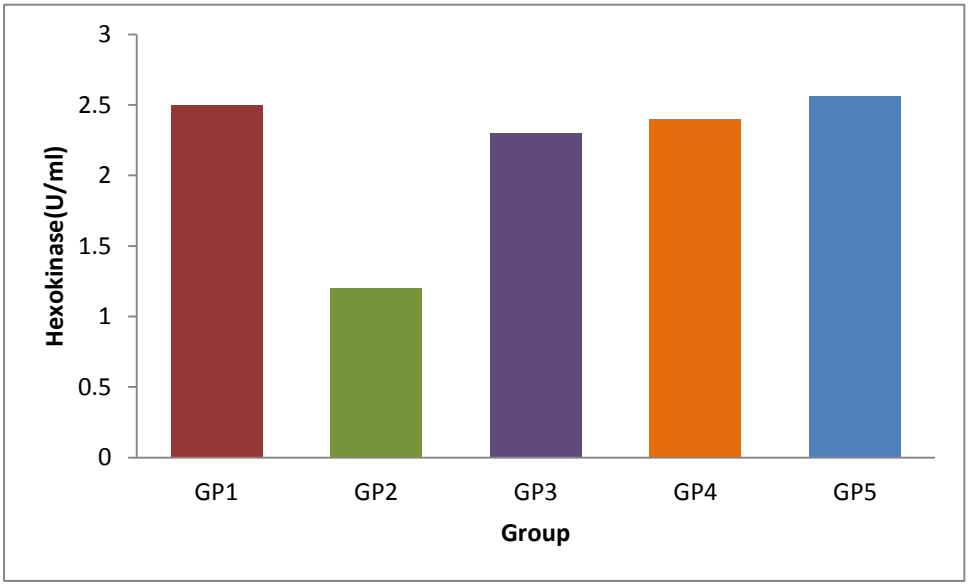



**Figure 8 is the graphical representation of Hexokinase activity.**

**Table 10 ESTIMATION OF G6PD (U/ml) ACTIVITY**

| Group | Treatment | G6PD(U/ml) |
|---|---|---|
| 1 | Normal control | 10.63±0.67 |
| 2 | Diabetic control (STZ 5mg/kg) | 2.4±0.22 |
| 3 | Glibenclamide (5mg/kg) | 3.56±0.2 |
| 4 | Pq (200mg/kg) | 3.36±0.6 |
| 5 | Pq (400mg/ml) | 3.43±0.25 |

The values are expressed as mean ± SD, n=6

In GP2 the mean G6PD level was lower than GP1, GP3, GP4 & GP5.

GP1 Vs GP2, GP3, GP4 & GP5- A significant ($p<0.05$) decrease in the G6PD level was observed in GP2

**Figure 9 G6PD ESTIMATION**

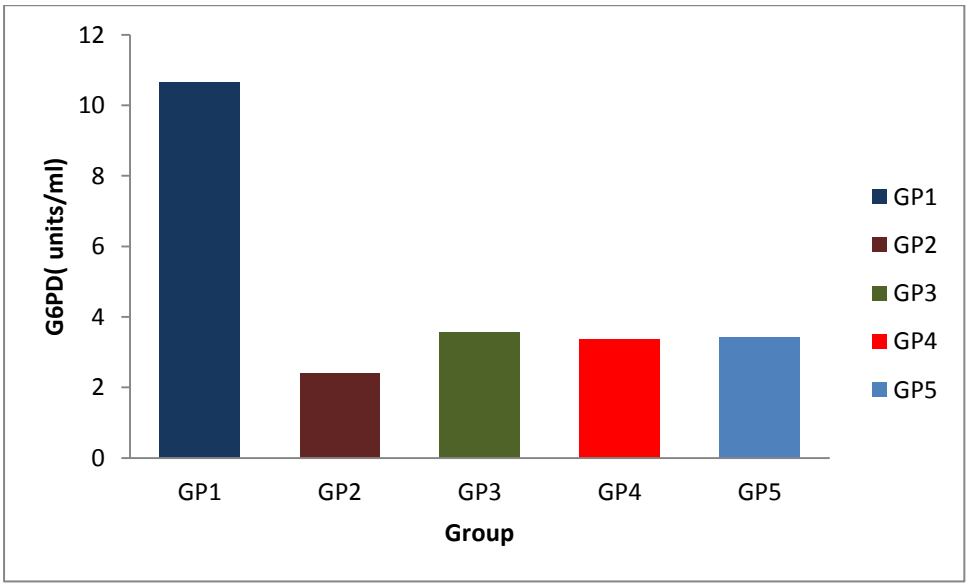



**Figure 9 the graphical representation of G6PD levels**

**Table 11 ESTIMATION OF LDH (U/ml) ACTIVITY**

| Group | Treatment | G6PD(U/ml) |
|---|---|---|
| 1 | Normal control | 13.63±0.7 |
| 2 | Diabetic control (STZ 5mg/kg) | 25.76±1.3 |
| 3 | Glibenclamide (5mg/kg) | 16.7±1.02 |
| 4 | Pq (200mg/kg) | 18.9±0.4 |
| 5 | Pq (400mg/ml) | 11.8±0.73 |

The values are expressed as mean ± SD, n=6

In GP2 the mean G6PD level was higher than GP1, GP3, GP4 & GP5.

A significant increase was observed in LDH level when compared to other groups.

GP1 Vs GP2, GP3, GP4 & GP5 - A significant ($p<0.05$) rise in the G6PD level was observed in GP2

**Figure 10 LDH ESTIMATION**

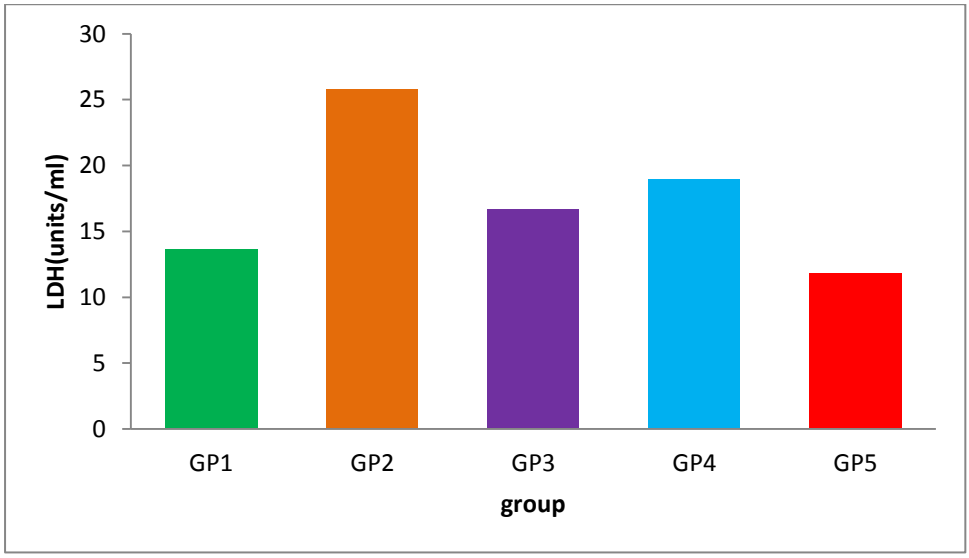



**Figure 10 is the graphical representation of LDH level**

**Table 12 ESTIMATION OF SGOT**

| Group | Treatment | SGOT(U/ml) |
|---|---|---|
| 1 | Normal control | 144.05±2.0 |
| 2 | Diabetic control (STZ 5mg/kg) | 205.1±3.15 |
| 3 | Glibenclamide (5mg/kg) | 172.9±1.84 |
| 4 | Pq (200mg/kg) | 163.23±2.4 |
| 5 | Pq (400mg/ml) | 159.38±2.52 |

The values are expressed as mean ± SD, n=6

In GP2 the mean SGOT level was higher than GP1, GP3, GP4 & GP5.

A significant increase was observed in SGOT level when compared to other groups.

GP1 Vs GP2, GP3, GP4 & GP5 - A significant ($p<0.05$) rise in the SGOT level was observed in GP2

**Figure 11 SGOT**

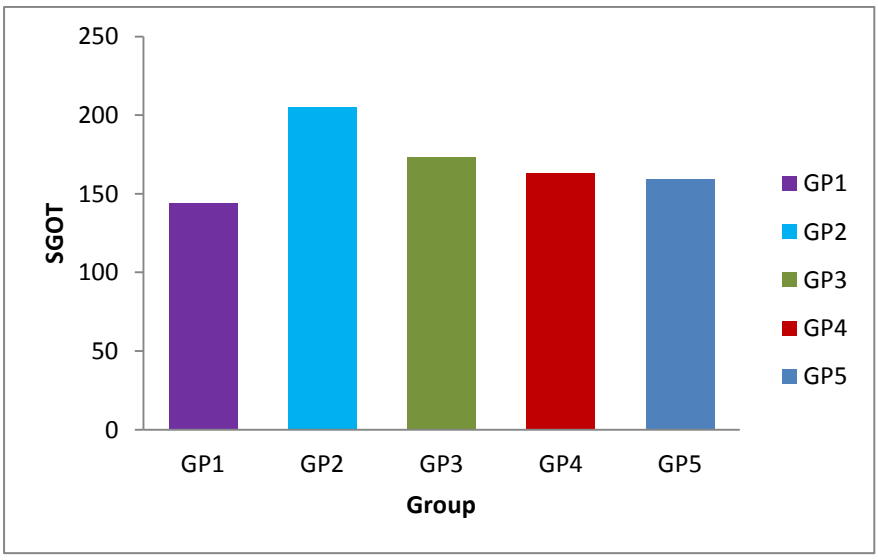



**Figure 11 is the graphical representation of SGOT level**

**Table 13 ESTIMATION OF SGPT**

| Group | Treatment | SGPT(U/ml) |
|---|---|---|
| 1 | Normal control | 103.68±2.0 |
| 2 | Diabetic control (STZ 5mg/kg) | 157.91±1.23 |
| 3 | Glibenclamide (5mg/kg) | 116.75±0.9 |
| 4 | Pq (200mg/kg) | 135.97±2.7 |
| 5 | Pq (400mg/ml) | 122.27±0.89 |

The values are expressed as mean ± SD, n=6

In GP2 the mean SGPT level was higher than GP1, GP3, GP4 & GP5.

A significant increase was observed in SGPT level when compared to other groups.

GP1 Vs GP2, GP3, GP4 & GP5 - A significant ($p<0.05$) rise in the SGOT level was observed in GP2

**Figure 12 SGPT**

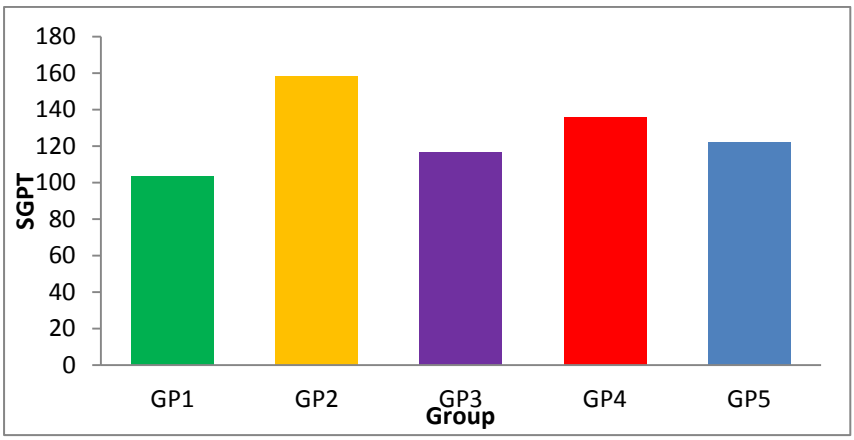



**Figure 12 is the graphical representation of SGPT**

**Table 14 TOTAL PROTEIN**

| Group | Treatment | Total protein |
|---|---|---|
| 1 | Normal control | 5.96± 0.55 |
| 2 | Diabetic control STZ(150mg/kg) | 4.31± 0.62 |
| 3 | Glibenclamide (5mg/kg) | 6.0±0.44 |
| 4 | Pq (200mg/kg) | 6.05± 0.62 |
| 5 | Pq (400mg/kg) | 5.98± 0.62 |

The values are expressed as mean± SD

On comparing GP1 with GP2, GP3, GP4 & GP5, there was a significant ($p<0.05$) decrease in total protein.

**Figure 13**

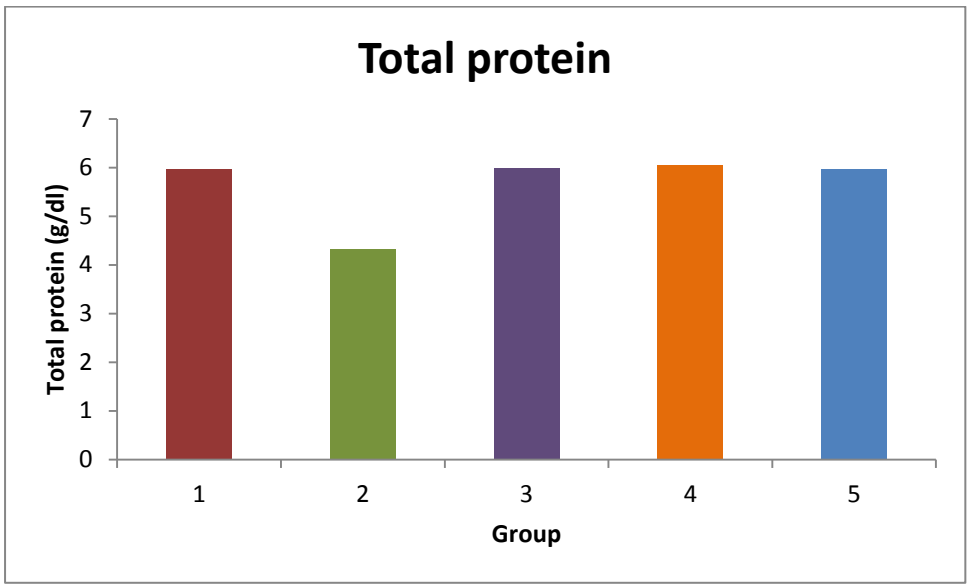



**Figure 13 is a graphical representation of Total protein**.

**Table 15 ESTIMATION OF UREA (mg/dl)**

| Group | Treatment | Urea |
|---|---|---|
| 1 | Normal control | 40.17± 1.74 |
| 2 | Diabetic control STZ(150mg/kg) | 48.08± 0.82 |
| 3 | Glibenclamide (5mg/kg) | 36.35±1.58 |
| 4 | Pq (200mg/kg) | 41.8± 2.59 |
| 5 | Pq (400mg/kg) | 35.11±0.66 |

The values are expressed as mean± SD

The mean blood urea level was higher in GP2 than other groups. A significant increase was observed in blood urea in GP2.

**Figure 14**

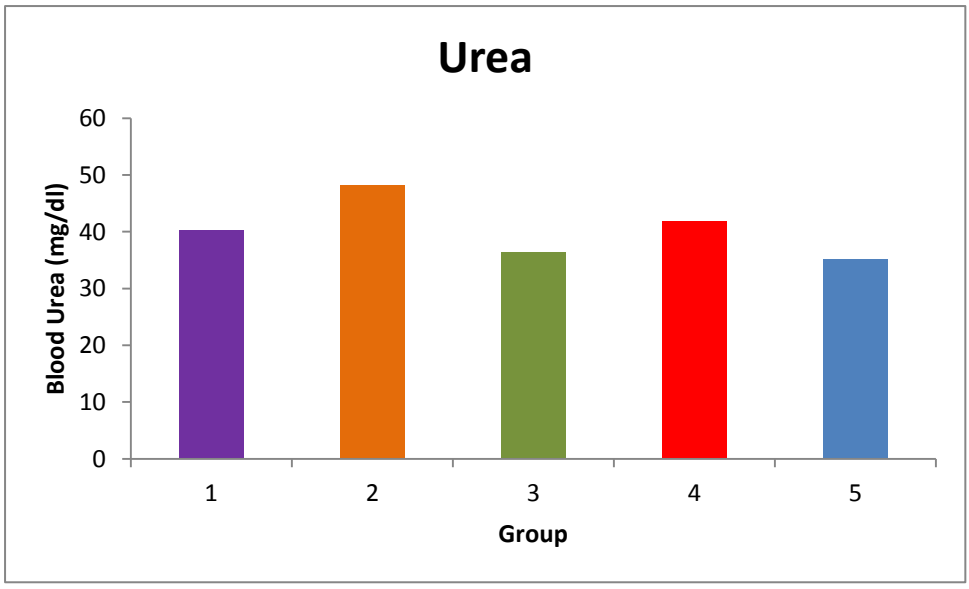



**Figure 14 is a graphical representation of blood urea**

**Table 16 ESTIMATION OF CREATININE**

| Group | Treatment | Creatinine |
|---|---|---|
| 1 | Normal control | 0.77± 0.17 |
| 2 | Diabetic control STZ(150mg/kg) | 1.58± 0.15 |
| 3 | Glibenclamide (5mg/kg) | 0.86± 0.12 |
| 4 | Pq (200mg/kg) | 1.1± 0.21 |
| 5 | Pq (400mg/kg) | 0.8± 0.16 |

The values are expressed as mean± SD

The mean blood urea level was higher in **GP2** than other groups. A significant increase was observed in blood urea in **GP2.**

On comparing GP4 Vs GP5, there was a significant difference in creatinine ($p< 0.05$).

**Figure 15**

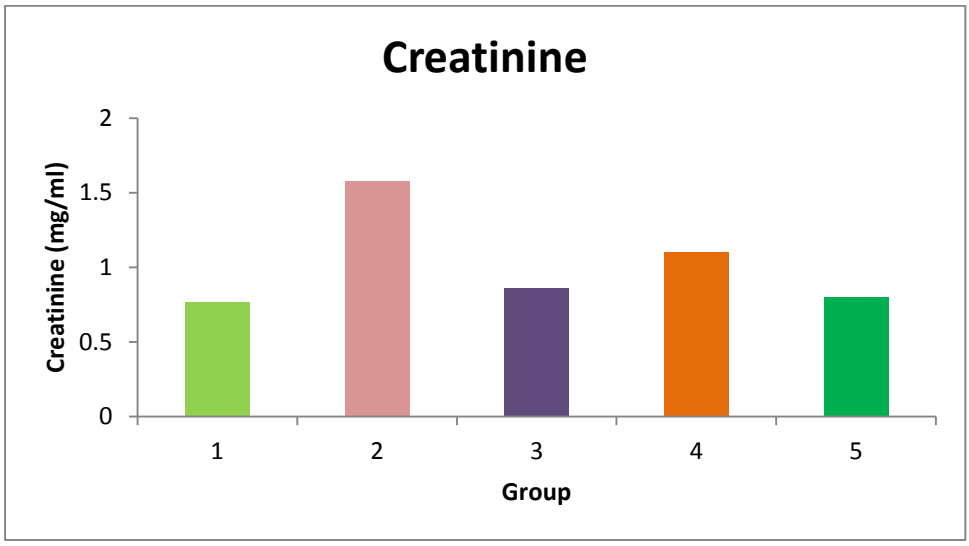
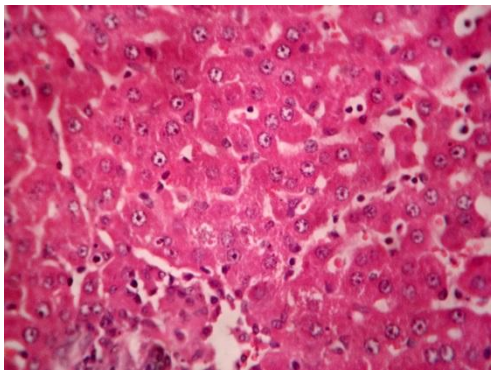
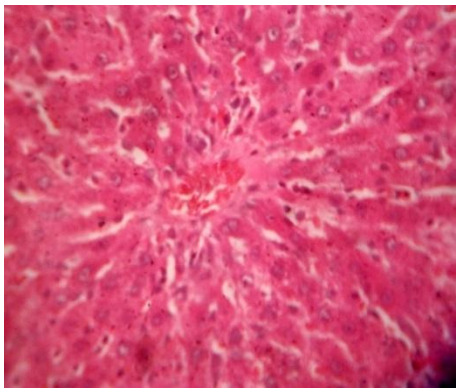



**Figure 15 is the graphical representation of Creatinine.**

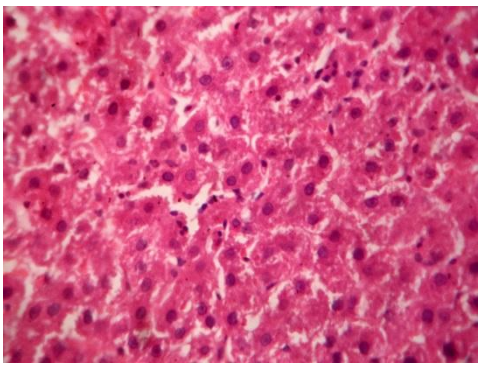
**HISTOPATHOLOGICAL STUDY OF LIVER**



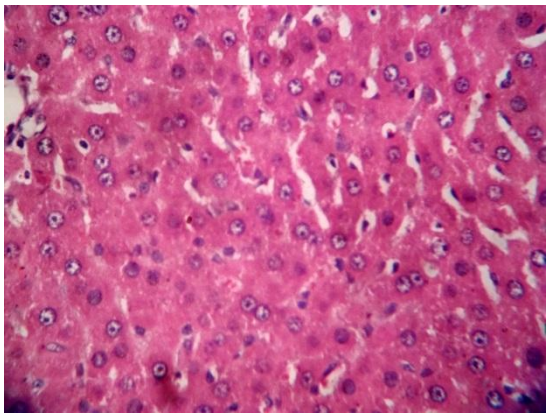
NORMAL CONTROL



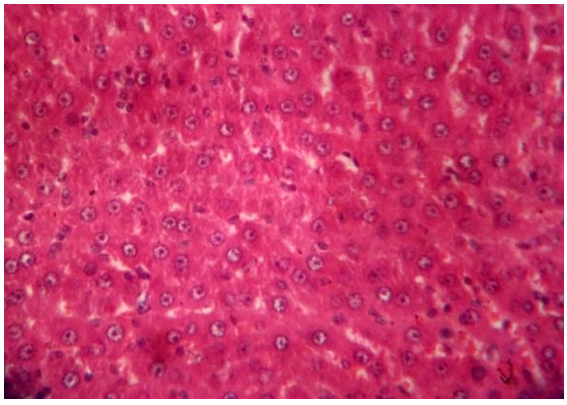
DIABETIC CONTROL



GLIBENCLAMIDE

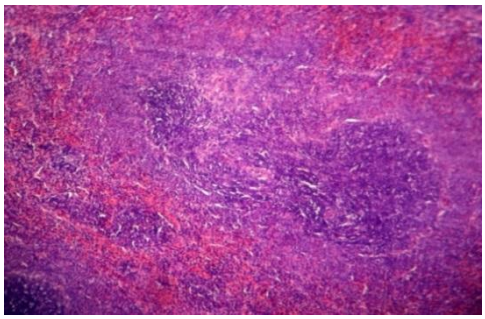


*Portulaca quadrifida L (200mg kg)*

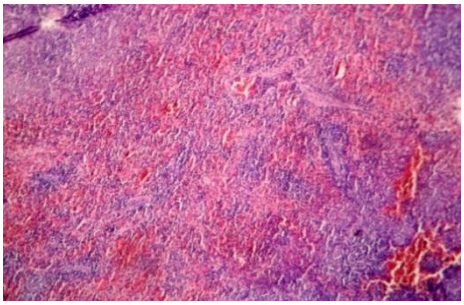


*Portulaca quadrifida L (400mg kg)*

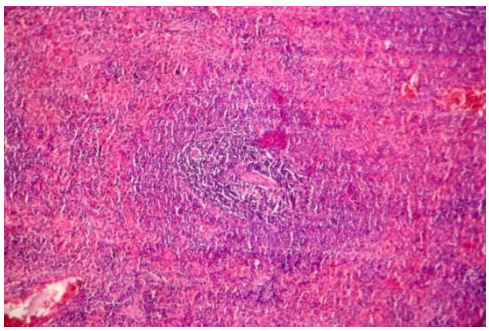
## HISTOPATHOLOGICAL STUDY OF PANCREAS



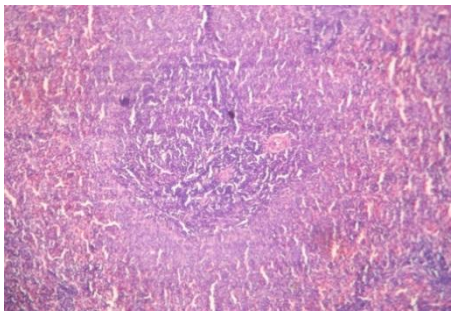
NORMAL CONTROL



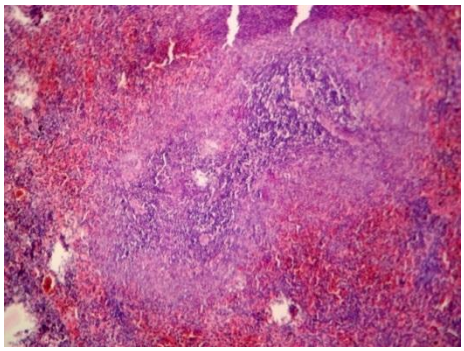
DIABETIC CONTROL



GLIBENCLAMIDE



*Portulaca quadrifida L (200mg kg)*



*Portulaca quadrifida L (400mg kg)*

## Discussion

Diabetes mellitus is a metabolic disorder, characterized by chronic hyperglycemia with disturbance of carbohydrate, fat and protein metabolism resulting from defects in insulin secretion, insulin action or both. Chronic hyperglycemia during diabetes leads to accelerated generation of reactive oxygen species (ROS) through oxidative phosphorylation during anaerobic glycolysis, glucose autoxidation, glucosamine pathway and a decline in the antioxidant defense. The ROS formed during hyperglycemia causes damage to the membrane lipids and proteins by lipid peroxidation and subsequent development of diabetic complications. Recently, there has been considerable interests in finding natural hypoglycemic agents from plant materials to replace synthetic ones to combat diabetic complications are going on relentlessly.[49]

The goals of treatment for diabetes are to reduce and control blood glucose levels, relieve the symptoms of disease, and prevent complications. The use of herbal medicine by the suffers of chronic diseases is encouraged by the concern about the adverse effects of chemical drugs and treatment using medicines of natural origin appears to offer gentler means of managing such diseases[50].

Over 150 plant extracts and some of the active principle including flavonoids are known to be used for the treatment of diabetes. In our study the whole plant extract of chemical constituents such as Carbohydrates, Flavonoids, Terpenes, Glycosides, Tannins, Saponins, Mucilage, Proteins and Sterols were used.

Acute toxicity study revealed that the ethanolic extract of *Potulaca quadrifida L.* was relatively nontoxic upto 2000mg/kg/bw p.o indirectly pronouncing safety profile of the extract.

*Portulaca quadrifida L.* is a tropical herb/forb which has been traditionally used in the management of DM. this present study has been undertaken to evaluate its antidiabetic property by preliminary phytochemical analysis, *in vitro,* Toxicological and *in vivo* studies in Wistar albino rats.

The phytochemical analysis of ethanolic extract of *Portulaca quadrifida L.* showed the presence of Alkaloids, Flavonoids, Tannins, Protein, Carbohydrates, Mucilage, Phytosterols, Saponins, Glycosides and Terpenoids.

Glucose adsorption capacity of *Portulaca quadrifida L.* extract showed maximum glucose bound at 50mM of glucose solution (53nm). Published research suggests that there is a direct relationship between a plant's ability to inhibit glucose absorption and viscosity of the plant's constituents such as soluble polysaccharides, supported by Gallagher *et al.,*(2003) and Wood *et al.,* (1994)[51]

The *invitro* antidiabetic evaluation was assessed by glucose diffusion assay. *Portulaca quadrifida L.* ethanolic extract showed maximum decrease in glucose diffusion when compared to control values. Glucose diffusion is useful *invitro* index to predict the effect of plant fibers on the delay in glucose absorption in GI tract. In addition to glucose adsorption, the retardation in glucose diffusion might be attributed to the physical obstacle presented by fiber particles towards glucose molecules and entrapment of glucose within the network formed by fibers. Based on this *invitro* study result, ethanolic extract of *Portulaca quadrifida L.* was selected for *invivo* study.

Vehicle control animals were found to be almost stable in their body weight but diabetic induced rats showed significant reduction in body weight. The administration of *Portulaca quadrifida L.* whole plant extracts (200,400 mg/kg) and Glibenclamide (5mg/kg) to the diabetic rats restored the changes in the body weight. On comparing 200mg Vs 400mg,

there was a significant difference in body weight (P<0.05). The dose dependent antidiabetic property of the ethanolic extract of *Portulaca quadrifida L.* exhibited improvement in body weight.

Plants may act on blood glucose through different mechanisms, some of them may have insulin-like substances.[52] Some may stimulate β-cells to produce more insulin and others may increase β-cells in the pancreas by activating regeneration of pancreatic cells. The fiber of the plants may also interfere with carbohydrate absorption; thereby affecting blood glucose.

In our study, after the administration of extract (200,400mg/kg), the fall in fasting blood glucose was evident at the end. This fall in fasting blood glucose level progressively increases till the end of 3rd week.

When the reduction glucose level with 200mg/kg & 400mg/kg were compared, there was a statically significant reduction in blood glucose levels (P<0.05) with 400mg of the extract.

The integrity of the lysosomal membrane is crucial to the maintenance of normal levels of lysosomal glycohydrolases in tissue and body fluids. A compromise of lysosomal membrane integrity may lead to an undesirable elevation of lysosomal enzymes in both intra- and extra-cellular space, which could pave the way for cellular and tissue disorders. Eg. Diabetes, arthritis, allergic response etc.[53]. In the present study the diabetic control group, there was an increase in alkaline phosphate and acid phosphate. This may due to the damage of the lysosomal membrane in the diabetic animals. But in the *Portulaca quadrifida L.* extract treated group, there was a significant reduction of these enzyme level which reveal the protective nature of the plant source.

The hexokinase involved in the phosphorylation step of glucose in glycolysis. Hepatic hexokinase is the most sensitive indicator of the glycolytic pathway in diabetes and its increased level can increase the utilization of blood glucose for glycogen storage in liver. Glucose-6-phosphate dehydrogenase activity was decreased in DM, resulting in diminished functioning of HMP pathway and there by the production of reducing equivalent such as NADH and NADPH.(54) In the present study, the hexokinase and Glucose-6-phosphate dehydrogenase activity were reduced in diabetic control group. The *Portulaca quadrifida L.* extract treated group, there was an increase in the activity of both hexokinase and Glucose-6-phosphate dehydrogenase. This shows that the *Portulaca quadrifida L.* improves the activity of carbohydrate metabolizing enzymes (HK & G6PD).

Lactate dehydrogenase (LDH) is a cytosolic enzyme that catalyzes the conversion of pyruvate to lactate in anaerobic glycolysis, which is subsequently converted to glucose in glucogenic flux. Increased activity of lactate dehydrogenase interferes with normal glucose metabolism and insulin secretion secretary defects in diabetes.(55) In the present study, the LDH activity was significantly elevated in diabetic control group. An increase in LDH activity is observed in diabetes due to impairment in glucose stimulated insulin secretion. In the *Portulaca quadrifida L.* extract treated group, the LDH activity was suppressed significantly. The suppression of LDH activity shows the beneficial effects of plant source.

The levels of total serum cholesterol, triglycerides and HDL were raised in diabetic rats which were lowered significantly with the treatment of *Portulaca quadrifida L.* On comparing groups 3,4,5 with group 2 there was a significant reduction in total cholesterol, triglycerides and HDL level ($P<0.05$). This shows that ethanolic extract of *Portulaca quadrifida L.* is effective in reducing the total cholesterol, triglycerides, HDL levels ($P<0.05$) in streptozotocin induced diabetic rats and its hypolipidemic effect could represent a

protective mechanism against the development of atherosclerosis which is usually associated with diabetes.

Ghosh and Suryawanshi, 2001 observed elevation in transaminase activity (SGOT and SGPT) in liver and kidney in diabetic rats.[56] Increased gluconeogenesis and ketogenesis observed in diabetic may be due to the high level in the activities of these transaminases. Similarly in our study also there was an increase in SGOT and SGPT in group 2 animals (Diabetic control). The restoration of SGOT and SGPT to their respective normal levels was seen after the treatment with both Glibenclamide and ethanolic extract of *Portulaca quadrifida L.* This supports the antidiabetogenic effect of *Portulaca quadrifida L.* Moreover, SGOT and SGPT levels also act as indicators of liver function and restoration of these parameters to normal levels indicate normal functioning of liver.

On comparing groups 3, 4 and 5 with group 2, there was a significant difference ($P<0.05$) in total protein. In diabetic control rats, there was a decrease in serum protein. *Portulaca quadrifida L.* extract prevented the reduction of serum protein in groups 4& 5. Group 5 showed a statistically significant difference when compared to group 4 ($P<0.05$).

Blood urea and creatinine was elevated in group 2 (Diabetic control). When comparing the groups 3, 4 & 5 with group 2, there was a significant difference ($P<0.05$) in blood urea and creatinine. This shows that in the groups which received *Portulaca quadrifida L.* extract, there was no significant increase in urea and creatinine.

The renewal of β cells in diabetes has been studied in different animal models. The total β-cells mass reflects the balance between the renewal and loss of these cells. It has been suggested that regeneration of islet β-cells following destruction by streptozotocin, may be the primary cause of the recovery of streptozotocin injected guinea pigs from the effect of the drug[57].

In our studies, the damage of pancreas in STZ treated diabetic control (Figure17) and regeneration of β-cells by Glibenclamide (Figure16) was observed. The regeneration in ethanolic extract of *Portulaca quadrifida L.* was comparable with Glibenclamide; *Portulaca quadrifida L.* extract (400mg/kg) was showed better regeneration than (200mg/kg) of *Portulaca quadrifida L.* extract.

Photomicrographical data in our studies reinforce healing of pancreas by ethanolic extract of 200mg/kg and 400mg/kg as a plausible mechanism of their antidiabetic activity.

The data of our studies suggests that *Portulaca quadrifida L.* has beneficial effects in diabetes mellitus holding the hope of a new generation for anti-diabetic drugs.

# CONCLUSION

- From the present study we conclude that the preliminary phytochemical analysis of *Portulaca quadrifida L.* indicated the presence of alkaloids, flavonoids, proteins, saponins, carbohydrates, terpenoids, mucilage, glycosides, tannins and phytosterols.
- *Invitro* Glucose adsorption assay of *Portulaca quadrifida L.* extract was evaluated using GOD-POD kit.
- *Invitro* Glucose diffusion properties of *Portulaca quadrifida L.* extract was evaluated using dialysis membrane and GOD-POD kit.
- Ethanolic extract of *Portulaca quadrifida L.* was safe in dose upto 2000mg/kg by acute toxicity study and there was no toxic effects produced.
- The ethanolic extract of *Portulaca quadrifida L.* exhibited significant antidiabetic activity in Streptozotocin induced diabetic rats.
- *In vivo* studies of *Portulaca quadrifida L.* ethanolic extract revealed the glucose lowering effect& improved activity of carbohydrate metabolizing enzymes. Thus it was concluded that the ethanolic extract of *Portulaca quadrifida L.* has anti-diabetic activity.

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