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ANTIDIABETIC ACTIVITY OF ETHANOLIC EXTRACT OF *PHYLLANTHUS VIRGATUS* IN STREPTOZOTOCIN AND HIGH FAT DIET INDUCED DIABETIC RATS

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ABSTRACT

Diabetes mellitus is a metabolic disorder characterized by hyperglycemia and alterations incarbohydrate, fat and protein metabolism, associated with the continuous destructionof β-cell leads to the disturbance inglucose homeostasis. Liver and pancreas are the two main organs which plays important role in maintaining the glucose level by regulating alternative pathways between glucose uptake and gluconeogenesis (Ferre*et al*., 1996). Diabetes is induced by streptozotocin (STZ) in animal model. Currently available drugs for the treatment of Diabetes mellitus have a number of limitations, such as adverse effects and a high rate of secondary failure Although there is a growing trend towards using natural remedies adjunct to conventional therapy, traditionally used plants might providea useful source of new hypoglycemic compounds. Although *Phyllanthus virgatus* may be described as a medicinal plant used for various purposes, no scientific reports exist on its antihyperglycemic effect.

KEYWORDS: Antidiabetic, Ethanolic Extract, Plant extracts, High Fat Diet, Phyllanthus virgatus.

INTRODUCTION

Diabetes mellitus is reaching as epidemic proportion across the globe, a metabolic disorder characterize by hyperglycemia resulting from defect in insulin secretion, insulin action or both (ADA, 2009). Currently, there are 40 million people with diabetes in India estimated to rise to almost 70 million by 2025 (IDF, 2006). It is estimated that 25% of the world population isaffected by this disease. Hyperglycemia is the most important factor in the onset and progress of diabetic complications mainly by producing oxidative stress (Giugliano *et al*., 1996). Altered cellular metabolism caused by hyperglycemia has been suggested to play an important role in increasing the risk of cardiovascular, renal, ophthalmic and neurological complications of diabetes mellitus (Brownlee and Cerami, 1981). Diabetes is a disease condition in which blood glucose level concentrations elevated i.e hyperglycemia due to loss of insulin secretion by pancreatic β-cells called type-I diabetes or loss of insulin responsiveness in targeted tissues referred as type-II diabetesor both (Schwarz, P.E *et al*., 2009).

It is one of the most common metabolic syndromes, since there are 200 million diabetic individuals in the world it is needed to understand the etiology and factors influencing onset of the disease(WHO, 1985). Pathogenic factors that are involved in the development of disease are auto-immune destruction of the β-cells of pancreas with consequent insulin deficiency to abnormalities results in insulin resistance. Deficient action of insulin on target tissues and hyperglycemia are the basis of the abnormalities in fat, carbohydrate, and protein metabolism, causing diabetic characteristic clinical features, increased risk of cardiovascular disease, micro and macro vascular complications (WHO, 1985). Diabetes mellitus is a metabolic disorder characterized by hyperglycemia, Polyuria, glycosuria. Reasons for this rise include sedentary lifestyle, obesity, consumption ofenergy rich diet, higher life span.

Obesity is a medical condition of a global concern, particularly due to consistent association with diabetes mellitus, some types of cancer and hypertension. The passage from obesity to diabetes is by progressive defect in insulin secretion coupled with a progressive rise in insulin resistance.

1.1 Etiologic Classification of Diabetes Mellitus: (Card JW and Magnuson BA, 2011).

i. Type I diabetes (β-cell destruction, insulin deficiency)

A. Immune-mediated

B. Idiopathic

Type-1 diabetes mellitus is insulin dependent diabetes mellitus and is seen in children so called as Juvenile diabetes. It is genetically predisposed which involves environmental factors such as toxic substances, viral infections; and autoimmune factors such as lymphocyte infiltration, insulitis. This results in the stimulation of immunology system like cell and antibody mediated immunity. As a result destruction of beta cell leads to lack of insulin secretion results in insulin dependent diabetes mellitus.

ii. Type II Diabetes (insulin resistance, insulin sensitivity)

Type-II Diabetes mellitus is a non-insulin dependent Diabetes mellitus also called as adult onset diabetes, which affects mainly due to hereditary factors, obesity, incomplete suppression of hepatic glucose output, impaired triglyceride uptake by fat and sedentary lifestyle. Type-II diabetes mainly occurs due to insulin sensitivity or resistance results in beta cell dysfunction and exhaustion due to which impairment of insulin secretion occur. Beta cells increase the secretion of insulin at this stage high insulin levels will overcome the independence to the action of insulin which persists for many years. This ultimately leads to non insulin dependent diabetes mellitus.

iii. Other specific types of diabetes

A. Genetic defects of beta cell function characterized by mutations in:

1. Hepatocyte nuclear transcription factor (HNF) 4 (MODY 1)
2. Glucokinase (MODY 2)
3. HNF-1 (MODY 3)
4. Insulin promoter factor-1 (IPF-1; MODY 4)
5. HN F-1 (MODY 5)
6. NeuroD1 (MODY 6)
7. Proinsulin or insulin
8. Mitochondrial DNA
9. Subunits of ATP-sensitive potassium channel

B. Genetic defects in insulin action

1. Type A insulin resistance
2. Rabson-Mendenhall syndrome
3. Leprechaunism
4. Lipodystrophy syndromes

C. Diseases of the exocrine pancreas

Pancreatitis, Pancreatectomy, Cystic fibrosis, Fibrocalculous pancreatopathy, Hemochromatosis, Neoplasia, Mutations in carboxyl ester lipase.

D. Endocrinopathies

Glucagonoma, Cushing's syndrome, Hyperthyroidism, Pheochromocytoma, Somatostatinoma, Aldosteronoma, Acromegaly.

E. Drug or chemical induced

Glucocorticoids, Vacor (a rodenticide), Pentamidine, Diazoxide, Nicotinic acid, Adrenergic agonists, Hydantoins, Thiazides, Asparaginase, Interferon, Epinephrine, Protease inhibitors, Antipsychotics (atypicals and others).

F. Infections: Congenital rubella, coxsackievirus, cytomegalovirus.

G. Uncommon forms of immune-mediated diabetes

"Stiff-person" syndrome, Anti-insulin receptor antibodies.

H. Other genetic syndromes sometimes associated with diabetes

Down's syndrome, Friedreich's ataxia, Huntington's chorea, Klinefelter's syndrome, Laurence-Moon-Biedl syndrome, Myotonic dystrophy, Porphyria, Prader-Willi syndrome, Turner's syndrome, Wolfram's syndrome.

iv. Gestational diabetes mellitus (GDM)

Gestational diabetes mellitus is a condition in which women without previously diagnosed diabetes mellitus exhibits elevated blood glucose levels in the 3rd trimester caused by the not proper working of the insulin receptors. As with diabetes mellitus in pregnancy in general, babies born to mother with untreated gestational diabetes are typically at increased risk of problems such as large for gestational age (leads to delivery complications), jaundice and low blood sugar. It may prolong or terminate after the delivery.

1.2 SYMPTOMS

It is important to remember that diabetes can occur without anysymptoms. Diabetes is a complex disease and not everyone has thesame symptoms. In general, excess sugar in blood is removed bythe kidneys, using up large amounts of water in the process: soone of the most

common symptoms is that you may pass moreurine and drink a lot more in order to compensate for the loss of liquid.

i. Type-I diabetes mellitus

These symptoms may occur suddenly and must receive immediate medical attention.

- Excessive thirst
- Frequent urination, sometimes exhibited by return of bedwetting in previously trained
- children (urination in large quantities day and night)
- Sudden vision changes
- A sweet, fruity odor may be present in urine, on one's breath/body (caused by
- high amounts of keytones in the blood and/or urine)
- Extreme hunger (increased appetite)
- Rapid or unexplained weight loss
- Fatigue (weak and tired)
- Irritability and mood changes
- Drowsiness, lethargy
- Nausea and/or vomiting
- Abdominal pain
- Rapid, hard breathing (heavy, labored)
- Confusion, Stupor, Unconsciousness

ii. Type-II diabetes mellitus

These symptoms occurs gradually, however, they must receive immediate medical attention.

- Blurred vision
- Tingling or numbness in the legs, feet or fingers
- Frequent infections of the skin
- Recurring skin, gum or urinary tract infections
- Darker patches of skin usually in neck folds
- Itching of skin and/or genitals
- Drowsiness
- Slow healing of cuts and bruises
- Any of the symptoms listed under type 1 diabetes

(www.diabetes.org , www.jdrf.org , www.joslin.org or www.JBWfund.org)

1.3 PATHOPHYSIOLOGY

Insulin is the principal hormone that regulates the uptake of glucose from the blood into most of the cells such as muscles and fat cells, but not into the cells of central nervous system. So, the deficiency of insulin or insensitivity of insulin receptors plays a crucial role in all forms of diabetes (Madhava Chetty K, *et al*., 2007).

Humans are capable of digesting carbohydrates that are in food; starch and some disaccharides like sucrose, are converted into simple forms in few hours as monosaccharide like glucose, the principal carbohydrate energy source used by the body. The rest of the food matter passed on for processing by gut flora mainly in colon. Insulin is released in to the blood by the beta cells that are in the islets of langherhans of pancreas, in response to raising blood glucose levels, typically after eating. About two-thirds of the insulin is utilized by the body cells to absorb glucose from the blood as fuel, for conversion into other molecules and for storage.

Insulin is also the principal signal that controls the conversion of glucose to glycogen for internal storage in liver and muscle cells. Reduction in the insulin release from the beta cells and reverse conversion of glycogen to glucose when fall in glucose level results in lowering glucose levels that is controlled by the glucagon hormone which counter acts the action of insulin (Rama Rao Naidu B.V.A, 2005). Glucose that is produced from the internal liver cells stores as glycogen which re-enters the blood stream; when muscle cells lack the necessary export mechanism. Normally when the insulin levels are low liver cells do this which normally correlates with low levels of blood glucose (Yoganarasimhan, S.N, 2005).

Higher insulin levels increase in anabolic process, such as protein synthesis, fat storage, growth and duplication. Presence or lack of insulin is the principal signal in converting many of the bidirectional process of metabolism i.e from catabolism to anabolism and *vice versa.* Particularly low insulin level is the trigger for entering or leaving ketosis which is the fat burning metabolic phase.

If there is shortage of insulin availability, cells respond poorly to the effects of insulin called insulin sensitivity or resistance. Glucose will not have its usual effect when there is defect in insulin, so there will be no proper absorption by the body cells that require it, nor stored appropriately in the liver and muscles. The net effect is persistent high glucose levels in blood, other metabolic derangements, such as acidosis and poor protein synthesis.

Blood glucose levels are raises to about 9-10mmol/L beyond its renal threshold in certain conditions except during pregnancy, in such condition re-absorption of glucose in the proximal renal tubuli is incomplete and part of the glucose remains in the urine referred as glycosuria. Due to this the osmotic pressure of the urine increases that inhibits the re-absorption of water by the kidney results in increased urine production, excess urination known as polyuria and increased loss of fluid. Lost blood volume will be replaced from water by the body cells and other body compartments osmotically results in dehydration and increased thirst (Silverstein and Robert M, 1991).

A. Pathogenesis of insulin dependent DM (Type-I)

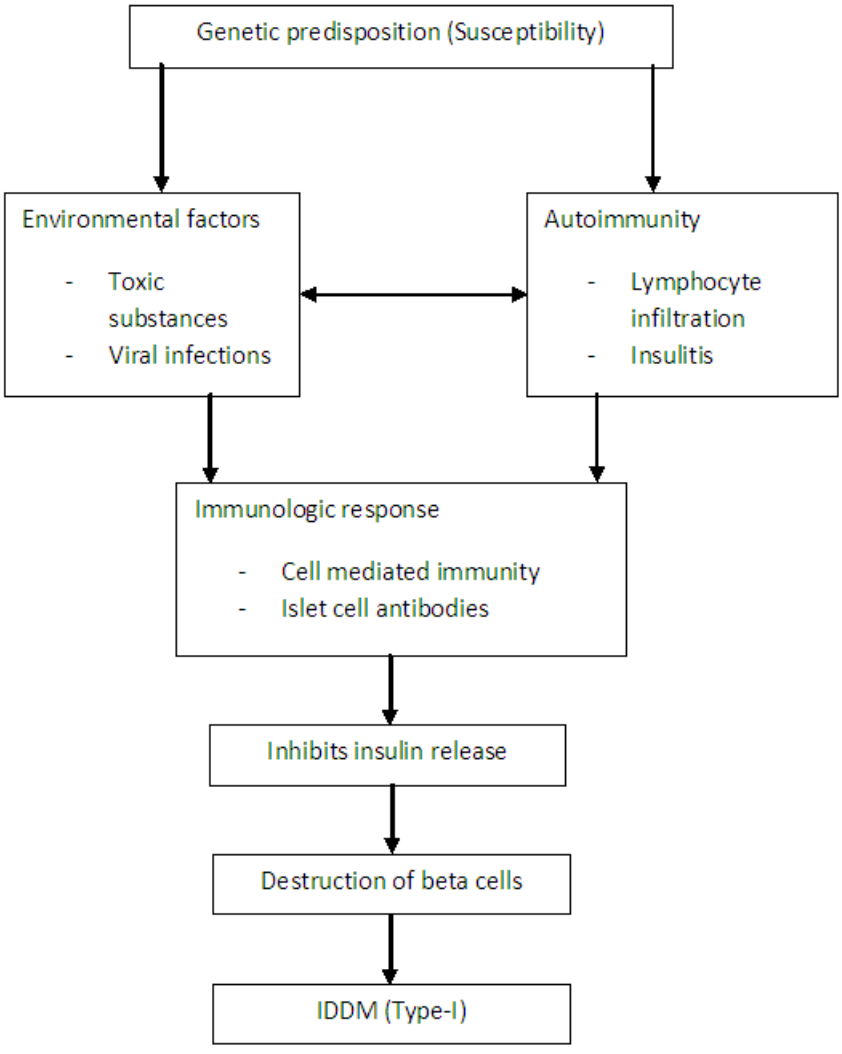

Fig. 1.1: Pathophysiology of type-I Diabetes mellitus

B. Pathogenesis of Non-Insulin dependent DM (Type-2)

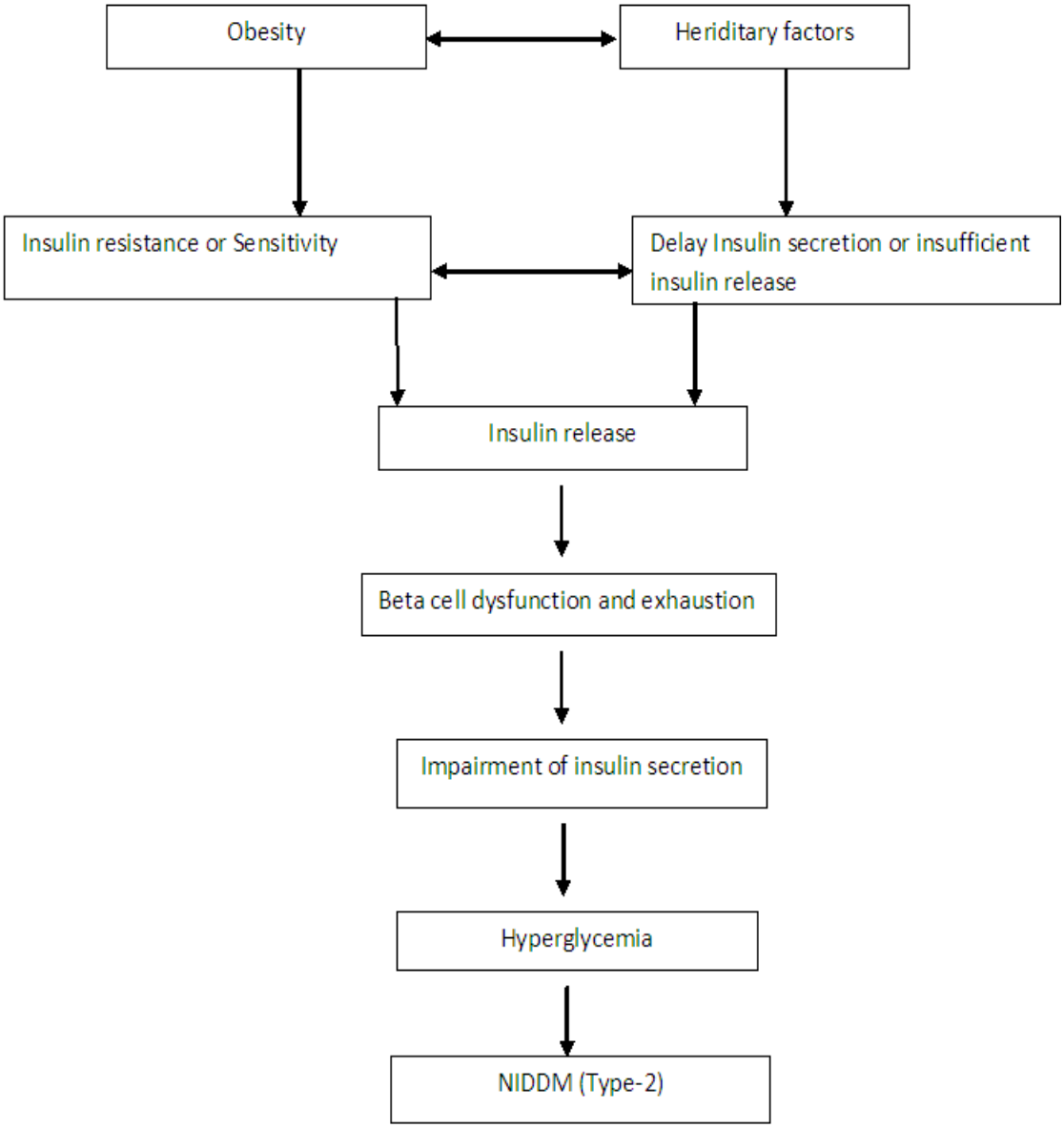

Fig. 1.2: Pathophysiology of type-II Diabetes mellitus

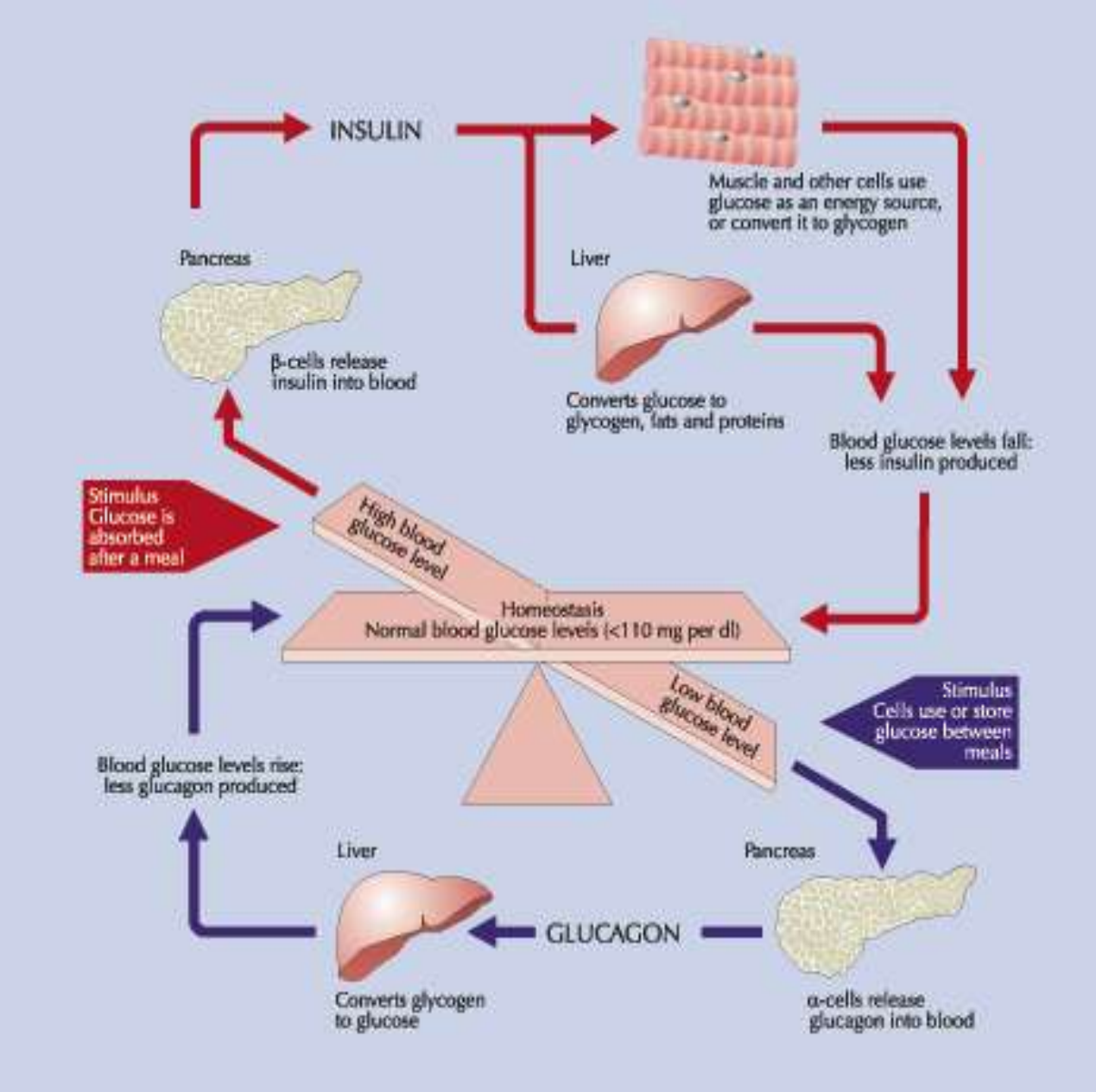

Fig.1.3: Pathophsiology of Diabetes mellitus

i. Risk Factors

The predisposing factors thatare associated with diabetes include both modifiable as well as non-modifiable factors. Residence seems to be the major determinant among the modifiable risk factors. Since urbanization have a 1.5 to 4 fold higher prevalence of diabetes compared to rural areas due to life style changes associated with urbanization and westernization, diet, obesity and physical inactivity. Non-modifiable determinants of diabetes prevalence are ethnicity, age, history of gestational diabetes and family history of type-2 diabetes (Libman IM and Arslanian SA, 2007).

ii. Free Radicals and Complications of Diabetes

After the advent of insulin therapy by Banting and Best in 1992 the causes of death in the diabetic population changed drastically. While insulin and many other treatments can control many aspects of diabetes, numerous complications are not uncommon. The neuropathic, macrovascular and microvascular complications are the major health problems for NIDDM or IDDM patients. Oxidative damage has been involved in the pathogenesis of longterm complications of diabetes. Enzymatic and non-enzymatic oxidation of lipids and carbohydrates results in reactive carbonyl compounds, including aldehydes derived from lipid peroxidation. Superoxiodes, hydrogen peroxides and hydroxyl radicals are the Oxygen free radicals that are involved in the pathophysiology of ischemia or reperfusion injury atherosclerosis (Colagiuri RN*et al*., 2006).

iii. Genetics and Family History

Certain genes are known to cause Wolfram syndrome and maturity onset diabetes of the young (MODY). Genes also contribute to other types of diabetes including IDDM and NIDDM.

iv. Family Medical History

According to American Diabetes Association if both the parents of a person have type-1 diabetes he has a 10 to 25% chance of developing that disease and if both the parents have type-2 diabetes then that person has a 50% chance of developing that disease.

v. Weight and Body type

Obesity and overweight are the leading factors of developing type-2 diabetes and gestational diabetes. Excess fat especially around the abdomen (central obesity), promotes metabolic syndrome and insulin resistance. Most people with type-1 diabetes are of normal weight and excess weight has not been traditionally considered in these conditions. Recent researches indicate that obesity may hasten the development of type-1 diabetes and the increasing rate of type-1 diabetes is due to childhood obesity. People those who gain weight along with autoimmune diabetes may susceptible to insulin resistance and double diabetes.

vi. Sex

Little or no research had been conducted on this trend. Most of the women are suffering with diabetes but actually men are more susceptible to diabetes than women. One factor that may

be the documented increase in recent years of low testosterone levels which scientists had linked to insulin resistance.

vii. Diet

Eating food containing sugar do not cause the disease where as some experimental studies have linked heavy consumption of soft drinks and other simple carbohydrates to risk of metabolic diabetes and foods low in glycaemic index like whole grains, to reduced risk. Weight gain due to sedentary habits and excess intake of calories are the main reason for diabetes. Further scientific research is on the diet related in causing diabetes.

Viii. Other diseases

Medical conditions such as asthma, sleep apnea, high blood pressure, hyperlipidemia, pancreatitis, hemochromatosis, endocrine disorders like hyperthyroidism, cushing's disease, acromegaly, genetic conditions like polycystic ovarian syndrome, down syndrome are linked to type-2 diabetes. Celiac disease (gluten intolerance) and other autoimmune diseases have been linked to type-1 diabetes.

ix. Hormones

These are the chemical messengers that can contribute to diabetes in many ways. Stress hormone such as cortisol causes fluctuating glucose levels in type-2 diabetes. Injected contraceptives, estrogens, androgen deprivation therapy for prostate cancer and corticosteroids have been linked to secondary diabetes.

x. Medical treatments

In addition to hormones, medications including beta blockers, immunosupressants, diuretics, antipsychotics, antidepressants, antiretroviral drugs may results in type-2 diabetes. Radiation therapy and pancreatectomy may also results in type-2 diabetes. Drugs such as L-asparginase and pentannidine may results in type-1 diabetes.

xi. Other chemicals

Exposure to agricultural pesticides during pregnancy may results in gestational diabetes. Pesticides, pollutants, common consumer plastics and plastic ingredients may results in insulin resistance. Rat poison called pyriminal may cause type-1 diabetes.

MATERIALS AND METHODS

Plan of Work

1. Plant collection and authentification.
2. Extraction
3. Phytochemical screening and
4. *In-vivo* studies

- Acutetoxicology studies
- Experimental protocol

EXPERIMENTAL ANIMALS

Healthy Wister rats, weighing between 150-200gm of either sex are used for the study. Animals procured from the animal house of Sree Vidyanikethan College of Pharmacy, Tirupati. The animals were housed individually in clean polypropylene cages in air-conditioned room where the temperature was 22±2 **o** C with 50%±10% relative moisture with a 12 h light and dark cycle all over the study, animals were maintained at normal laboratory circumstances and were given commercial laboratory animal feed and water with libitum. Prior to conducting experiments, ethical permission was taken from Institute's Animal Ethics Committee (IAEC) of CPCSEA Reg.no.930/PO/a/2006/CPCSEA.

CHEMICALS

Streptozotocin (Sigma Pvt. Ltd. India). Glibenclamide (10mg/kg) reagent kits for alkaline SGOT, SGPT, TG's, (EX-cel life science Pvt.Ltd), Sodium citrate, HCl, CMC and all other chemicals worn in the study are of analytical grades procured from respective manufacturers.

INSTRUMENTS

- Electronic balance
- Centrifuge
- Auto analyzer

EXPERIMENTAL PROTOCOL

Plant material collection and authentification

The fresh whole plants of *Phyllanthus virgatus*were obtained from the thalakona hills, tirupati. A.P, India in the month of February and they were authentified by Prof. Madhavsettey, Botanist, SVU, Tirupati.

Extraction procedure

The whole plants were shade dried, powdered, and sieved (mesh no. 40) to get coarse powder. Then this powdered coarse plant material was packed into soxhelet apparatus subjected to soxhlation using ethanol as solvent for 72 hours at a temperature of 40°c. After filtration, it was evaporated using water bath at a temperature not exceeding 40°c until the crude extract was obtained as a semi solid and air dried to get solid mass.

Selection of dose

Plant extract was prepared according to ED50 of individual herbs. ED50 of individual plants was found to be as, *Phyllanthus virgatus* (200 mg/kg). Plant extractat a dose of 200mg/kg and 400mg/kg were used for the present study according to ED_{50} values from previous studies.

Experimental Design

The healthy female animals were randomly selected into five groups, each group containing 6 animals were treated for 28 days, as given in following treatment schedule ethanolic extract of *Phyllanthus virgatus* was freshly suspended in distilled water and administered to animals by oral feeding needle. The standard drug Glibenclamide (5mg/kg, *p.o*) was prepared by suspending in a 1% CMC using mortar and pestle, made as a suspension and administered immediately.

Table 1: Treatment schedule for assessing anti antidiabetic activity of Ethanolic Extract of Phyllanthus virgatus

S.No	GROUPS	TREATMENT	PURPOSE
I	Normal	Normal saline	Serves as normal
II	Negative control	Steptozotocin(40mg/kg i.p)	Serves as disease control
III	Standard	Steptozotocin + Glibenclamide(5mg/kg p.o)	To study the effect of Glibenclamide in disease condition
IV	Test-I	Steptozotocin + Low dose of Plant extract	To study the effect of *Phyllanthus virgatus* at low dose in disease condition
V	Test-II	Steptozotocin + High dose of Plant extract	To study the effect of *Phyllanthus virgatus* at high dose in disease condition

Preliminary phytochemical analysis

The crude ethanolic extract of*Phyllanthus virgatus* had been screened for the presence of phytochemicals like alkaloids, carbohydrates, glycosides, sterols, phenolic compounds and saponins, tannins, flavonoids, proteins and amino acids by using the standard procedures.

i. Detection of Carbohydrates and Glycosides

Small quantity of aqueous extract along with alcohol was dissolved and filtered. The filtrate was subjected to various tests for the presence of carbohydrates.

***In-vivo* Studies**

The in-vivo testing was performed on albino wistar rats of either sex (150-220gm) obtained from Sree Vidyanikethan College Of Pharmacy animal house which were housed under standard laboratory conditions and fed with commercial rat feed (Lipton India Ltd., Mumbai, India) and boiled water, ad libitum. All the animal experiments were carried out according to NIH guidelines, after getting the approval from the Institute's Animal Ethics Committee (IAEC) of CPCSEA Reg.no.930/PO/a/2006/CPCSEA.

a. Acute toxicity studies

Acute toxicity studies were performed to test the lethal or toxic dose of the test drug on animals to avoid any complications while administering the plant extract. The acute oral toxicity study was carried out according to the OECD 423 guidelines protocol. In this study female rats (n=3) weighing about 180-220gm were used in the testing dose of 2000mg/kg body weight. Then they were observed for signs of toxicity and mortality for 14 days from the initial day of dosing.

It was observed that there were no signs of toxicity and mortality for 14 days at a testing dose of 2000mg/kg body weight. Therefore one-tenth of the maximum no mortality dose of extract were selected low dose 200mg/kg and high dose 400mg/kg.

b. Experimental procedure

i. Blood glucose estimation after food treatment

Blood glucose levels are estimated after the food administration to the animals at specific time intervals in order to determine hyperglycemia caused due to assimilated food using one touch glucometer. Induction of diabetes to the animals is an essential step prior the test to be conducted.

ii. Inducing Diabetes

Diabetes was induced in rats by injecting 40mg/kg of streptozotocin i.p and also by feeding the rats with high fat diet. Rats were then kept for next 1 week on 10% glucose solution bottles to prevent hypoglycaemia. After that rats with hyperglycemia (fasting blood glucose levels >250mg/dl) were selected and used for study. The selected diabetic animals were divided into five groups (n=5). The blood glucose levels were measured just prior to 2, 4 and 6 hours after drug administration. Treatment was continued for 28 days. The fasting blood glucose levels were estimated on 0, 7, 14 and 28 days.

The animals were divided into five groups each group containing five animals.

- Group 1: Control group (normal saline)
- Group 2: Negative control (streptozotocin 40mg/kg i.p)
- Group 3: Standard group STZ (40mg/kg i.p) + Glibenclamide (5mg/kg p.o)
- Group 4: Plant extract (low dose 200mg/kg p.o) and STZ (40mg/kg i.p)
- Group 5: Plant extract (low dose 400mg/kgp.o) and STZ (40mg/kg i.p)

The anti-diabetic effect of Ethanolic Extract of *Phyllanthus virgatus* will be evaluated by estimation of

- Blood glucose levels,
- Biochemical estimations like SGOT, SGPT.
- Estimation of serum lipids
  - Cholesterol, Triglycerides, HDL, LDL, and VLDL.
- Histopathology studies

EXPERIMENTAL RESULTS

Preliminary Phytochemical Studies

Table 2: Results of Phytochemical analysis of Ethanolic Extract of Phyllanthus virgatus.

S.no	Phytoconstituents	Present/Absent
1	Alkaloids	-
2	Glycosides	+
3	Carbohydrates	+
4	Fats& oils	+
5	Saponins	+
6	Tannins	+
7	Proteins & aminoacids	-
8	Gums& mucilage	-

9	Flavonoids	+
10	Phenols	+
11	Phytosterols	-

Whereas + (Present), - (Absent).

The chief phytochemicals present in the extract were alkaloids, glycosides, carbohydrates, terpinoids, saponins, fats and oils, tannins, flavanoids, and phenols. These Phytochemical constituents might be responsible for the anti-diabetic activity of the plant.

***IN-VIVO* STUDIES**

The in-vivo testing was performed on albino wistar rats of either sex (150-220gm) obtained from Sree Vidyanikethan College Of Pharmacy animal house which were housed under standard laboratory conditions and fed with commercial rat feed (Lipton India Ltd., Mumbai, India) and boiled water, ad libitum. All the animal experiments were carried out according to NIH guidelines, after getting the approval from the Institute's Animal Ethics Committee (IAEC) of CPCSEA Reg.no.930/PO/a/2006/CPCSEA.

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It was observed that there were no signs of toxicity and mortality for 14 days at a testing dose of 2000mg/kg body weight. Therefore one-tenth of the maximum no mortality dose of extract were selected low dose 200mg/kg and high dose 400mg/kg.

Table 3: Results of Acute toxicity studies.

S.no	Initial weight	Final weight	Signs of toxicity			Mortality
			Behavioral	Somatic	Neurological	
1.	185	210gm	No signs	No signs	No signs	Nil
2.	190	204gm	No signs	No signs	No signs	Nil
3.	199	214gm	No signs	No signs	No signs	Nil

Blood Glucose Levels

Table 4: Results of Blood Glucose Levels.

S.No	Groups	0th day	7th day	14th day	28th day
1.	Control	102.3±2.24	101.2±1.51	101.5±1.33	102.2±1.10
2.	Negative control	227.2±2.91*a	224.0±2.29*a	229.0±2.11*a	235.5±2.01*a
3.	Standard	225.5±2.63**b	159.0±1.59**b	125.3±0.71**b	114.8±0.87**b
4.	Low dose	224.5±2.18*a	176.0±1.71*a	157.5±1.47*a	146.2±1.32*a
5	High dose	224.7±2.62**b	162.0±1.86**b	131.5±0.76**b	124.3±0.88**b

Values are expressed in mean ±SEM, (n=6), when compared with control, *P<0.01, **P<0.001, oneway ANOVA followed by Dunnet's t – Test. STZ (40mg/kg) was injected to all groups except control. [a]STZ induced diabetic group vs normal group, [b]Extract treated group vs STZ induced diabetic group.

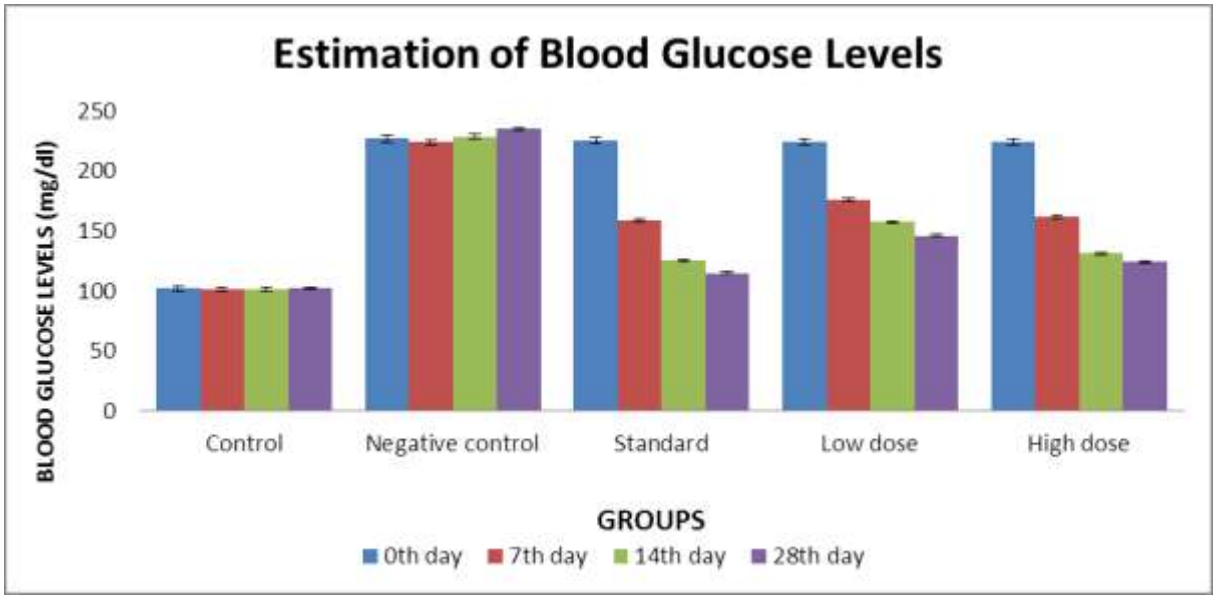

Fig. 4: Estimation of Blood Glucose Levels.

Antioxidant Studies

Table 5: Results of Antioxidants Estimation

S.no	Groups	SGOT	SGPT
1.	Control	98.83±1.07	44.67±0.88
2.	Negative control	132.3±0.98*a	68.67±0.66*a
3.	Standard	103.7±0.80**b	50.33±0.71**b
4.	Low dose	110.5±1.14*b	58.00±0.81*b
5.	High dose	105.7±0.88**b	51.33±0.84**b

Values are expressed in mean ±SEM, (n=6), when compared with control, *P<0.01, **P<0.001, oneway ANOVA followed by Dunnet's t – Test. STZ (40mg/kg) was injected to

all groups except control. [a]STZ induced diabetic group vs normal group, [b]Extract treated group vs STZ induced diabetic group.

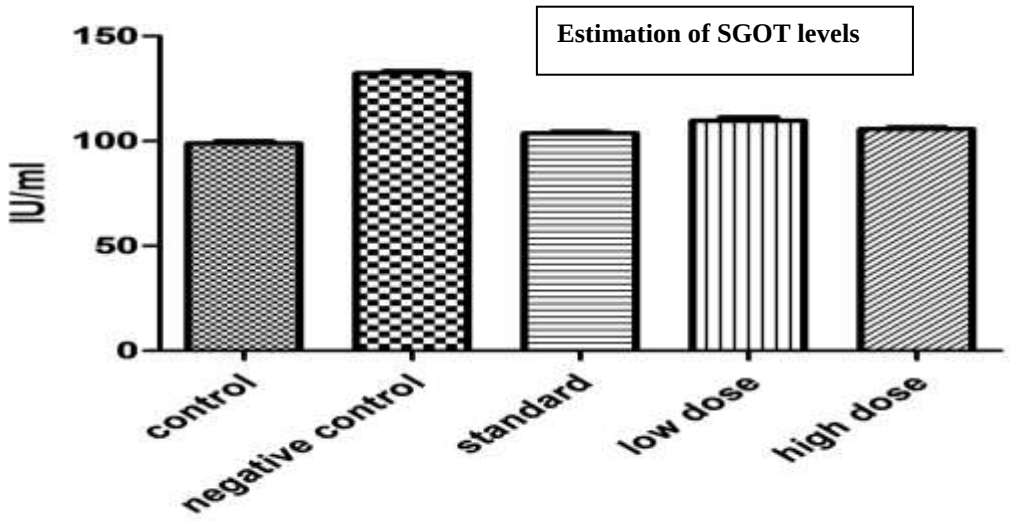

Fig. 5: Estimation of SGOT Levels.

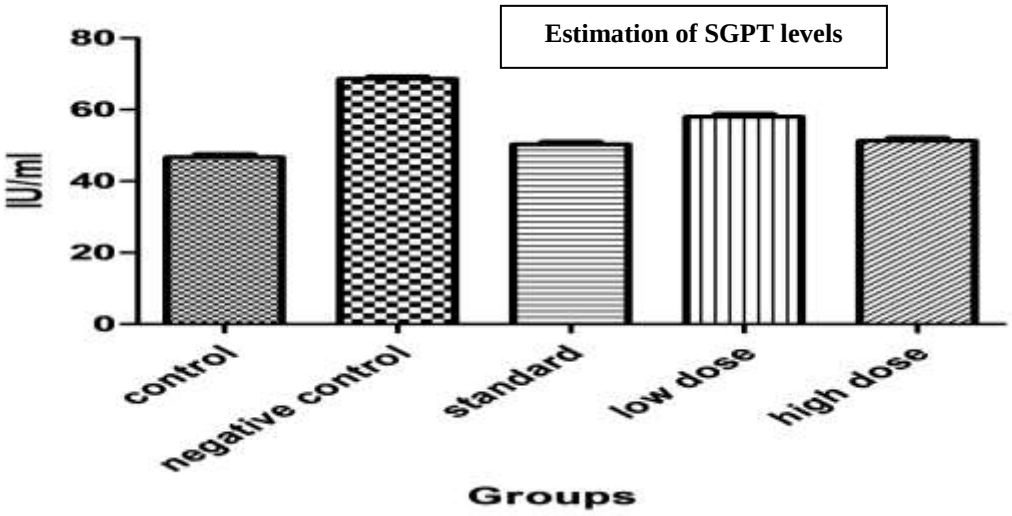

Fig. 6: Estimations of SGPT Levels.

Estimation of Serum Lipid Levels

Table 6: Results of Serum Lipid Levels Estimation.

S.No	Groups	Cholesterol	Triglycerides	VLDL	LDL	HDL
1.	Control	68.18±0.78	67.17±0.78	13.43±0.19	26.67±0.66	32.17±1.07
2.	Negative control	133.7±1.78*a	164.8±1.04*a	32.97±0.20*a	79.67±0.49*a	18.83±0.66*a
3.	Standard	74.17±0.94**b	79.83±1.41**b	15.57±0.54**b	31.33±0.98**b	27.67±0.49**b
4.	Low dose	84.83±1.16*a	91.67±1.54*a	18.33±0.30*a	40.33±0.71*a	25.67±0.49*a
5.	High dose	71.83±0.79**b	84.00±1.73**b	16.77±0.34**b	34.33±0.80**b	27.83±0.47**b

Values are expressed in mean ±SEM, (n=6), when compared with control, *P<0.01, **P<0.001, oneway ANOVA followed by Dunnet's t – Test. STZ (40mg/kg) was injected to all groups except control. [a]STZ induced diabetic group vs normal group, [b]Extract treated group vs STZ induced diabetic group.

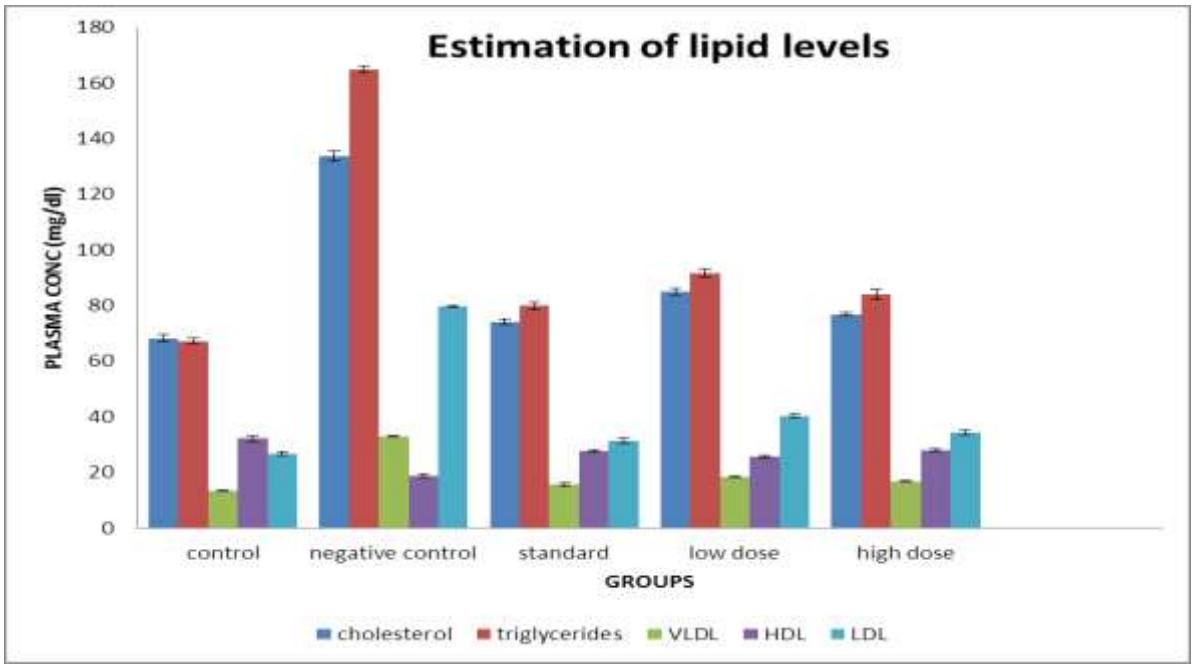

Fig. 7: Estimations of Serum Lipid Levels

Histopathological studies

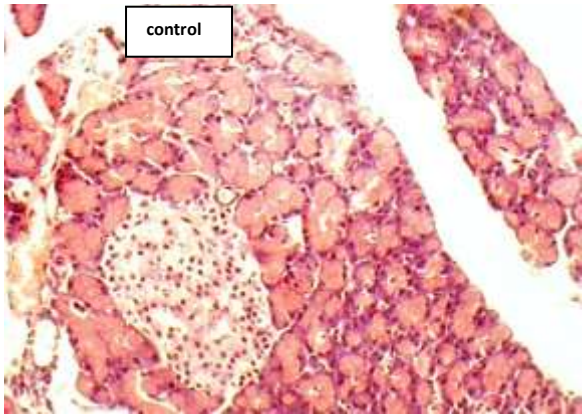

Fig. 8 Control

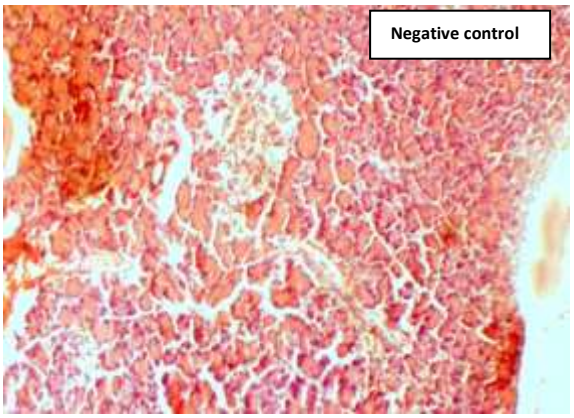

Fig. 9 Negative control

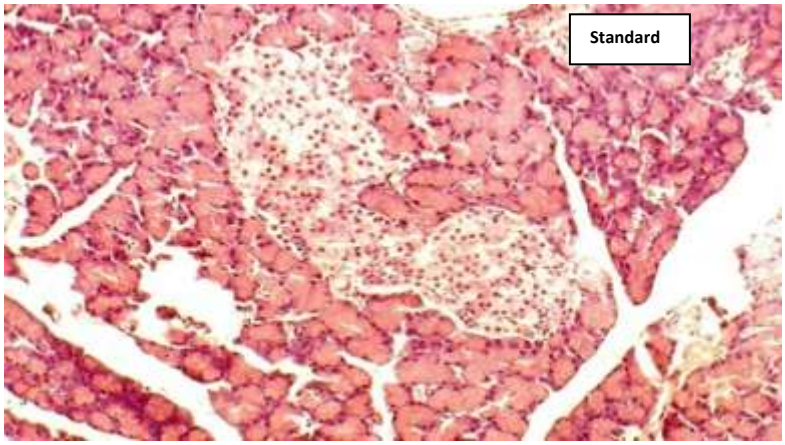

Fig. 10 Standard

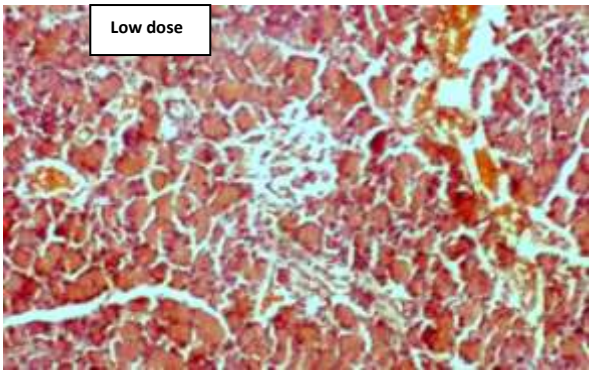

Fig. 11 Low dose

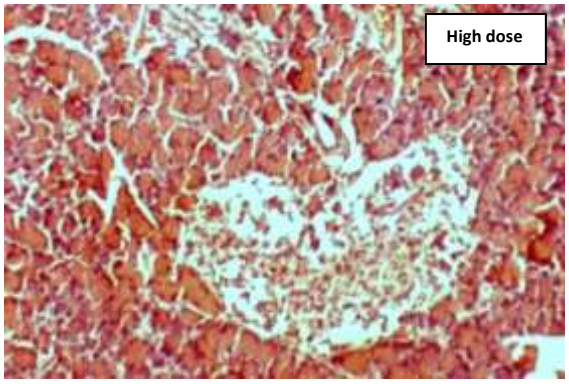

Fig. 12 High dose

DISCUSSION

Diabetes mellitus is a metabolic disorder characterized by hyperglycemia and alterations incarbohydrate, fat and protein metabolism, associated with the continuous destructionof β-cell leads to the disturbance inglucose homeostasis. Liver and pancreas arethe two main organs which plays importantrole in maintain the level by regulating alternativepathways between glucose uptake andgluconeogenesis (Ferre*et al*., 1996).Diabetes is induced by streptozotocin (STZ) in animal model.

Currently available drugs for the treatment of Diabetesmellitus have a number of limitations, such as adverseeffects and a high rate of secondary failure Although there isa growing trend towards using natural remedies adjunct toconventional therapy, traditionally used plants might provideа useful source of new hypoglycemic compounds. Although *Phyllanthus virgatus* may be described as a medicinal plant used for various purposes, no scientific reports exist on itsantihyperglycemic effect.

STZ is known to induce diabetes in animal models along with hyperlipidemia/atherosclerosis, diabetic nephropathy, retinopathy and neuropathy. STZ mediatedtoxicity is might be due to their proposed siteof action at nuclear DNA. The decompositionof STZ leads to formation of highly reactivecarbonium ions, which cause DNAbases alkylation and also damage pancreatic-cell membrane and break the DNA strandwhich guide to the activation of poly (ADPribose) synthetase and NAD depletion,which ultimately leads to cell death (Portha *et al*., 1989). It has been previously reportedthat STZ selectively destroy the pancreaticcells that secrete insulin and results in substantialhyperglycemia as well as also resultin weight loss, ketosis and a high rate ofmortality (Arulmozhi*et al*., 2004 and Szkudelski,2001). Since, it has been shown thathyperglycemia is directly associated withdecreased body weight of diabetic animals(Zafar and Naqvi, 2010), our data is inagreement with this study and also demonstrateda decline in body weight in diabeticrats, that could be due to muscle destructionor degeneration of structural proteins (Salahuddinand Jalalpure, 2010). Insulin secretion or insulin action after inducing the disease liver damage will occur it leads to altered in SGOT, SGPT, levels and also change in blood glucose and triglyceride levels.

The present work was designed to investigate theantihyperglycaemic effects of Ethanoic extract of *Phyllanthus virgatus* (EEPV)extract in streptozotocin diabetic albino rats. PV is having the antioxidant properties it might be responsible for the antidiabetic activity.

The antioxidant enzymes SOD and CAT play an importantrole in reducing cellular stress. SOD scavenges thesuperoxide radical by converting it to hydrogen peroxide and molecular oxygen, while CAT brings about the reductionof hydrogen peroxides and protects higher tissues from thehighly reactive hydroxyl radicals. In the present investigationboth these enzymes registered low levels of activity indiabetic controls indicating diabetes-induced stress. Sucha decline in these enzyme activities has also been reported earlier. EEPV when administered to the diabeticanimals improved both SOD and CAT activities substantially, reflecting the antioxidant potency of *Phyllanthus virgatus* antioxidants(GSH, SOD, CAT and LPO) were found to be better thanthose of glibenclamide administered diabetic animals.

Ethanolic extract of *phyllanthus virgatus*in diabetic rats,reduced blood glucose levels and increased glycogenesis andglycolysis, reduced gluconeogenesis and brought the glucosemetabolism towards normal levelsonthe carbohydrate metabolism in diabetic rats is found to besimilar to that of glibenclamide.

In the present study, the liver enzymes like SGOT, SGPT in plasma was markedly increased in the STZ treated control group, compared to the normal group indicates a damage of liver. This enzymes activity was reduced by the Ethanolic extract of *Phyllanthys virgatus* treatment groups with doses 200mg/kg and 400mg/kg respectively. Decreased levels of SGOT, SGPT might be due to *P.virgatus* containghepatoprotectective active activity.

The induction of experimental diabetes inrats by STZ had a reflective impact on lipidparameters when compared to normal rats. After 28 days of treatment the levels of TGs, Cholesterol, LDL, and VLDLwere significantly decreased and the levels of HDL are increased in allthe treated groups.

CONCLUSION

Phyllanthus virgatus possess various pharmacological activities such as Diabetes Mellitus, Tonic, Jaundice, Diuretic, Scabies, Gonorrhea and Antioxidant.

In the present study, ethanolic extract of *Phyllanthus virgatus* showed a promising anti diabetic effect in STZ and high fat diet induced diabetes in rats. Hence oral administration of EEPV has the potential of antidiabetic activity. Further the active principles of *Phyllanthus virgatus* responsible for this property is too isolated phytochemically and studies with these purified constitutions are waiting to understand the complete mechanism of anxiety activity.

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