"Effect of Aqueous extract of *Cyperus rotundus* roots on Diabetic complications in Alloxan induced diabetic Rats".

Dissertation submitted to

THE TAMILNADU Dr. M.G.R MEDICAL UNIVERSITY

CHENNAI

In partial fulfillment of the requirements for the degree of

MASTER OF PHARMACY in PHARMACOLOGY

BY

Mr.ANANTHULA PRUTHVIDHAR (Reg. No.261226003)

Under the guidance of

J.Gunasekaran M.Pharm

Associate Professor

Department of Pharmacology

MOHAMED SATHAK A.J. COLLEGE OF PHARMACY,

SHOLINGANALLUR, CHENNAI - 600119.

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MOHAMED SATHAK A.J. COLLEGE OF PHARMACY

(Affiliated to The Tamil Nadu Dr.M.G.R. Medical University, Chennai)
Approved by AICTE & P.C.I. New Delhi.
Medavakkam Road, Sholingnallur, Chennai - 600 119.
Email: msajcpharm@gmail.com Web: www.msajcpharm.in
Ph: 044-24502572, Fax: 24502573.
Sponsored by: **MOHAMED SATHAK TRUST**

CERTIFICATE

This is to certify that the dissertation entitled **"'Effect of Aqueous extract of *Cyperus rotundus* roots on Diabetic complications in Alloxan induced diabetic Rats"** submitted to The Tamilnadu Dr. M.G.R. Medical university, Chennai, in partial fulfillment for the award of degree of **Master of Pharmacy in Pharmacology** is a bonafide individual research work done by **Mr.ANANTHULA PRUTHVIDHAR** Mohamed Sathak A.J. College of Pharmacy, Chennai, under the guidance and direct supervision of **J.GUNASEKARAN M.Pharm, Associate Professor, Department of Pharmacology** during the academic year 2013-2014.

Place: Chennai

Date:

Dr.R.SUNDARARAJAN,M.pharm.,Ph.D.,

Principal,

MOHAMED SATHAK A.J. COLLEGE OF PHARMACY

(Affiliated to The Tamil Nadu Dr.M.G.R. Medical University, Chennai)
Approved by AICTE & P.C.I. New Delhi.
Medavakkam Road, Sholingnallur, Chennai - 600 119.
Email: msajcpharm@gmail.com Web: www.msajcpharm.in
Ph: 044-24502572, Fax: 24502573.
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M. JAGADEESAN, M.Pharm

Professor and Head

Department of Pharmacology.

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Place: Chennai

Date: **(M. JAGADEESAN, M.Pharm)**

Head, Department of Pharmacology

MOHAMED SATHAK A.J. COLLEGE OF PHARMACY

(Affiliated to The Tamil Nadu Dr.M.G.R. Medical University, Chennai)
Approved by AICTE & P.C.I. New Delhi.
Medavakkam Road, Sholingnallur, Chennai - 600 119.
Email: msajcpharm@gmail.com Web: www.msajcpharm.in
Ph: 044-24502572, Fax: 24502573.
Sponsored by: **MOHAMED SATHAK TRUST**

J.GUNASEKARAN, M.Pharm

Associate Professor

Department of Pharmacology

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Place: Chennai

Date: (**J.GUNASEKARAN, M.Pharm)**

Guide and Supervisor

Dedicated to my

Parents, Teachers

&

Friends

Mr.ANANTHULA PRUTHVIDHAR

II year- M.Pharm, Pharmacology (Reg.no: 261226003),

Department of pharmacology,

Mohamed Sathak A. J. College of Pharmacy.

DECLARATION OF THE CANDIDATE

I hereby declare that the thesis titled **"''Effect of Aqueous extract of *Cyperus rotundus* roots on Diabetic complications in Alloxan induced diabetic Rats"** submitted in partial fulfillment for the award of degree Master of Pharmacy to The Tamilnadu Dr. M.G.R. Medical University and carried out at Mohamed Sathak A.J.College of Pharmacy, Chennai, is my original and independent work done under the direct supervision and guidance **J.GUNASEKARAN M.Pharm, Associate Professor, Department of Pharmacology** during the academic year 2013-2014 and this thesis contains no material which has been accepted for the award of any degree or diploma of other Universities.

Place: Chennai

Date:

[ANANTHULA PRUTHVIDHAR]

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Ananthula Prudhvidhar

LIST OF ABBREVATIONS

- **AAP:** American Academy of Pediatrics
- **AHA:** American Heart Association
- **AMA:** American Medical Association's
- **ATP III :**Adult Treatment Panel III
- **ACAT:** Acyl coenzyme A cholesterol O-acyl transferase
- **ACOA:** Acetyl coenzyme A
- **ANOVA:** Analysis of variance
- **ATP:** Adenosine triphosphate
- **BSCL2:** Berardinelli-Seip congenital lipodystrophy, type 2
- **BMI:** Body mass index
- **CAT:** Catalase
- **CETP:** Cholesteryl ester transfer protein
- **CHD: Coronary heart disease**
- **CHE:** Cholesteryl ester
- **CHOD POD:** Cholesterol oxidase peroxidase
- **CMC:** Carboxy methyl cellulose
- **CPCSEA:** Committee for the purpose of control and supervision of experimental animals
- **CRP:** C-reactive protein
- **DMSO:** Dimethyl sulfoxide
- **FFA:** Free fatty acid
- **GPO:** Glycerol phosphate oxidase
- **GPx:** Glutathione peroxidase
- **GSH:** S-glutathiolation
- **HDL-C:** High density lipoprotein cholesterol
- **HFD:** High fat diet **HL:** Hepatic lipase

- **HNE:** 4-hydroxy-2-nonenal
- **HOCL:** Hypochlorous acid
- **IDL:** Intermediate density lipoprotein
- **JNC7:** Joint National Committee 7
- **LCAT:** Lecithin cholesterol acyl transferase
- **LDL-C: Low density lipoprotein cholesterol**
- **LLD:** Lipid lowering drugs
- **LPL:** Lipoprotein lipase
- **LRP:** LDL receptor related protein
- **MDA:** Malondialdehyde
- **MI:** Myocardial infraction
- **MVA:** Mevalonate
- National Cholesterol Education Program (NCEP)
- National Heart Blood and Lung Institute (NHLBI)
- **NNT** :number needed to treat
- **NNH:** number needed to harm
- **OTC:** over-the-counter
- **OECD:** Organization for economic co-operation and development
- **P.O:** Per oral
- **PPARα:** Paroxisome proliferator activated receptor α
- **SEM:** Standard error mean
- **SOD:** Superoxide dismutase
- **SJM :** Syzygium jambos(L) alston
- **TBA:** Trichloro butyric acid
- **TC:** Total cholesterol
- **TCA:** Trichloro acetic acid
- **TG: Triglycerides**
- **TLC:** Thin layer chromatography
- **TLC:** Therapeutic lifestyle change
- **VLDL: Very low density lipoprotein cholesterol**
- **WHO**: World health organization

CONTENTS

Chapter: I

Introduction

INTRODUCTION

Diabetes mellitus

The term Diabetes is a multifarious group of disorders characterized by hyperglycemia that has reached epidemic proportions in the current century. Infection is a leading cause of morbidity and mortality among the diabetic population. Diabetes is involved with vascular and renal damage characterized by hypertension, dyslipidemia, micro-albuminuria, macro-albuminuria and glomerular mesangial rise[5]. Diabetes mellitus may present with characteristic symptoms such as polyphagia, polydypsia, polyuria, blurring of vision, and weight loss. In its severe forms, ketoacidosis or a non-ketonic hyperosmolar state may develop and lead to stupor, coma and in the absence of effective treatment to death[6].

Epidemiology

Type-1 diabetes mellitus account for 10 % of all cases of diabetes mellitus and results from an autoimmune destruction of the pancreatic β-cells. The prevalence of β-cell autoimmunity appears proportional to the incidence of Type-1 diabetes mellitus. In population groups. In countries like Sweden, Sardinia and Finland have the highest number prevalence of islet cell antibody (3%-4.5%) and are associated with the high incident of Type-1 diabetes mellitus, 22-35 per 100,000.

Type-2 diabetes mellitus is heterogeneous disorder of glucose metabolism. Type-2

diabetes mellitus accounts for much as 90% of all cases of diabetes mellitus and usually results from defects in insulin sensitivity and a relative defect in insulin secretion[7].

According to WHO (World health organization) predictions, the prevalence of diabetes is to increase by 35%. Currently, there are over 150 million diabetic patients worldwide and this is likely to increase to 300 million or more by the year 2025. Statistical estimations about India reveals that the cases of diabetics will increase from 15 million in 1995 to 57 million in the year 2025, the highest number of diabetic cases in the world [8].

Fig.no.1

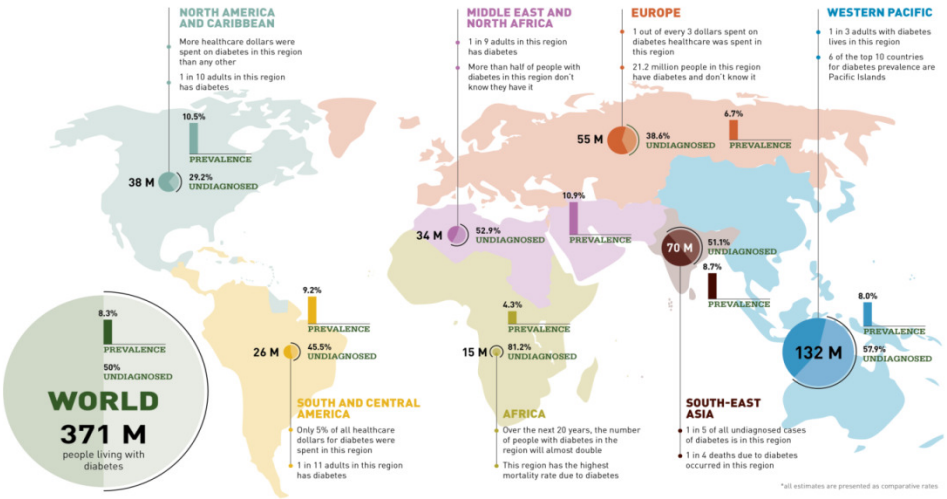


Table 1 : Top ten countries for estimated number of adults with diabetes mellitus in million

Sr.No.	Country	1995	Country	2025

1.	India	19.4	India	57.2
2.	China	16.0	China	37.6
3.	U.S.	13.9	U.S.	21.9
4.	Russian	8.9	Pakistan	14.5
5.	Japan	6.3	Indonesia	12.4
6.	Brazil	4.9	Russian	12.2
7.	Indonesia	4.5	Mexico	11.7
8.	Pakistan	4.3	Brazil	11.6
9.	Mexico	3.8	Egypt	
10.	Ukraine	3.6	Japan	8.5
All other countries		49.7		103.6
Total		**135.3**		**300.0**

THE PANCREAS:

Pancreas is a flattened organ lying to the posterior wall of the upper abdomen. The bulk of the gland consists of acinar cells that synthesize and secrete digestive enzymes that enter the duodenum via the pancreatic duct system, involving the exocrine function. Scattered more or less randomly through the pancreas and accounting for only 1-2% of its weight are many microscopic nests of cells, the 'islets of langerhans', which constitute the endocrine pancreas, secreting several critically essential hormones directly into the blood stream[9].

Pancreas of human has 1-2 million islets of langerhans, each having a diameter of 0.3mm and organized in circular round small capillaries in to which its cells secrete hormones[10]. The pancreatic islet includes four types of hormone secretions,

1. Alpha (α) or A cells constitute about 20% of pancreatic islet cells and secrete glucagon

2. Beta (β) or B cells constitute about 70% of pancreatic islet cells and secrete insulin

3. Delta (δ) cells or D cells constitutes about 5% of pancreatic islet cells and secrete somatostain .

4. F cells constitute the remainder of pancreatic islet cells and secrete pancreatic Polypeptide[11]. Regulates release of pancreatic digestive enzyme. Two minor cell types, called D_1 cells and enterochromaffin cells are seen in islets.

D_1 cells elaborate vasoactive intestinal polypeptide, a hormone that results glycogenolysis and hyperglycemia[12].

Diagnostic Criteria[13]

The clinical diagnosis of diabetes is of prompted by symptoms such as increased thirst and urine volume, recurrent infections and weight loss in severe cases, drowsiness and coma; high levels of glycosuria are usually present.

Table:2. Values for diagnostic of diabetes mellitus and other categories of hyperglycemia according to the WHO

	Glucose concentration (mmol / L)			
	Whole blood		**Plasma**	
	Venous	**Capillary**	**Venous**	**Capillary**

Diabetes mellitus				
Fasting or load	≥ 6.1	≥ 6.1	≥ 7.0	≥ 7.0
2-h post glucose of both	≥ 10.0	≥ 11.1	≥ 11.1	≥ 12.2
Impaired glucose tolerance (IGT)				
Fasting (if measured) and	< 6.1 and	< 6.1 and	< 7.0 and	< 7.0 and

The revised diagnostic criteria for diabetes (according to the American diabetes Association ADA) are as follows.[14]

Random plasma glucose : 11.1 mmoL / l (200 mg/dL) Fasting plasma glucose : 7.0 mmoL / l (126 mg/dL)

Classification of Diabetes mellitus[15]

The classification reveals both clinical stages and etiological types of diabetes mellitus and categories of hyperglycemia. Diabetes progresses with several clinical stages. Individual subjects progress from stage to stage. Diabetes mellitus can be categorized by stage according to the clinical characteristics, even in the absence of in concerning etiology information. The classification by a etiological type results from understanding the causes of diabetes mellitus.

(1) Type-1 diabetes

Islet beta-cell destruction, usually leading to absolute insulin deficiency

A. Autoimmune B. Idiopathic

(2) Type-2 diabetes

Heterogenous-may range from predominantly insulin resistance with relative insulin deficiency to a predominantly secretary defect with or without insulin resistance.

(3) Genetic defects of beta-cell function

1. Chromosome 20, HNF4α
   (Formerly MODY 1)
2. Chromosome 7, glucokinase
   (Formerly MODY 2)

(5) Diseases of the exocrine pancreas

1. Fibrocalculous pancreatopathy
2. Pancreatitis
3. Trauma / pancreatectomy
4. Neoplasia
5. Cystic fibrosis
6. Haemochromatosis

(6) Endocrinopathies

1. Cushing's syndrome
2. Acromegaly
3. Phaeochromocytoma
4. Glucagonoma
5. Hyperthyroidism
6. Somatostatinoma

(7) Drug- or chemical-induced

1. Nicotinic acid
2. Glucocorticoids
3. Thyroid hormone
4. Alpha-adrenergic agonists
5. Beta-adrenergic agonists
6. Thiazides
7. Dilantin
8. Pentamidine

9. Vacor
10. Interferon-alpha therapy

(8) Infections

1. Congenital rubella
2. Cytomegalovirus

(9) Uncommon forms of immune-mediated diabetes

1. Insulin autoimmune syndrome

(Antibodies to insulin)

(10) Other genetic syndromes

1. Down's syndrome
2. Friedreich's ataxia
3. Huntington's chorea
4. Klinefelter's syndrome
5. Lawrence-Moon-Biedel syndrome
6. Myotonic dystrophy
7. Porphyria
8. Prader-Willi syndrome
9. Turner's syndrome
10. Wolfram's syndrome

Type-1 diabetes mellitus

Type 1 indicates the processes of beta–cell destruction that may ultimately lead to diabetes mellitus in which "insulin is required for survival" to prevent the development of ketoacidosis, coma and death.[16]

Type-2 diabetes mellitus

Type-2 diabetes mellitus is a heterogeneous disorder characterized by some degree of insulin resistance with variable insulin secretion. Insulin secretion is said to be relatively deficient because many patients may have normal to elevated levels of insulin. However their blood sugars remain elevated because of tissue resistance to the action of the insulin that is not usually life threatening.[17]

Genetics

Genetic factors account for about one-third of the susceptibility to Type-2 diabetes. Over 20 different regions of the human genome show some linkage with Type-1 diabetes, but more interest has been focused on the Human Leucocyte Antigen (HLA) region within the major histocompatibility complex on the short arm of chromosome 6. This locus is designated IDDM-1. The HLA haplotype DR3 and / or DR4 are associated with increased suspectibility to Type-1 diabetes in Caucasians.

Genetic factor are more important in the etiology of Type-2 than Type-1 diabetes as shown by studies in monozygotic twins where concordance rates for Type-2 diabetes approach 100%.

The majority of cases of Type-2 diabetes are multifactorial in nature, with interaction of environmental and genetic factor. The nature of the genetic contribution is largely unknown but it is evident that several genes are involved.[18]

Maturity onset-diabetes of young

MODY is associated with autosomal dominant inheritance and is characterized by age of onset in at least one family member prior to age 25 years, and absence of ketosis. At least four genetically different types of MODY have been described. Some patients will ultimately require insulin to control glycemia.[19]

Other genetic defects in insulin action[21]

A partial list of the genetic defects in insulin action include a number of well described conditions, including Type-A insulin resistance, leprechaunism, Rabson – menden hall syndrome and the lipoatrophic syndromes.

Pancreatic Disease[20]

Acute Pancreatitis: Transient hyperglycemia may require insulin therapy. Permanent diabetes is unlikely to follow a single episode of pancreatitis unless massive pancreatic destruction occurs.

Chronic Pancreatitis: Chronic pancreatitis is frequently complicated by glucose intolerance or diabetes and at least in western countries, is often attributable to alcoholism. Pancreatic calcification may be evident on radiographs or computerized tomography.

Endocrinopathies[20]

Several hormones (e.g. growth hormone, cortisol, glucagon, epinephrine) antagonize insulin action. Diseases associated with excess secretion of these hormones can cause diabetes (e.g.Acromegaly, Cushing's syndrome, Glucagonoma and Phaeochromocytoma). These forms of hyperglycemia typically resolve when the hormone excess is removed. Somatostatinoma, and aldosteronoma–induced hypokalemia, can cause diabetes, at least in part by inhibiting insulin secretion. Hyperglycemia generally resolves following successful removal of the tumor.

Gestational diabetes mellitus complicates roughly 4% of all pregnancies. Clinical recognition is important to reduce associated morbidity and mortality. Most women will return to a normoglycemia postpartum but 30%-50% will develop diabetes mellitus or glucose intolerance later in life.

Pathogenesis of Type-1 Diabetes mellitus[21]

Three interlocking mechanisms are responsible for the islet cell destruction:

1. Genetic Susceptibility
2. Auto-Immunity
3. Environmental

Genetic Predisposition

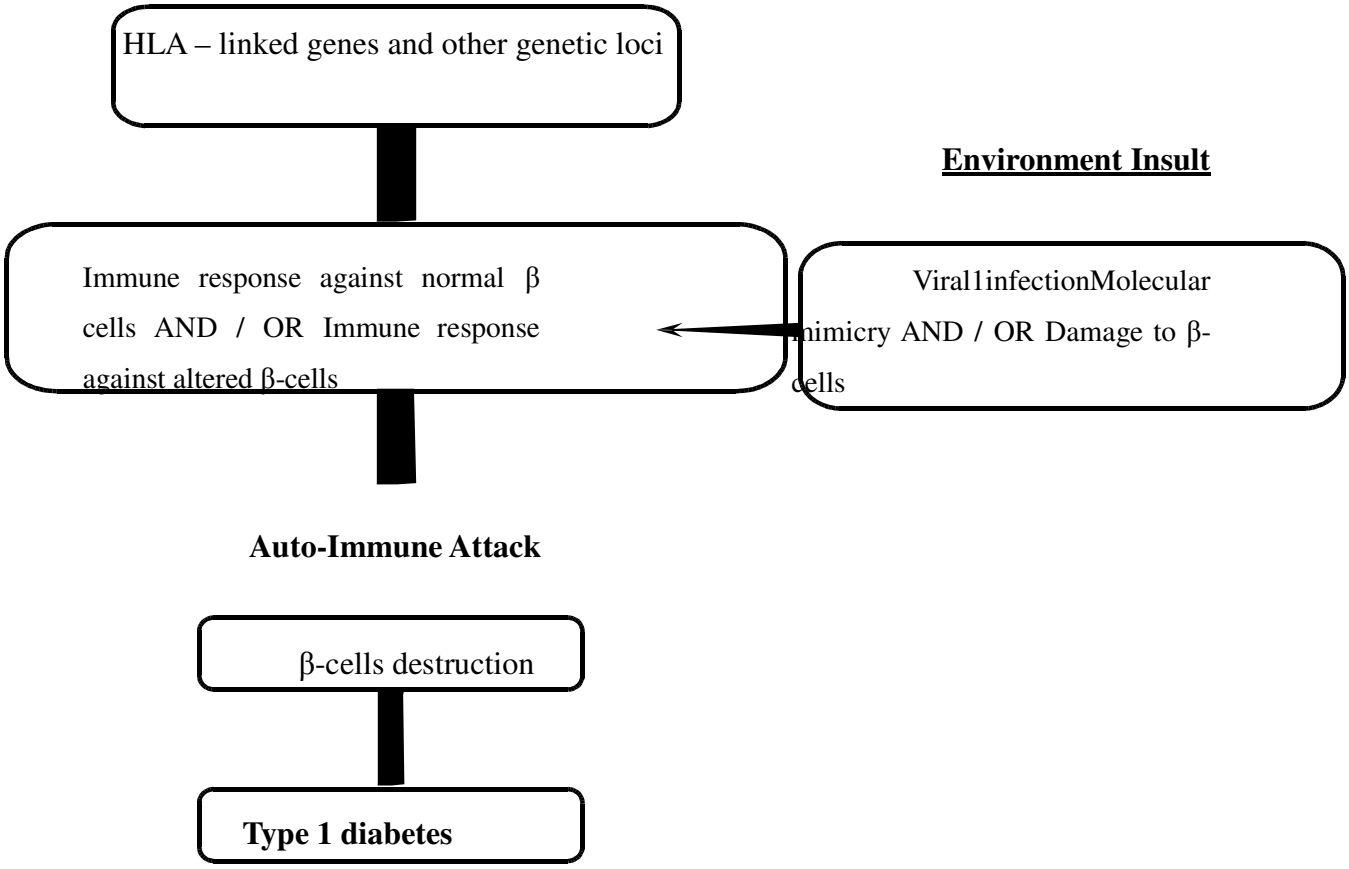


Fig: Pathogenesis of Type-1 diabetes mellitus

1. Genetic Susceptibility

At least one of the susceptibility gene for Type-1 diabetes resides in the region that encodes the class II antigens of the Major Histo compatibility Complex (MHC) on chromosome GP21 (HLA-D). The HLA-D region contains three classes of genes (DP, DQ and DR). The class II molecules are highly polymorphic and each has numerous alleles.

About 95% of white patient with Type-1 diabetes have either HLA-DR3 or HLA- DR4 alleles or both where as in the general population the prevalence of these antigens is only 45%. It is thought that genetic variations in the HLA class II molecules may alter recognition by the T-cell receptor, or may modify the presentation of the antigen because of variations in the antigen-binding cleft, thus, class II HLA gene may effect the degree of immune responsiveness to a pancreatic β-cell auto

antigen or a β-cell auto antigen may be presented in a manner that promotes an abnormal immunologic reaction.

2. Auto-Immunity

Clinical onset of Type-1 diabetes is abrupt; this disease in fact results from a chronic auto-immune attack of β-cells that usually exists for many years before the disease becomes evident.[22] A lymphocyte with rich inflammatory infiltrate (insulitis) is observed in the islets of patients in early diabetes. The infilteration consists mostly of CD8 T- lymphocytes, plus variable numbers of CY4T cells and macrophages. CD4T cell from animals with auto immune diabetes can transfer diabetes to normal animals, thus establishing the primary of T-cell auto-immunity in Type-1 diabetes.

The insulitis is associated with increase expression of class I MHC molecules and aberrant expression of class II MHC molecules on the β-cells. This abberrant expression is mediated in part by locally produced cytokines [eg. Interferon-gamma (IFN-γ) derived from activated T-cells]. Genetic dysregulation of a cytokine that induce IFN-γ production promotes the development of diabetes in a mouse model.[23]

About 70%-80% of patients with Type-1 diabetes have islet cell auto antibodies against intracellular islet cell antigens, such as GlutamicAcid Decarboxylose (GAD) "islet auto antigen 2" (1a-2a tyrosine phosphatase), insulin and gangliosides.

3. Environmental Factors

Viruses

Epidemiologic studies suggest the action of viruses. A viral infection has long been noted in the diagnosis of new cases, and has the association between coxsackie viruses of group B and pancreatic diseases including diabetes. Other implicated viral infections include mumps, measles, cytomegalovirus, rubella and infectious mononucleosis.

It has been postulated that one of that viruses causes mild β-cells injury, which is followed by an auto-immune reaction against previously sequestered antigens in virally altered β-cells in persons with HLA-linked susceptibility. Another is that an immune response develops against a viral protein that shares amino acid sequences

with a β-cell protein (molecular mimicry).

Others

Antigenic exposure may also come from other sources. Children who ingest cow's milk products early in life (before age of 4 months) have a 1.5 fold increase risk for Type-1 diabetes relative to those who do not, raising the spectrum of a cross-reacting antigen in cow's milk.

Normal insulin production and effects[24]

Insulin is a protein composed of 51 amino acids in two chains (A and B chains), connected by two disulfide bonds. Insulin is synthesized and stored in the β-cells of the islets of Langerhans, which are located in the pancreas. The pancreas produces a parent protein called pre pro-insulin. Pre pro-insulin is claved to form a smaller protein, proinsulin. Proinsulin is claved to form equimolar amounts of C-peptide and insulin. The normal pancreas contains approximately 200U insulin, and a basal amount of insulin is secreted continuously at a rate of approximately 0.5 to 1.0 U/h. Additional insulin is also released in response to blood glucose levels of 100 mg/dL or more. The average daily insulin secretory rate in the adult is 25 to 50 U/day. Insulin is cleared metabolically by the liver, peripheral tissues, and kidneys. Insulin follows first-order elimination kinetics, and the serum half-life is approximately 4 to 5 minutes.

The important metabolic sites that are sensitive to insulin include the liver, where glycogen is synthesized, stored and broken down; skeletal muscle, where glucose oxidation produces energy; and adipose tissue, where glucose can be converted to fatty acids, glyceryl phosphate and triglycerides. Insulin affects carbohydrate, protein and lipid metabolism.

Carbohydrate metabolism

In patients without diabetes, insulin acts in concert with glucagon, somatostain, growth hormone, corticosteroids, epinephrine and parasympathetic innervation to maintain blood glucose between 40 and 160 mg/dL at all times. Three cell Types, α-cells, β-cells and δ-cells, have been identified in the islets of Langerhans of the pancreas. The α-cells produce glucagons, a hormone that acts to increase blood glucose levels. The β- cells produce, store and release insulin. The δ-

cells produce somatostain, which inhibits both insulin and glucagon secretion and suppresses growth hormone. The suppression of glucagon by somatostain decreases blood glucose levels and its effect persists for roughly 60 to 120 minutes.

Euglycemia is maintained by the three previously identified hormones working in concert. Ingestion of a carbohydrate load results in a prompt increase in the amount of insulin release and a concomitant decrease in plasma glucagon. Glucagon is released in response to low blood glucose levels and protein ingestion. Glucagon release stimulates insulin secretion; insulin in turn inhibits glucagon release. The presence of insulin favors the uptake and use of glucose by insulin-sensitive sites. In skeletal muscle, glucose uptake and subsequent energy production increase. In the liver, glucose uptake and the formation of glycogen increase in the presence of insulin. A minimum blood glucose level of 40 mg/dL is needed to provide adequate fuel for the brain, which can use only glucose as fuel and does not depend on the presence of insulin for its use. Glucose spills into the urine, resulting in energy and water loss, when blood glucose levels exceed the renal threshold (180 mg/dL).

Protein metabolism

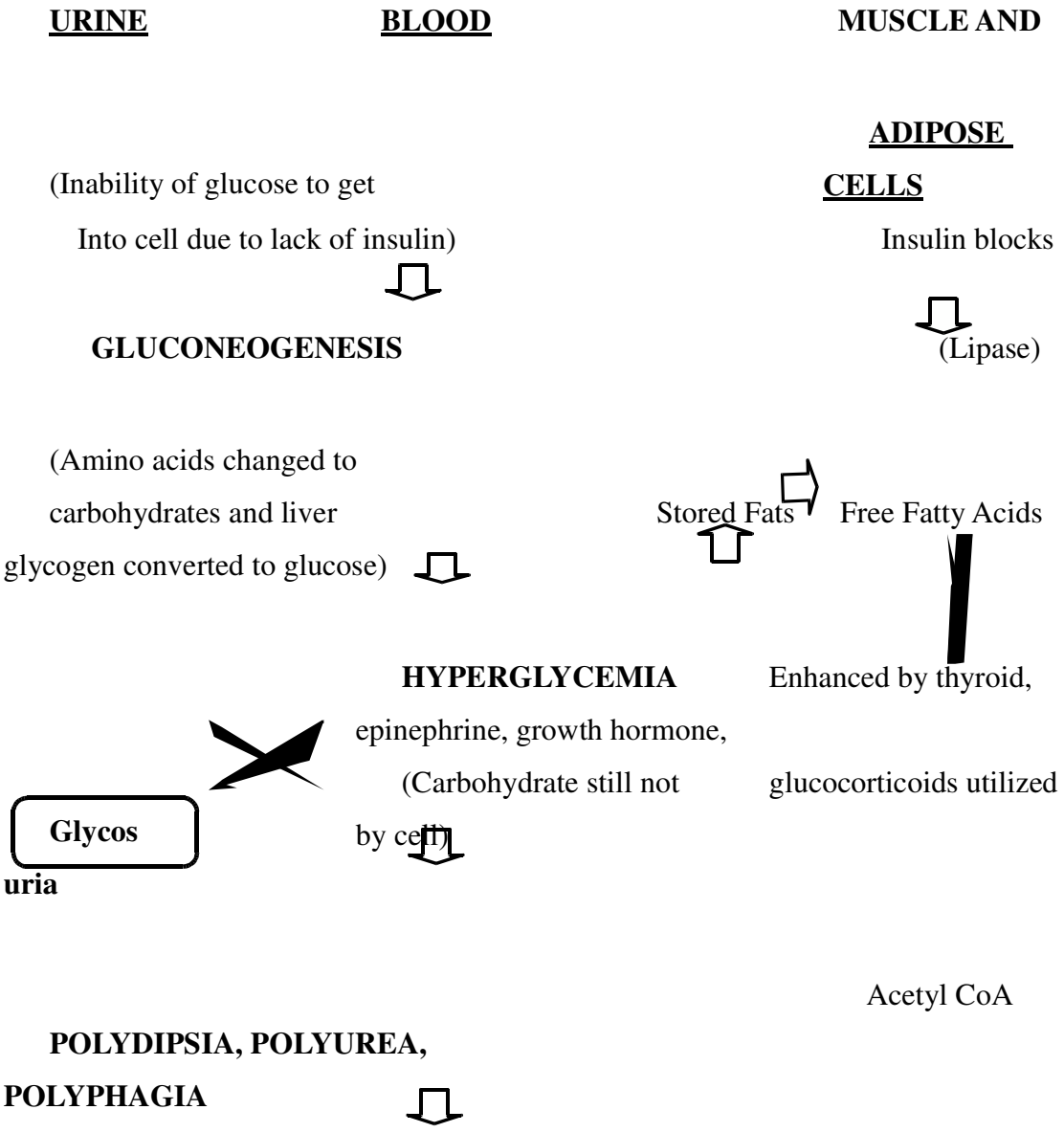
The presence of insulin favors the production of structural proteins from constituent amino acids. When glucose is present intra cellularly in sufficient quantities for needed energy production, most structural proteins retain their integrity. In the absence of insulin, structural protein production is not favored, and intracellular glucose levels are insufficient to match energy demands. In attempt to produce energy, skeletal muscle converts its structural proteins to constituent amino acids. The liberated amino acids are transported to the liver, where they are converted to glucose via gluconeogenesis. In patients with diabetes, glucose enters the blood but is not taken up by tissues because of a true or relative lack of insulin. Thus, hyperglycemia is escalated, and structural proteins are wasted.

Fat metabolism

The presence of insulin favors the production of triglycerides from free fatty acids (FFAs). When insulin deficiency causes an energy deficit, FFAs are oxidized to β-Hydroxybutyric acid, acetoacetic acid and acetone. β -Hydroxybutyric acid can be used as an energy source, but in the absence of insulin the production of the keto acids

eventually is greater than their metabolism and excretion.

If insulin is not given to the patient, metabolic ketoacidosis ensues. The keto acids cause the blood pH to decline and diuresis secondary to the elimination of ketones and glucose causes dehydration. The body's neutralizing factors eventually are depleted, and the patient continues to deteriorate to the point of coma and possibly death. Fig: shows the clinical manifestations of the untreated patient with Type-1 diabetes who is insulinopenic (complete lacks insulin).



INCREASED FAT METABOLISM

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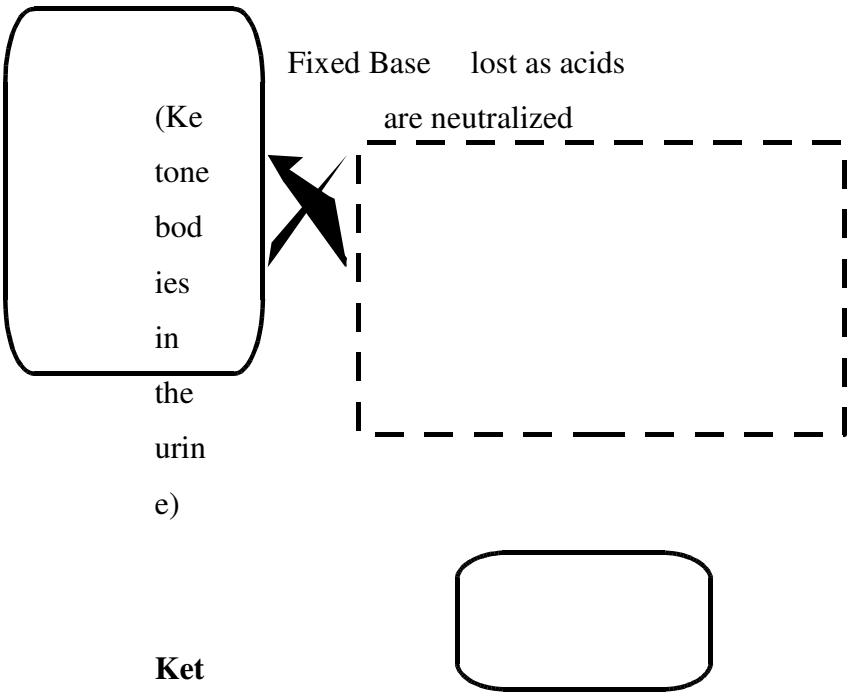
Krebs cycle for Oxidation (Energy)



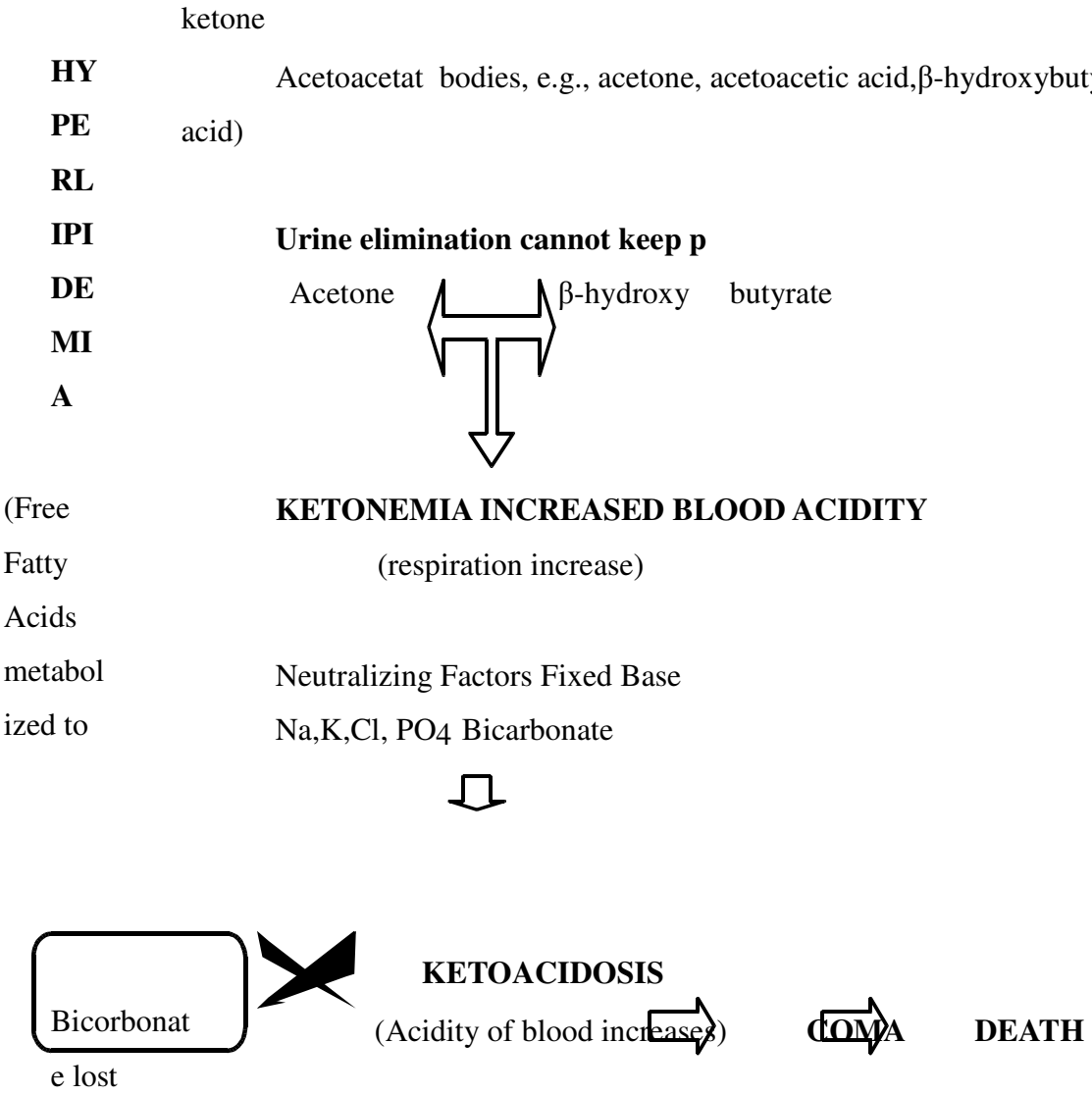


Fig: Clinical manifestations of a complete lack of insulin

Pathogenesis of Type-2 Diabetes mellitus[25]

The two metabolic defects that characterize Type-2 diabetes are:

1. A derangement in β-cell secretion of insulin
2. A decrease response of peripheral tissue to respond to insulin

(Insulin resistance)

1. Deranged β-cell secretion of insulin

A modest hyper insulinimia may be observed, attributed to β-cell hyper responsiveness to physiologic elevations in blood glucose, with the development of overt disease. The pattern of insulin secretion exhibits a subtle change. Early in the

course of Type-2 diabetes, insulin secretion appears to be normal and plasma insulin levels are not reduced.

However, the normal pulsatile oscillating pattern of insulin secretion is lost and the rapid first phase of insulin secretion triggered by glucose is obtunded. Collectively, these and other observations suggest derangements in β-cell response to hyperglycemia early in Type-2 diabetes, rather than deficiencies in insulin synthesis per se. Later in the cause of Type-2 diabetes a mild to moderate deficiency of insulin develops which is less severe than that of Type-1.

2. Insulin Resistance

Insulin resistance is a common pathologic state in which target cells fail to respond to ordinary levels of circulating insulin. It is frequently associated with a number of diseases, including chronic infection, human obesity and Type-2 diabetes.[26] At the molecular level, impaired insulin signaling results from mutations or post translation modification of the insulin receptor itself or any of its down-stream effector molecules. Insulin resistance could be accounted for by a defect in insulin binding to its receptor, there may be a decrease in the number of insulin receptor and more important, post receptor signaling by insulin is impaired.

The binding of insulin to its receptor leads to translocation of glucose transporter (GLUT's) to the cell membrane, which in turn facilitates cellular uptake of glucose. It is suspected that reduced synthesis and translocation of glucose transporter (GLUT's) in muscle and fat cells underlies the insulin resistance noted in obesity as well as in Type-2 diabetes.

Obesity

Approximately 80% of Type-2 diabetic are obese with abdominal obesity having a greater impact. Intra-abdominal fat catabolism delivers free fatty acids to the liver, yet is relatively resistant to the modulating effects of insulin. Although abdominal obesity and insulin resistance could be coincidental expressions of a third unknown factor, the possibility that they are casually related must be considered.

Amylin

Amylin, a 37 amino acid peptide is normally produced by the β-cells co-packaged with insulin and co secreted with insulin in response to food ingestion. In patients

with Type-2 diabetes, Amylin tends to accumulate in the sinusoidal space outside the β-cells, in close contact with their cell membrane, eventually acquiring the tinctorial characteristics of amyloidal.[27] It is unknown whether Amylin deposition contributes to the disturbance in glucose sensing by the β-cells, noted early in the course of Type-2 diabetes or is instead a result of disordered β-cell function.

Patients with Type-2 diabetes may have an elevated, normal or low level of circulating insulin, depending on the chronicity of their disease, and have a relative lack of effective insulin. In patients with Type-2 disease, insulin and glucose levels usually are adequate to prevent ketoacidosis development. However, glucose can accumulate in the blood and can reach extremely high levels (more than 400 mg/dL), resulting in non ketotic hyper osmolar syndrome (NKHS). [25]

Risk factors for complication of Diabetes mellitus[28]

Fixed risk factor:

Duration of diabetes: A linear relationship with the duration of the diabetes.

Age: The older the individual with, the greater the chance for complication, including cardiovascular and neuropathic complications.

Genetics: A relationship exists but is not really predictive.

Race: African-American individuals are more at risk for macro vascular and neuropathic changes but have a lower prevalence rate of myocardial infarction than Caucasians, Mexicans-american individual seem to hence an increased risk for peripheral vascular disease.

Gender: Premenopausal women without diabetes are less at risk than males without diabetes for cardiovascular events and complications, but not females without diabetes.

Height: Taller individuals seem to experience more neuropathy than other patients with

diabetes.

Modifiable risk factors:

Hyperglycemia:

Treating hyperglycemia lowers the incidence of many complications, including

neuropathy, retinopathy. Suggestive evidence is present to support the protective effect for cardio vascular disease, though it is not conclusive.

Hypertension: Plays a role in cardiovascular complication and neuropathy, nephropathy and retinopathy. Blood pressure maintainance to normal is vital.

Dyslipidemia: This concerns with decreased high density lipoprotein cholesterol (HDL), increased low density lipoprotein (LDL), increased triglyceride level and increased lipoprotein levels center primarily on cardiovascular complications but also is linked to neuropathy.

Platelet adherence: Patients with diabetes have an increased platelet adherence and

(PAI-1) aggregation that can cause significance micro vascular problems. Plasminogen activator inhibitor-1 can play a role in altering the blood's ability to clot also.

Smoking: Smoking is a direct risk for increased cardiovascular event in both patients

Fat intake: High fat dietary intake enhances the risk for vascular problems in patients with diabetes.

Obesity: Central or truncal obesity has been linked to atherogenesis, though no causal relationship is yet established for patients with diabetes.

Alcohol use: Alcohol intake enhances the potential for patients with diabetes to develop neuropathy.

Complications of diabetes[29]

Both types of diabetes mellitus may develop complications which are broadly divided into two major groups.

1. Acute metabolic complications:

These include diabetic ketoacidosis, hyperosmolar nonketonic coma an n hypoglycaemia.

Late systemic complications:

These are atheroscelerosis, diabetic microangiopathy, diabetic nephropathy, diabetic retinopathy and infections.

Acute metabolic complications

Metabolic complications develop acutely, while ketoacidosis and hypoglycemic episodes are primarily complications of Type-1 diabetes. Hyperosmolar non-ketotic coma is chiefly a complication of Type-2 diabetes.

Diabetic ketoacidosis[30]

It is a state of accelerated starvation caused by insulin deficiency. Insulin deficiency causes excessive lipolysis and decrease in peripheral utilization of glucose. Free fatty acids produced from lipolysis are oxidized into ketones in liver due to elevated glucagons and decreased insulin concentration. Ketosis produces acidosis which causes further loss of electrolyte, loss of vascular tone and shock. Clinically, the condition is characterized by anorexia, nausea, vomiting, deep and fast breathing, mental confusion and coma. Most patients of ketoacidosis recover.

Hyperosmolar non-ketotic coma[31]

Hyperosmolar non-ketotic coma occurs in elderly Type 2 diabetic patients. It is caused by severe dehydration resulting from sustained hyperglycemic diuresis. The loss of glucose in urine is so intense that the patient is unable to drink sufficient water to maintain urinary fluid loss. Blood sugar is extremely high and plasma osmolality is high. Thrombotic and bleeding complications are frequent due to high viscosity of blood. The mortality rate in hyperosmolar non-ketotic coma is high.

Hypoglycaeia [32]

Mainly occur in patients of Type-1 diabetes. This may result from excessive administration of insulin missing a meal or due to stress. Hypoglycemic episode are harmful as they produce permanent brain damage or may result in worsening of diabetic control and rebound hyperglycemia so called Somogyi's effect.

2. Late systemic complications[33]

Systematic complication may develop after a period of 15-20 years in either type of diabetes. These late complications are largely responsible for morbidity and premature mortality in diabetes.

Atherosclerosis:

Diabetes mellitus of both Type-1 and Type-2 accelerate the development of atherosclerosis and are more extensive. More often it is associated with

complication plaques such as ulceration, calcification and thrombosis. The cause for these accelerate atherosclerotic plaques are possible because of contributory factors like hyperlipidemia, reduced HDL levels, non-enzymatic glycosylation, increase platelet adhesiveness, obesity and associated hypertension. In diabetes, the possible ill effects of accelerated atherosclerosis are easily onset of coronary artery disease, silent myocardial infarction, cerebral stroke and gangrene of lower extremities.

Diabetic Microangiopathy:

It is characterized by basement membrane thickening of small blood vessels and capillaries of different organs and tissue such as the skin, skeletal muscle, eye and kidney. Increase glycosylation of haemoglobin and other proteins (e.g. Collagen and basement membrane material) results in thickening of basement membrane.

Diabetic Nephropathy:

Renal involvement is a common complication and leads cause of death. Four types of lesions are described in diabetic nephropathy.

1. Diabetic glomerulosclerosis which includes diffuse and nodular lesions of glomerulosclerosis, which is termed as Kimmelstiel – Wilson (KW) lesions.
2. Vascular lesions those include hyaline arteriolosclerosis of afferent and efferent arterioles and atheromas of renal arteries.
3. Diabetes pyelonephritis and necrotizing renal papillitis.
4. Tubular lesion or Armanni – Ebstein lesions characterized by development of glycogen vacuoles in the epithelial cells of proximal convoluted tubules.

Diabetic Neuropathy:

Diabetic neuropathy may affect all parts of the nervous system but symmetric peripheral neuropathy is most characteristic. The basic pathologic changes are segment demyelination, Schwann cell injury and axonal damage. The pathogenesis of neuropathy is not clear but may be related to diffuse microangiopathy as already explained or may be due to accumulation of sorbitol and fructose as a result of hyperglycemia.

Diabetic Retinopathy:

Diabetic retinopathy is a leading cause of blindness.

There are two Types of lesions involving retinal vessels.

1. Background
2. Proliferative

Background retinopathy is the initial retina capillary microangiopathy. Proliferative retinopathy or retinitis proliferens occurs eventually due to severe ischemia and hypoxia of the retina. The major pathologic changes are the neovascularisation at the optic disc or along the retinal veins. Besides retinopathy diabetes patients are also prone to early development of cataract and glaucoma.

Infections:

Diabetics have enhanced susceptibility to various infections such as tuberculosis, pneumonias, pyelonephritis, otitis, carbuncles and diabetic ulcers. This could be due to various factor such as impaired leucocyte functions, reduced cellular immunity poor blood supply due to involvement and hyperglycemia per se.

Mechanisms of hyperglycemia-induced damage[34]

Four major hypotheses about how hyperglycemia causes diabetic complications have generated a large amount of data on specific inhibitors of these mechanisms.

1. Increased polyol pathway flux
2. Increased intracellular AGE
3. Activation of protein kinase-C
4. Increased hexosamine pathway flux formation.

1. Increased polyol pathway:

Increase activity of this ubiquitous biochemical pathway lead to intracellular accumulation of osmotically active sorbitol and fructose through the action of the enzyme aldose reductase. A number of mechanisms have been proposed to explain the potential detrimental effects of hyperglycemia induced increases in polyol pathway flux. These include sorbitol-induced osmotic stress, decreased Na^+/ K^+ ATPase activity, increased cytosolic NADH / NAD^+ and decreased cytosolic NADPH. Depletion of myo-inositol and impairment of Na^+ / K^+ ATPase are the associated disturbances those have been implicated, particularly in the pathogenesis of diabetic neuropathy.

2. Increased intracellular AGE Formation

AGEs are found in increased amounts in extracellular structure of diabetic retinal vessels and renal glomeruli, where they can cause damage by following mechanisms. Intracellular production of AGE precursors damages target cells by three general mechanisms:

- Intracellular proteins modified by AGEs have altered function
- Extracellular matrix components modified by AGE precursor interact abnormally with other matrix components and with matrix-receptor (integrins) on cells.
- Plasma proteins modified by AGE precursors bind to AGE receptor on cells such as macrophages, inducing receptor-mediated ROS production. This AGE receptor ligation activates the pleiotrophic transcription factor NFκB, causing pathologic changes in gene expression.

Activates protein kinase C - β in endothelial cells. This alters vascular permeability and increases basement membrane synthesis (an important early histological feature of the micro vascular complications of diabetes) and pathological changes results.

↑ Glucose → ↑ Di acyl glycerol → ↑ Protein Kinase C - β

Secondary increase in the expression of growth factors with effect on vascular permeability and may contribute to neovascularization in the retina.

4. Increased hexosamine pathway flux

In this mechanism glucose is shunted into the hexosamine pathway. In this pathway, fructose-6-phaosphate is diverted from glycolysis to provide substrates for reactions that required UDP-*N*- acetyl glucosamine, such as proteoglycan synthesis and the formation of *O*-linked glycoproteins. Inhibition of the rate limiting enzyme in the conversion of glucose to glucosamine, blocks hyperglycemia-induced increases in the transcription of both TGF- α and TGF-β_1. This pathway has previously been shown to play an important role in hyperglycemia-induced and fat-induced insulin resistance.

Treatment of diabetes mellitus

Treatment of diabetes mellitus involves changes in lifestyles and pharmacologic intervention with insulin or oral glucose lowering drugs. In Type-1 diabetes mellitus, the primary focus is to replace insulin secretion; lifestyle changes are required to facilitate insulin therapy and optimize health. For most patients with Type-2 diabetes, changes in lifestyle are the cornerstone of treatment, particularly in the early stages of the disease.

Pharmacologic intervention is a secondary treatment strategy.[32]

Drugs for Type-1 diabetes mellitus[32]

Insulin remains the mainstay of therapy for the treatment of Type-1 diabetes. Various forms of insulin dosage forms are available.

Insulin Types

Four types of insulin are classified according to the three criteria.

1. Onset: How quickly the insulin starts to work after it is injected.
2. Peak time: The period of time when insulin is most effective in lowering blood sugsr levels.
3. Duration: How long the insulin is most effective in lowering blood sugar levels.

Only NPH insulin is mixed with regular insulin to allow the action of both types of insulin at the same time. Other types of insulin cannot be mixed together and may required separate injections. Insulin is indicated in the following conditions in diabetes.

Insulin preparation	Onset (h)	Peak (h)	Duration (h)
Short acting			
Lispro	< 0.25	0.5 – 1.5	4 –6
Intermediate acting			
NPH Lente	2 -4	6 -10	14 -18
Long acting			
Ultralente	6 -10	10 -16	20 -24
Combination			
75 % NPH, 25 % Regular	0.5 -1	Dual	14 -18
70 % NPH, 30 % Regular	0.5 -1	Dual	14 -18
50 % NPH, 50 % Regular	0.5 -1	Dual	14 -18

Table: Insulin types

Oral hypoglycemic agents[32]

Type-2 diabetes patients requiring insulin are those cases who have failed to achieve optimal glycemic control on maximal doses of oral agents. In this situation addition of evening or bedtime intermediate acting insulin to the oral agent is an easy and highly effective method to regain optimal glucose control. When evening insulin is added, total dose of oral agent can be given in the morning before breakfast. In this manner, evening insulin can be used to control early morning hyperglycemia whereas oral agents exert their effects on day time glycemia.

Table: Targeted drugs therapy for Type-2 diabetes

1. Insulin secretogogues:

a. Sulphonylureas:

First generation :Tolbutamide, Chlorpropamide, Acetohexmide, Tolazolamide

Second generation : Glibenclamide, Glipizide, Gliclazide, Glimeperide

b. Meglitinides : Repaglinide

2. Insulin sensitizers : Biguanides, Thiazolidines
3. α-glucosidase inhibitors: Acarbose, Miglitol
4. Indian drugs : Guar gum, Fenugreek seeds

Sulphonylureas

Sulphonylureas have been the mainstay in the treatment of Type-2 diabetes and functions by stimulating insulin secretion from pancreas; hence they are called insulin secretogogues. The net effect is increased responsiveness of β-cells of pancreas resulting in more insulin. 1^{st} generation sulphonylureas have a lower binding affinity to sulphonylureas receptor and must be given in higher doses than 2^{nd} generation sulphonylureas.

Mode of action: The sulphonylureas stimulate insulin secretion through a direct effect on the pancreatic β-cells. They bind to sulphonylurea receptor (SUR) on these cells, which is a component of the trans membrane complex that houses the ATP-sensitive potassium channels (K-ATP channels) binding of the sulphonylurea closes K-ATP channels, reducing potassium efflux and favoring membrane depolarization. In turn, depolarization opens voltage-dependent calcium channels, increasing calcium influx, raising intracellular calcium concentrations and activating calcium dependent proteins that control the release of insulin granules. In this way, the sulphonylureas lead to a prompt release of pre-formed insulin granules adjacent to the plasma membrane (first phase insulin release). This class of drug also seems to exert some weak extra pancreatic effects which suppress

hepatic gluconeogenesis and potentiate insulin mediated glucose uptake into muscle and adipose tissue.

Clinical use of sulphonylureas

Indications:

Patients with Type-2 diabetes inadequately controlled by non pharmacological measures of other types of oral antidiabetic agents.

Type of therapy: Monotherapy or in combination with other antidiabetic agents except other insulin-releasing agents.

Treatment schedule: A low dose to begin with escalated slowly. Schedule should be adjusted if necessary to minimize the risk of hypoglycemia. Maximum effect may be achieved before the maximum permitted dose is reached.

Cautions and contraindications: Should be used cautiously in patients with hepatic or renal disease of porphyria.

Side effect: Occasional, usually transient, sensitivity reactions. Chlorprapamide can lead to facial flushing after alcohol has been consumed rarely, it causes hyponatremia.

Adverse effects: Risk of hypoglycemia, especially with high doses of longer acting preparations.

Note: Always start with a low dose of sulphonyl urea. Monitor blood glucose by an appropriate method and increase dosages at 2-4 week interval as necessary until the glycemic target is achieved.

Non-Sulphonylureas

Brief history: The non sulphonyl urea portion of glibenclamide, a benzamido compound termed meglitinide was shown to stimulate insulin secretion in the early 80's.

Mode of action: The mechanism through which benzamido prandial insulin releasers stimulate insulin secretion is essentially the same as that of the sulphonylureas. These drugs also bind to the SUR on the plasma membrane of the β-cell, but at a different site. This binding closes the ATP-sensitive potassium channel, leading to depolarization, voltage-dependant calcium influx, and activation of calcium dependant proteins that control insulin release.

Clinical use of Repaglinide

Indications: Patients with Type-2 diabetes inadequately controlled by non pharmacological measures or other Types of oral agents that do not stimulate insulin secretion. Patients prone to interprandial hypoglycemia on a sulphonylurea.

Type of therapy: Monotherapy or in combination with other antidiabetic agents (except other insulin-releasing agents).

Cautions and contraindications: Should be used with cautions in patients with hepatic or severe renal disease.

Side effects: Occasionally sensitivity reactions, usually transient.

Adverse reactions and precautions: Risk of hyperglycemia. Possible interactions with nerythromycin, rifampicin and some antifungal agents.

Futere therapies to improve insulin secretion

New sulphonylurea formulations:

A micronized formulation of glibenclamide (glyburide) introduced in the US increases the rate of absorption and enables an earlier onset of action. A longer acting (extended release) formulation of glipizide (glucotrol XL) has also been introduced.

A modified release formulation of gliclazide (diamicron MR) has been developed. While the duration of action of glicazide is unchanged; this new formulation uses a hydrophilic matrix to match progressive delivery of gliclazide with the hyperglycemic profile. Improved bioavailability enables once daily dosing while reducing the dosage from 80 mg per tablet to30 mg per tablet.

Novel insulin releasers

A potential future approach to synchronizing insulin secretion with meal consumption involves the intestinal hormone Glucagon-Like Peptide-1 (7-36 amide) (GLP-1). GLP-1 potentiate nutrient stimulated insulin secretion via receptors in the pancreatic β-cell membrane which activate cyclic adenosine monophosphate (cAMP) and this increases the insulin response to a meal. It also enhances proinsulin biosynthesis and may additionally slow gastric emptying and induce satiety.

A further novel approach to enhancing insulin secretion involves succinate esters which enhance the activity of the Krebs cycle in the β-cell. This stimulates proinsulin biosynthesis and insulin secretion. However, succinate esters can serve as a nutrient fuel in a range of tissues and provide a substrate for gluconeogenesis. Thus, this approach can only be exploited if the succinate esters are targeted predominately at β-cells.

Biguanides

Metformin is the only biguanide available. It is less widely used than the sulphonylureas because of a higher incidence of side effects, particularly gastrointestinal symptoms.

Mode of action: Metformin has a variety of metabolic effects. It acts partly by improving insulin action and partly by effects that are not directly insulin dependent. Metformin lowers blood glucose concentrations without causing overt hypoglycemia. Its clinical efficacy requires the presence of insulin, but the drug does not stimulate insulin release and typically causes a small decrease in basal insulin concentration in hyperinsulinemic patients.

It reduces hepatic gluconeogenesis mainly by increasing sensitivity of insulin. Additionally it reduces the hepatic extraction of certain gluconeogenic substrates (such as lactate) and opposes the effects glucagons. It decreases the rate of hepatic glycogenolysis, and the activity of hepatic glucose-6-phosphatase.

Insulin stimulated glucose uptake and glycogen formation in skeletal muscles are enhanced by metformin. These involved increases movement of insulin sensitive glucose transporters into the cell membrane and increased activity of glycogen synthase.

Metformin also acts in an insulin-independent manner to suppress fatty acid oxidation and reduce triglyceride levels in hypertriglyceridemic patients. This reduces the energy supply for gluconeogenesis and improves the glucose cycle.

Indications: Patients with Type-2 diabetes inadequately controlled by non pharmacological measures or other oral antidiabetic agents.

Type of therapy: Monotherapy or in combination with any other antidiabetic

agents. **Treatment schedule:** Metformin should be taken with meals and dose escalated slowly to a maximum of 2550 or 3000 mg/day.

Cautions and contraindications: Renal and hepatic disease, cardiac or respiratory disease or any other hypoxic condition, severe infections, alcohol abuse, history of acidosis, intravenous radiographic contrast media.

Side effects: Gastrointestinal symptoms (e.g. diarrhoea), metallic taste, possibly educed absorption of vitamin B and folic acid, intravenous radiographic contrast media.

Adverse reactions: risk of lactic acidosis if contraindications breached risk of hypoglycemia with combination therapy.

Precautions: Contraindications should be observed, especially renal function (e.g. creatinine level): interacts with cimentidine.

α **-Glucosidase inhibitors**

Mode of action: α-Glucosidase enzymes in the brush border of the small intenstine, along with pancreatic α-amylase are responsible for the hydrolysis of complex starches, oligosaccharides, trisaccharides and disaccharides. Acarbose, an α-Glucosidase and α- amylase inhibitor, works by reducing the rate of complex carbohydrate digestion and subsequent absorption of glucose, thereby lowering post prandial glucose excursions in patients with diabetes. Acarbose is particularly effective for patients who experience postprandial hyperglycemia. Meglitol is another α-Glucosidase inhibitor that works similarly to acarbose and like wise is effective in controlling postprandial hyperglycemia.

Indications: For patients with Type-2 diabetes inadequately controlled by non-pharmacological measures or other antidiabetic agents.

Treatment schedule: Should be taken with meals rich in digestible complex carbohydrate. Dose should be titrated slowly to be compatible with meals.

Cautions and contraindications: renal and hepatic disease, history of chronic intestinal disease.

Adverse reactions: Gastrointestinal disturbances are occasionally severe, very rarely associated with abnormal liver function.

Adverse effects: The most common problems experienced with the α-Glucosidase

inhibitors are gastrointestinal side effects.

Thiazolidinediones

Mode of action: These drugs (also called TZD drugs, glitazones or PPARγ agonists) bind and activate peroxisome proliferators activated receptor γ, a nuclear receptor that regulates the expression of several genes involved in metabolism and worked by enhancing the action of endogenous insulin. Insulin sensitivity (mainly in adipose tissue) is improved only in patients with insulin resistance: plasma insulin concentrations are not increased and hypoglycemia is not a problem. Its main action is on muscle tissue, where it increase glucose disposal, and to a lesser degree in the liver, where it decreases hepatic glucose production. To other thiazolidinediones, rosiglitazone and pioglitazone, act similarly to troglitazone.

Clinical use of the Thiazolidinediones

Indications: Type-2 diabetic patients inadequately controlled by non-pharmacological measure or other antidiabetic agents.

Type of therapy: Monotherapy or in combination with any other antidiabetic agents. **Treatment schedule:** Pioglitazone (15 or 30 mg/day), rosiglitazone (2 or 4 mg/day). Dose should be escalated gradually upto a typical maximum of 45 mg/day for pioglitazone and 8 mg/day for rosiglitazone.

Cautions and contraindications: Congestive heart disease, heart failure, oedema, anaemia, impaired liver function.

Side effects: Fluid retention, increased plasma volume, reduced haematocrit, decreased haemoglobin, ovulation in polycystic ovarian syndrome.

Adverse effect: Risk of oedema and anaemia, risk of hypoglycemia with combination therapy.

Precautions: Check for contraindications, monitor liver enzymes (e.g. alanine transaminase), and potential effect on oral contraceptive activity (pioglitazone).

Animal models for experimental Diabetes Mellitus [33, 34] There are many advantages of using animals models in research work on diabetes as various aspects of the disease like the etiology, its multifactorial genetics, pathogenesis of the disease and its complication can be explicitly understood. Secondly, it also helps in the

development and evaluation of newer agents for the treatment of diabetes. However, there are some limitations in the use of animal model for studies on diabetes.

Induction of diabetes in animals can be carried out by various ways – by using different chemical diabetogenic agents, surgically by partial pancreatectomy, by viral induction and genetic manipulation by selective in breeding.

Various diabetic chemicals

Induction of diabetes by various chemical diabetogenic agents is also dependent on the species, the strain, sex and the diet of the animals. Variations in susceptibility have also been observed amongst male and female mice of same strain, males being more susceptible to insulin dependent diabetes mellitus (IDDM) than females. Types of diabetes produced depend on the amount of diabetogenic agent used.

1. Alloxan[34]

Diabetogenic action of alloxan is mediated by reactive oxygen species. Alloxan and the product of its reduction, dialuric acid, establish a redox cycle with the formation-of superoxide radicals. These radicals undergo dismutation to hydrogen peroxide. Thereafter highly reactive hydroxyl radicals are formed by the Fenton reaction. The action of reactive oxygen species with a simultaneous massive increase in cytosolic Ca^{+2} concentration causes rapid destruction of β-cells. The action of alloxan in the pancreas is preceded by its rapid uptake by the β-cells. Since alloxan exhibits a high affinity to th SH-containing cellular compounds, reduced glutathione (GSH), cysteine and protein bound sulfhydryl groups (including SH-containing enzymes) are very susceptible to its action. The reaction between alloxan and dialuric acid is a process in which intermediate alloxan radicals ($HA^{\bullet}$) and an unidentified "compound 305" (maximum absorption at 305 nm) is formed. Alloxan is converted into unstable dialuric acid which is then re oxidised back to alloxan. This reaction establishes a redox cycle for the generation of superoxide radicals and also accompanied by reduction of oxygen to the OFR, O_2, and H_2O_2. The latter, through a Fanton Type reaction in the presence of transition metals generates the highly toxic OFR, OH. Increased production of OFR in the islets, together with inadequate

defence makes the β-islet cells susceptible to alloxan. Alloxan induces membrane lipid peroxidation and extensive DNA strand breakage in these cells. In normal non fasted animals, the blood glucose level after alloxan injection fluctuates in a triphasic pattern.

Triphasic response of alloxan

1. Early hyperglycemia of short duration (about 1-4 h) due to a sudden short lasting decrease or cessation of insulin release and a direct glycogenolytic effect on the liver.

2. Hyperglycemia phase lasting up to 48 h and often resulting in convulsion and death (which may be prevented by treatment by glucose) due to uncontrolled leakage of insulin from the damaged cells.

3. Chronic diabetes phase, consequence of insulin lack histologically only a few β- cells if any, are detectable in animals with fully developed alloxan diabetes. Exogeneous insulin readily restores normal blood glucose level.

2. Streptozotocin[35]

Streptozotocin [STZ,2-deoxy-2-{3-(methyl-3-nitrosoureido)-Dglucopyranose}] is synthesized by streptomycetes achromogenes and is used to induce both Type-1 and Type-2. It is freely soluble in water, unstable at room temperature and has to be stored below -20 ^{O}C.

Streptozotocin induces diabetes in almost all the species. Diabetes dose varies with the species and the optimal dose required to produce diabetes in rat was found to be (50 – 60 mg/kg i.p. or i.v.), in mice (175-200 mg/kg i.p. or i.v.) and in dogs (15 mg/kg, for 3 days). Due to its low stability the rapid i.v. injection appears to be the best route of administration. STZ induces diabetes in hamster, monkey and guinea pigs. STZ diabetes can be induced by two ways either by single injection of STZ or by multiple low dose injection of STZ. Like alloxan, it shows triphasic fluctuation pattern in diabetes.

Initial hyperglycemia is observed by 1 h after the injection followed by hyperglycemia and again a hyperglycemia state at 48 h, the elevated blood glucose level is observed by 48-72 h (peak effect) and is maintained thereafter.

Different mechanism of action on the β-cells destruction by STZ have been proposed. Its main actions through free redical generations. Other report proposed that STZ exerts lethal damage by alkylating DNA or its phosphate backbone as well as glycolytic or mitochondria enzyme. STZ also influence the immune system by suppressing the T-cell function associated with atrophy of the thymus and peripheral lymphoid tissue. Like alloxan, STZ also induces OFR induced lipid peroxidation and DNA strand breaking in pancreatic islet cells.

Streptozotocin enters the β-cell via a glucose transporter (GLUT 2) and cause alkylation of DNA. DNA damage induces activation of poly ADP-ribosylation leads to depletion of cellular NAD^+ and ATP. Enhanced ATP dephosphorylation after streptozotocin treatment supplies a substrate for xanthine oxidase resulting in the formation of superoxide radicals. Consequently, hydrogen peroxide and hydroxyl radicals are also generated. Furthermore, streptozocin liberates toxic amounts of nitric oxide that inhibits aconitase activity and participates in DNA damage.

Alloxan	**Streptozotocin**
1. Maximum blood glucose levels during phase of acute hyperglycemia occur in 45 minutes. 2. Liver glucogen depletes faster. 3. Elevated insulin levels during hypoglycemia phase. 4. Hypoglycemia less severe. 5. Sensitive to insulin 6. Mortality rate is 37%. 7. Reversible after 3 months.	1. Maximum blood glucose level during phase of acute hyperglycemia occurs in 120 minutes. 2. Liver glucogen depletes slowly. 3. Elevated insulin levels during hypoglycemia phase. 4. More severe. 5. Sensitive to insulin. 6. Mortality rate is 8%. 7. Irreversible.

Table: Comparison of alloxan and streptozotocin diabetes

3. Lithium salt[34]

It has been shown to inhibit glucose stimulated insulin release in rats, after either in vitro or in vivo administration leading to glucose intolerance. Lithium stimulates the α- adrenocepters leading to increase in plasma glucose level and inhibits the release of insulin from the pancreatic islet calls. I.V. infusion of lithium at a dose of 4 mg/kg into the jugular vein of anaesthetized rats produced an elevated blood glucose level within 30 minutes and 7-8 nmol/l and by 2 h the animal became normoglycemic. This hyperglycemia effect on the sympatho– adrenal function, both directly and indirectly, leading to decreased release of insulin from the β-cells of the pancreas

4. Cyclosporine A (C_SA)

Cyclosporine A has effects on the pancreatic endocrine functions and has been reported to cause hyperglycemia in humans, dogs and several different strains of rats. The exact mechanism of this hyperglycemic action is not known but in vivo studies suggest that C_SA administered orally at a dose of 40 mg/kg to Wistar rats for 7 days produced significant increase in fasting blood sugar levels.

5. Miscellaneous agents[36]

The other agents used for induction of NIDDM induce adrenaline (0.1 mg/kg, s.c.) administered in rabbits, the peak glycemia effects was noticed at 1 h and lasted up to 4 h. The increase in blood sugar levels was found to be 120-150 mg/100 ml. Oral hypoglycemic agents can be screened by this method. Diabetes can also be induced in animals with the chelating agents 8-hydroxyquinoline. Diphenylthiocarbazine EDTA has been reported to be diabetogenic in partially depancreatized rats. Injection of an antiserum made against ox insulin in guinea pigs or sheep caused diabetes in mice.

Administration of thiazides, chlorothiazides, hydrochlorothiazides, diazoxide and furosemide produced hyperglycemia and glucosuria in experimental animals, including rabbits, rats and mice. Diazoxide was found to be more effective either alone or in

combination with other drugs.

- Ethyl Alloxan 53-130 mg/kg in rat
- Oxime & Dithizone 53 mg/kg in rabbit
- Sodium Diethyldithiocarbonate 0.5-1 gm/kg in rabbit
- . Potassium Xanthate 200-350 mg/kg in rabbit
- Uric Acid 1 gm/kg in rabbit

Non-insulin dependent diabetes mellitus (NIDDM) resembling animal models

By altering the dose and the day of the STZ injection, the n-STZ models exhibit various stages of Type-2 diabetes mellitus, such as impaired glucose tolerance, mild, moderate and severe hyperglycemia.

Neonatal STZ-induced rat (n-STZ) model of Type 2 diabetes mellitus model is generated by injecting Wistar rats on the day of their birth (n0=birth) intravenously (sapheneous vein) or intraperitoneally with 100 mg/kg of STZ. Also, the n-STZ rat model is developed by varying the day of the STZ injection after the birth, such as 2^{nd} day or 5th day of the birth, and these are alternatively called n2-STZ and n5-STZ models respectively.

The rats treated with STZ on the day of birth, exhibit insulin deficient acute diabetes mellitus 3-5 days after birth. They showed high plasma glucose and about 93% decrease in plasma insulin and high plasma glucagon content. It was found that only by 8 weeks of age and thereafter n0-STZ rats showed mild hyperglycemia.

Sprague-Dawley pups were injected intraperitoneally on the 2nd day after birth with 90 mg/kg STZ and on 1.5 days after birth with 120 mg/kg STZ. By 6 weeks of age these animals showed basal hyperglycemia and abnormal glucose tolerance. The above two animal models are based mainly on β-cell deficiency and these models are useful for evaluating the effect of β-cell deficiency in the development of NIDDM.

NIDDM animal models can also be prepared by neonatal alloxan induced diabetes by injecting alloxan 200 mg/kg body weight i.p. to neonates of 6 days old. Non fasted blood sugar is determined after 8 weeks. The hyperglycemia produced is stable upto 6 months.

Insulin dependent diabetes mellitus (IDDM) resembling animal models[34]

IDDM both in human beings and in animals models of IDDM, is due to

destruction of β-islet cells, ultimately leading to an absence or decreased secretion of insulin, the destruction of the β-cell can be brought about by viral induction injection of diabetogenics or by introduction of transgenes. Streptozotocin and alloxan were found to be more selective in β-cell destructive than other chemical like ascorbic acid and its derivative and uric acid. These substances have also been referred to as β-cytotoxic substance or simply β-cytotoxic with the implication that the actions are restricted to the β-cell of the islet of Langerhans.

Pancreatectomy in dogs and rats

Either by total pancreatectomy or partial excision of pancreas, diabetes can be induced in dogs, cats and rats. The diabetes produced is very severe and persist at maxium intensity.

Virus induced diabetes

Juvenile-onset (Type-1) diabetes mellitus may be due to virus infection and β-cell specific auto-immunity. The D-variant of encephalomyocarditis virus (EMC-D) selectively infects and destroys pancreatic β-cells in susceptible mouse strains similar to human insulin dependent diabetes. Adult male ICR swiss mice are susceptible to the diabetogenic effect of the D-varient of encephalo myocarditis virus in contrast to adult CH/HCT male mice, which are relatively resistant.

Genetically diabetic animals

Several animal species, mostly rodents were described to exhibit spontaneously diabetes mellitus on a hereditary basis. Due to difficulty in breeding severely affected animals only a few species are used on a broader scale, such as Chinese hamster, obese mice, the obese hyperglycemic Zucker rat and various substrains of KK mice. **Spontaneously diabetic animals**

1. Spontaneously diabetic rats
2. Spontaneously diabetic mice

1. Spontaneously diabetic BB rat:

A BB rat is model of spontaneous diabetic associated with insulin deficiency due to auto-immune destruction of pancreatic β-cells.

2. WBN / KOB rat:

Spontaneous hyperglycemia glycosuria and glucose intolerance have been

observed in aged male of an in-bred Wistar strain.

3. Cohen diabetic rat:

Characterized by hyperglycemia, glycosuria and hyperinsulinemia with late development of hypoinsulinemia, insulin resistance and a decrease in the number and sensitivity of insulin receptors.

4. Zucker-Fatty rat:

This is a classic model of hyperinsulinemic obesity. These rats manifest mild glucose intolerance, hyperinsulinemia and peripheral insulin resistance similar to human NIDDM.

Chapter: II

Scope, Objective and Plan of Work

II. SCOPE, OBJECTIVE AND PLAN OF WORK

A study of Evaluation of aqueous extract of C*yperus rotundus* roots on diabetic complication in Alloxan induced diabetic rats.

Objectives of the study:

To evaluate the Aqueous extact of *Cyperus rotundus* in the management of type-II non-insulin dependent diabetes mellitus complications. The study was conducted by the following objectives.

1. To prepare aqueous extraction of *Cyperus rotundus* root
2. To study the phytochemical screening of ethanolic extract *Cyperus rotundus* root
3. To study the acute oral toxicity of *Cyperus rotundus* root
4. To study the diabetic complication of aqueous extract of *Cyperus rotundus* root in rats by the following model
    - Alloxan induced diabetic rats

Parameters to be studied:

1. Assessment of thermal hyperalgesia and cold allodynia
    - Hot plate method
    - Tail immersion (Hot water and cold water)
2. Estimation of serum biochemical parameters
    - SGOT
    - SGPT
    - ALP
    - Creatinine
    - Urea
    - uric acid

3. Histopathology of pancrease

Chapter: III

Review of Literature

III. REVIEW OF LITERATURE

1. **Nagulendran kr et al**[45] in (2007) has studied, In Vitro Antioxidant Activity and Total Polyphenolic Content of *Cyperus rotundus* Rhizomes

2. **Gupta MB et al** [46] in (1971) has studied Pharmacological studies to isolate the active constituents from *Cyperus rotundus* possessing anti-inflammatory, anti-pyretic and analgesic activities.

3. **Uddin SJ et al**[47] in (2006), has studied Antidiarrhoeal activity of *Cyperus rotundus*.

4. **Puratchikody A et al**[48] in (2006), had studied Wound healing activity of *cyperus rotundus linn.*

5. **KemprajVivek et al** [49] in (2008), had studied Ovicidal and larvicidal activities of *Cyperus giganteus Vahl* and *Cyperus rotundu*s Linn .

6. **Kilani Soumaya, et al**[50] had studied Investigation of extracts from (Tunisian) *Cyperus rotundus* as antimutagens and radical scavengers.

7. **Komai KV et al**[51] in (1975), has studied Chemical properties and behaviour of polyphenolic substances in *Cyperus rotundus.*

Chapter: IV

Plant profile

IV.PLANT PROFILE

Botanical Name : *Cyperus rotundus*

Scientific Classification

Kingdom : Plantae

(Unranked) : Angiosperms

(Unranked) : Monocots

(Unranked) : Commelidins

Order : Poales

Family : Cyperaceae

Genus : *Cyperus*

Species : C.**rotundus** [35]

General

Cyperus rotundus (Cyperaceae) is a traditional herbal medicine used as analgesic, sedative antispasmodic, antimalarial, stomach disorders and to relieve diarrhea[36.] Cyperus rotundus is a Perennial Plant that reach a height up to 140 cm. the leaves sprout in three from the base of the plant, around 5–20 cm long. The flowers stems have a triangular cross-section. The flower is Bisexual and has three stamina and a three-stigma carpels, with the flower head have 3-8 unequal rays. The fruit is a three-angled achene. The root of a young plant initially forms white, fleshy rhizomes, up to 25 mm, in chains. Some rhizomes grow upward in soil, form a bulb-like structure from which new shoots and roots grow[37.]

Distribution and habitat:

Cyperus rotundus is one of the most invasiveweed known, having a worldwide distribution in tropical and temperate regions. It has been called "the world's worst weed" [38]

Common Indian Names

Gujarati: Motha

Hindi: Motha, Mutha

Canarese: Koranarigadde, Tungegaddo, Tungehullu

Marathi: Bimbal, Nagarmotha, Motha

Sanskrit: Bhadramusta, Granthi, Kachhda, Mustako, Sugandhi-granthila

Tamil: Korai

Telugu: Tungagaddi .

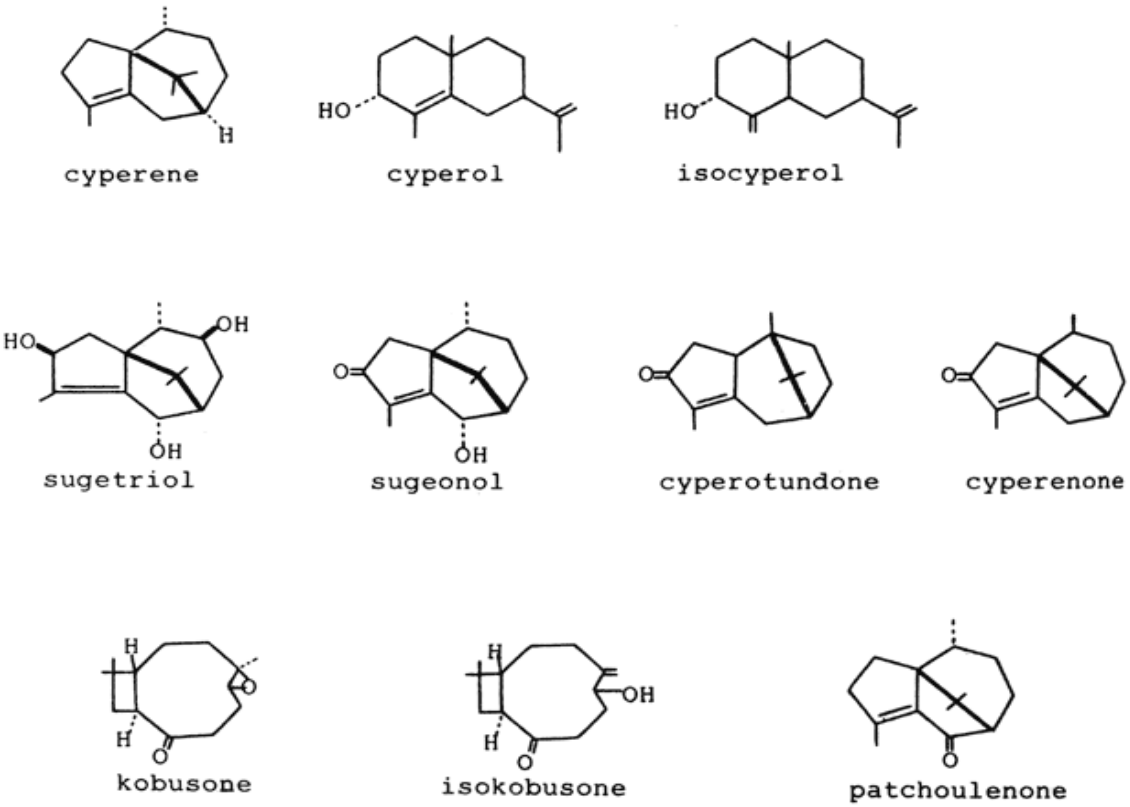
Morphology:

A perennial, stolaniferous, rhizomatus, halophytic sedge. Rhizome many, slender; Tuber-white, succulent when young, hard and black when mature;stem-leafy at base arising from a tuber. Culm-dark green, glabrous. Leaf dark green above, with reddish brown sheaths, clustered at the base of stem. Inflorescence 3-9 spreading rays bearing tassels of few, large spikelets; spikelet 20-40 flowered, red brown to almost black. Fruit oblong ovate[39].

ACTIVE CHEMICAL CONSTITUENTS:

*Cyperu*s has numerous chemical constituents, but the main active components appear to be the sesquiterpenes. These are aromatic, spicy tasting molecules. Among the main sesquiterpenes identified in cyperus rhizomes. α-cyperone, β-selinene, cyperene, cyperotundone, patchoulenone, sugeonol, kobusone, and isokobusone.

Cyperus also contains other terpenes, such as the commonly occurring plant component pinene (a monoterpene), and several derivatives of the sesquiterpenes, such as cyperol, isocyperol, and cyperone[40]



PHARMACOLOGYCAL USES:

The *Cyperus rotundus* rhizomes are pharmacologically used for promoting, nervine tonic, diuretic, antiperiodic, and traditionally used to treat diarrhoea, dysentery, leprosy, bronchitis, amenorohoea, dysmenorrhoea, renal and vesical calculi, ophthalmic disorders, blood disorders and general debility[41] Pharmacological studies rvealed the rhizomes as analgesic, anti-inflammatory, antipyretic[42]. This antidibetic activity can be attributed to its antioxidant activity as it showed the strong DPPH radical scavenging action in vitro[43]. The Antibacterial activity of Cyperus oil was studied for various microorganisms (Staphylococcus aureus, Klebsiella pneumoniae, Proteus vulgaris, Streptococcus pyogenes, Eschirichia coli and Pseudomonas aeruginosa, activity against gram-positive bacteria[44].

Chapter: V


S


SUCCESS

Materials and Methods

V. MATERIAL AND METHODS

Borosilicate Soxhlet extractor, Alloxan monohydrate (Sigma Aldrich, usa), Biochemical Analyser-ROBONIK (prietest easylab, Mumbai), ROBONIC diagnostic kit (creatinine, total protein, urea, uric acid) Centrifuge Biofuse pico (Heraeus), electronic digital weighing (Apex), EDDY's Hot plate analgesometer MK-111, (Sisco, Thane, Maharastra), Glucose check monitoring system (Aspen diagnostics ltd, Delhi, India.) micropippet.

DRUGS AND CHEMICALS

Alloxan monohydrate (Sigma Aldrich, USA), Metformin (Alembic Pharma) Chloroform (Fisher scientific), Diethyl ether (Fisher scientific) and all other chemicals were used AR grade.

PLANT MATERIAL

Plant Authentication

Plant sample of *Cyperus rotundus* roots were collected in march 2013 from S.V. University, Tirupathi, India. Verified by prof. Dr.M. Madhava Shetty Department of Botony.

Preparation of extract:

Plant material of *Cyperus .rotundus* dried roots were collected and powdered by pulverize. The powdered drug then was passed through sieves no. #44 and used for extraction process. Approximately 100 gm of powder was placed in soxhlet extractor with water 400 ml (1:4) for 72 hrs. the final yield is found to be 20.2 gm. The extract was dried and concentrated at 45 c in the water bath and residue of extract was analysed for experiment. The extract was dissolved with distill water and given orally at the time of animal dosing.

Experimental animals

The Wister albino rats of either sex (200-250g) were obtained from the central animal house of Sigma institute of clinical research & administration pvt. ltd, Hyderabad. Animals were housed at a temperature of 24±2°C and relative humidity of 30-70%. A 12:12 hr light :dark cycle was followed. All animals had free access to water and standard pellet laboratory animal diet. Animals were acclimatized to laboratory conditions before the experiment procedure used in this study were reviewed and carried out in with that described by Zimmerman et al(1). Institutional Animal Ethics Committee (769/2010/CPCSEA) approved the study protocol.

Acute oral toxicity study[52]

Procedure: Acute toxicity studies were performed according to OECD-423 guidelines category IV substance (acute toxic class method). Albino mice (n=3) of either sex selected by random sampling technique were employed in this study. The animals were fasted for 4 hrs with free access to water only. The root extracts of *Cyperus. rotundus* were administered orally with maximum dose of 2000 mg/kg body weight. The mortality was observed for three days. If mortality was observed in 2/3 or

3/3 of animals, then the dose administered was considered as a toxic dose. However, if the mortality was observed only one mouse out of three animals then the same dose was repeated again to confirm the toxic effect. If mortality was not observed, the procedure was then repeated with higher dose (Organization for economic Co-operation and development, 2001).

Results

The plant root extracts of *Cyperus rotundus* didn't shown any mortality and toxicity even at highest dose of 2000 mg/kg body weight employed. The present research study was carried out using two different doses (low and high) aqueous extract's of *Cyperus rotundus* such as 200 and 400 mg/kg body weight for diabetic complication.

Toxicity record sheet: Toxicity record sheet is shown as follows for the *Cyperus rotundus*

Acute Toxicity Record Sheet of *Cyperus rotundus*

Title: Evaluation of LD50 ***Cyperus rotundus***

Drug*: Cyperus rotundus* Dose: 2000 mg/kg BW Species: Albino mice Sex: Male & Female

Date: Duration: 24 hours

S.no.	code	Toxicity		Time Of Death	Observation										
		Onset	Stop		Skin colour	Eyes	Resp	CNS	Tre	Con	Sali	Diah	Sleep	Leth	Com
1.	ECR	x	x	x	x	x	x	x	X	x	x	x	x	x	X

(*TRE-Tremor, CON-Convulsions, SALI- Salivation, Diah - Diarrhea, LET-Lethargy) **x** = Negative, ☙ = Positive

AECR: Aqueous extract of *Cyperus rotundus*

Qualitative chemical test[53]:

Preliminary phyto chemical studies: Aqueous extract of the plant of root of *Cyperus rotundus* were subjected to chemical tests for the identification of their active constituents.

4.2.3 Tests for carbohydrates and glycosides

A small quantity of the extract was dissolved separately in 4 ml of distilled water and filtered. The filtrate was subjected to Molisch's test to detect the presence of carbohydrates.

A. Molisch's Test

Filtrate was treated with 2-3 drops of 1% alcoholic α-naphthol solution and 2ml of con. H2SO4 was added along the sides of the test tube. Appearance of violet coloured ring at the junction of two liquids shows the presence of carbohydrates. Another portion of the extract was hydrolysed with HCl for few hours on a water bath and the hydrolysate was subjected to Legal's and Borntrager's test to detect the presence of different glycosides.

B. Legal's Test

To the hydrolysate, 1ml of pyridine and few drops of sodium nitroprusside solution were added and then it was made alkaline with sodium hydroxide solution. Appearance of pink to red colour shows the presence of glycosides.

C. Borntrager's Test : Hydrolysate was treated with chloroform and then the chloroform layer was separated. To this equal quantity of dilute ammonia solution was added. Ammoniacal layer acquires pink colour showing the presence of glycosides.

4.2.4 Tests for alkaloids: A small portion of the methanol extract was stirred separately with few drops of dil. HCl and filtered. The filtrate was treated with various reagents as shown for the presence of alkaloids.

Mayer's reagent - Creamy precipitate

Dragandroff's reagent - Orange brown precipitate

Hager's reagent - Yellow precipitate

Wagner's reagent - Reddish brown precipitate

4.2.5 Tests for phytosterol

The extract was refluxed with solution of alcoholic potassium hydroxide till complete saponification takes place. The mixture was diluted and extracted with

ether. The ether layer was evaporated and the residue was tested for the presence of phytosterol.

Libermann Burchard Test

The residue was dissolved in few drops of acetic acid, 3 drops of acetic anhydride was added followed by few drops of con. H2SO4. Appearance of bluish green colour shows the presence of phytosterol.

4.2.6 Tests for fixed oils

Spot test

Small quantity of extract was separately pressed between two filter papers. Appearance of oil stain on the paper indicates the presence of fixed oil. Few drops of 0.5N alcoholic potassium hydroxide were added to a small quantity of extract along with a drop of phenolphthalein. The mixture was heated on water bath for 1-2 hours. Formation of soap or partial neutralization of alkali indicates the presence of fixed oils and fats.

4.2.7 Tests for gums and mucilages

Small quantity of the extract was added separately to 25 ml of absolute alcohol with constant stirring and filtered. The precipitate was dried in air and examined for its swelling properties for the presence of gums and mucilages.

4.2.8 Tests for Saponins

The extract was diluted with 20 ml of distilled water and it was agitated in a graduated cylinder for 15 minutes. The formation of 1cm layer of foam shows the presence of saponins.

4.2.9 Tests for proteins and free amino acids

Small quantity of the extract was dissolved in few ml of water and treated with following reagents.

A. Millon's reage Appearance of red colour shows the presence of protein and free amino acids.

B. Ninhydrin reagent - Appearance of purple color shows the presence of proteins and free amino acids.

C. Biuret test - Equal volumes of 5% NaOH solution and 1% copper sulphate solution were added. Appearance of pink or purple colour shows the presence of proteins and free amino acids.

4.2.10 Tests for phenolic compounds and tannins

Small quantity of the extract was taken separately in water and tested for the presence of phenolic compounds and tannins using following reagents.

A. Dil.FeCl3 solution (5%) -violet colour

B. 1% solution of gelatin containing 10% NaCl - white precipitate

C. 10% lead acetate solution - white precipitate.

4.2.11 Tests for flavonoids

A. With aqueous Sodium hydroxide solution:

Blue to violet colour (anthocyanins), yellow colour (flavones), yellow to orange (flavonones)

B. With Con. H2SO4:

Yellow orange colour (anthocyanins), yellow toorange colour (flavones), orange to crimson (flavonones)

C. Shinoda's test

Small quantity of the extract was dissolved in alcohol and to that a piece of magnesium followed by Con. HCl drop wise was added and heated. Appearance of magenta colour shows the presence of flavonoids.

The results of preliminary phytochemical studies of the plant root extract are presented in **Table 1.**

Phytoconstituents	**Presence or Absence**
Carbohydrates	+
Glycosides	+
Fixed oils and fats	-
Gums & mucilage	-
Potein & amino acids	-
Saponins	+
Tannins	+
Phytosterols	+
Flavonoids	+
Alkaloids	+

Steroids	+

Presence: +, Absence:-

Selection of Dose: In the present study we have selected three doses i.e. 200 mg/kg and 400 mg/kg of body weight, selection of doses is based upon toxicity study. In the toxicity studies up to 2000 mg/kg body weight animals have not shown any signs of toxicity, morbidity and mortality so 10% of the maximum dose have been chosen i.e 200 mg/kg body weight, other two doses were selected one is submax and other is supramax of 200 mg/kg body weight.

Preparation of the drug solution

Metformin: Solution was prepared by dissolving Metformin (Alembic) in distilled water. The drug was prepared daily and was stored at room temperature away from sunlight and moisture. The volume of drug solution were calculated based upon the body weight of the animal

Experimental induction of Diabetic in rats[54]

After a 48 hr fast, the animals was given intraperitoneal (i.p) injections of alloxan monohydrate (150mg/kg) dissolved in 0.9% sodium chloride. Control animals received i.p injections of 0.9% sodium chloride. Within 48 hrs following alloxan administration, blood glucose concentration were estimated by (Glucose check monitoring system) diagnostic kit (Apex). The rat with serum glucose levels more than 250mg/dl were selected for the present study. After the induction of diabetes in the animals diabetic rats were randomly selected into five groups of 6 animals each. ie. Normal, control 0, (diabetic untreated) and diabetic animals treated with Metformin (10mg/kg,O.D) with AECR (200mg/kg, O.D) AECR (400mg/kg, O.D) treated. Treatment begin from the day of blood sugar levels (BSL) detection after the alloxan treatment. Body weight was recorded daily and serum glucose level measured on 1^{st}, 7^{th}, 14^{th} day of study.

Animal Groupings

ESTIMATION OF NOCICEPTION

Tail immersion (Hot water) test:

Tail of rat was immersed in a hot water bath (52.5 ± 0.5 °C) until tail withdrawal (flicking response) or signs of struggle were observed (cut-off 12 s). Shortening of the tail withdrawal time indicates hyperalgesia[55,56] (Kannan *et al*., 1996; Ramabadran *et al*., 1989).

Hot plate test

The hyperalgesic response on the hot-plate is considered to result from a combination of central and peripheral mechanisms[55] (Kannan *et al*., 1996). In this test, animals were individually placed on a hot-plate (Eddy's Hot-Plate) with the temperature adjusted to 55 ± 1 °C. The latency to the first sign of paw licking or jump response to avoid the heat was taken as an index of the pain threshold; the cut-off time was 10 s in order to avoid damage to the paw.

Assessment of Cold allodynia

Rationale

Allodynia is hallmark of neuropathic pain, and diabetic neuropathy pain in experimental animals.

Procedure

The method was performed as described earlier[57,58] (Kumar *et al*., 2011; Naik *et al*., 2006). In brief, 2 h after assessment of thermal hyperalgesia cold allodynia was assessed by measuring paw (ipsilateral) withdrawal latency (PWL). Ice cold water (4±10C) was taken in beaker. The paw of rat was submerged gently in water and the withdrawal time was measured on 1, 7th, 14th day after chronic constriction injury and in the case of diabetic neuropathy it was assessed weekly after confirmation of diabetes. A cut off 20 sec was maintained throughout the experiment.

EVALUATION OF BLOOD GLUCOSE LEVELS AND BODY WEIGHT

Blood glucose levels were measured with a portable glucometer (CONTOUR TS). In brief, blood was withdrawn from the rats using tail vein rupture method, and a drop of blood was placed on the glucometer strip loaded in the glucometer for blood glucose determination. During the experiment, blood glucose levels and body weights were verified in the interim of each week.

BIOCHEMICAL ESTIMATION

On day 14, blood was collected by retrobital punture under mild ether anesthesia from overnight fasted rats and fasting blood sugar[59] (Giordano et al., 1989) was estimated. Serum was separated and analyzed for serum creatinine[60] (Bowers, 1980), serum urea[61] (Wilson, 1966), serum uric acid, SGOT and SGPT were estimated.

Serum Creatinine (Mod. Jaffe's kinetic method)[62]

Principle

Picric acid in alkaline medium reacts with creatinine to form a orange coloured complex with the alkaline picrate. Intensity of colour formed during the fixed time measured at 520 nm is directly proportional to the amount of creatinine present in sample.

Creatinine + alkaline picrate ——————> Creatinine picrate complex

Pipette in to test tubes	**Standard**	**Sample**
Working reagent	1000 µl	1000µl
Standard	100 µl	……..
Sample	……	100 µl

Mix and read the variation of absorbance (ΔA) between 30 seconds and 90 seconds

Calculation :

With standard or calibrator

$$\text{Concentration in sample (mg/dl)} = \frac{\text{Concentration of Standard} \times \Delta A \text{ Sample}}{\Delta A \text{ Standard}}$$

Uric acid[63]

Principle:

Uric acid is oxidized to allantoin by uricase. The generated hydrogen peroxide reacts with 4-aminoantipyrine and DHPS to quinoneimine.

$$\text{Uric acid} + H_2O + O_2 \xrightarrow{\text{Uricase}} \text{Allantoin} + CO_2 + H_2O_2$$

$$\text{DHPS} + \text{4-Aminoantipyrine} + 2\,H_2O_2 \longrightarrow \text{Quinoneimine} + 3\,H_2O$$

Assay procedure

Pipette in to test tubes	**Blank**	**Standard**	**Sample**
Working reagent 1	1000 µl	1000 µl	1000µl
Distill water	25µl	……	……..
Standard	……	25 µl	……..
Sample	…….	……	25 µl

Mix and read the absorbance (A) after a 10 minutes incubation but within 30 minutes.

Calculation :

With standard or calibrator

$$\text{Conc. of unknown Sample} = \frac{\text{Concentration of Standard}}{\text{Abs. Standard - Abs. of Reagent Blank}} \times \text{Abs. of unknown Sample – Abs.of Reagent Blank}$$

Urea[64]

Principle

Enzymatic determination according to the following reactions :

$$\text{Urea} + 2H\,O \xrightarrow{\text{Urease}} 2NH_4^+ + Co_3^{-2}$$

$$NH_4^+ + \text{Tris Base} + \text{NADH} \xrightarrow{\text{GLDH}} \text{L-Glu} + NAD^+ + H_2O$$

ASSAY PROCEDURE: Two Reagent Procedure

	Standard	Sample / Control
R1	800 µl	800 µl
R2	200 µl	200 µl

Mix and Incubate at 37°C for 2 minutes then add

Pipette in to test tubes	Standard	Sample
Working reagent	1000 µl	1000µl
Standard	10µl	……..
Sample	……	10µl

Mix and read the variation of absorbance (ΔA) between 30 seconds and 60 seconds.

CALCULATION:

With standard or calibrator

$$\text{Concentration in Sample (mg/dl)} = \frac{\text{Concentration of Standard}}{\Delta A \text{ Standard}} \times \Delta A \text{ Sample}$$

SGOT/AST[65]

Principle

Kinetic determination of the aspartate aminotransferase (GOT) activity:

$$\text{L - Aspartate} + \alpha - \text{Ketoglutarate} \xrightarrow[\text{MDH}]{\text{GOT}} \text{Oxaloacetate} + \text{L-Glutamate}$$

Pipette in to test tubes	Sample/Control
R1	800µl
R2	200 µl

Mix and Incubate at 37°C for 2 minutes then add

Pipette in to test tubes	Sample/Control
Working reagent	1000µl
Sample/Control	100 µl

Mix and after a 60 seconds incubation at 37°C measure the change of absorbance per

minute (ΔA/minute) during 180 seconds.

Calculation :

Activity of Sample (U/L) = Δ A/Min X 1746

SGPT/ ALT[66]

Principle

Kinetic determination of the GPT activity

$$\text{L - Alanine} + \alpha\text{ – Ketoglutarate} \xrightarrow{\text{GPT}} \text{Pyruvate} + \text{L-Glutamate}$$

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

ASSAY PROCEDURE 1: Two Reagent procedure

Pipette in to test tubes	Sample/Control
R1	800µl
R2	200 µl

Mix and Incubate at 37°C for 2 minutes then add

Pipette in to test tubes	Sample/Control
Working reagent	1000µl
Sample/Control	100 µl

Mix and after a 60 seconds incubation at 37°C measure the change of absorbance per

minute (ΔA/minute) during 180 seconds.

Calculation :

Activity of Sample (U/L) = Δ A/Min X 1746

Histopathological studies:

At the end of the study period, animals from all the five groups were sacrificed and pancrease was dissected out, washed, 5µm thick section slides were prepared and stain with heamatoxyline-eosin and examined by light microscopy.

STATISTICAL ANALYSIS

Results were expressed as mean±SEM. The data were analyzed by one-way analysis of variance (ANOVA) followed by Dunnett's multiple comparison tests. Statistical significance was considered at $P<0.05$ in all the cases.

Chapter: VI

Results

RESULTS

Acute toxicity study

The overall study showed the LD50 of oral toxicity of extracts AECR to be above 2000 mg/kg b.w. in mice. So, the extracts are safe for long term administration.

Preliminary phytochemistry of the plant extract

The yield of aqueous extract of *Cyperus rotundus* root was found to be 7.9 % W/W. Preliminary phytochemical analysis revealed that the plant possessed phyto constituents alkaloids, steroids, saponins, tannins, Phytosterols, flavonoids, glycosides and carbohydrates.

Effect of AECR on blood sugar level (BSL) in alloxan induced diabetes in rats.

The effect of repeated oral administration of aqueous extract of AECR on blood glucose levels in alloxan-diabetic rats is presented in table-1, and the effect on body weight is presented in table-2. In an alloxan diabetic rats serum glucose level has significantly increased ($p<0.001$) in diabetic control (289.65±3.071) rats when compared to normal groups (87.76±2.056).

AECR, administered at doses of 200 (87.45±1.987) & 400 (80.55±1.998) mg/kg to alloxan-treated diabetic rats caused significant ($p<0.01$) and ($p<0.001$) reduction of blood glucose levels which was related to dose and duration of treatment as when compared to control diabetic group (289.65±3.071). Maximum reduction was observed on day 14. gradual increase in body weight was also observed. AECR 200

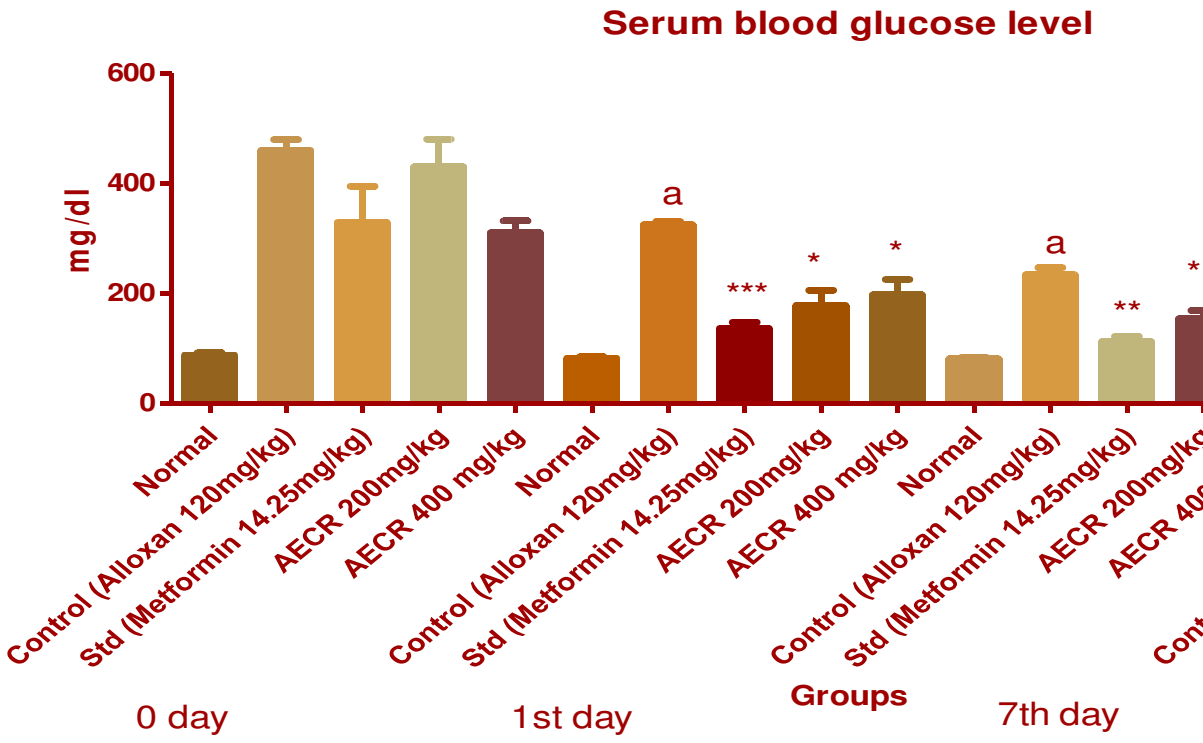
and 400mg/kg exhibited maximum glucose lowering effect in diabetic rats. Administration of Metformin 14.25 mg/kg (82.49±2.421) orally for 14 days

Treated groups	Serum blood glucose level			
Groups	**0-DAY**	**1st-DAY**	**7th-DAY**	**14th-DAY**
Normal	92.00±3.540	92.839±2.030	87.44±2.162	87.76±2.056
Control (Alloxan 120mg/kg)	243.00±7.739	248.68±6.89	275.57±4.986b	289.65±3.071a
Standard (Metformin 14.25mg/kg)	229.83±5.597	160.35±2.81	102.54±3.789**	82.49±2.421***
AECR 200 mg/kg	240.00±3.765	190.12±2.055	109.10±3.312*	87.45±1.987**
AECR 400 mg/kg	251.68±6.332	194.15±2.345	96.90±1.54**	80.55±1.998***

Table.1: Effect of AECR on blood sugar level (BSL) in alloxan induced diabetes in rats.

Data expressed in Mean ± S.E.M. n=6, ANOVA followed by Dunnett's multiple comparison test *p<0.05, **p<0.01, ***p<0.001 as compared to Control group.and ap<0.001, bp<0.01 as compared to Normal group.

Fig.no1: Effect of AECR on blood sugar level (BSL) in alloxan induced diabetes in rats



Data expressed in Mean ± S.E.M. n=6, ANOVA followed by Dunnett's multiple comparison test *p<0.05, **p<0.01, ***p<0.001 as compared to Control group.and [a]p<0.001 as compared to Normal group.

Effect of AECR on allodynia produced by tail immersion (cold water) in alloxan induced diabetes in rats.

Rats treated with alloxan (8.978±1.080) had significant ($p<0.01$) decrease in tail flick latency was observed after 14 days of diabetic complication induction in cold immersion test as compared normal group (12.53±0.588). This deficit in tail flick response latency was significantly ($p<0.001$) reversed on 14 days treatment with AECR 200 and 400mg/kg).

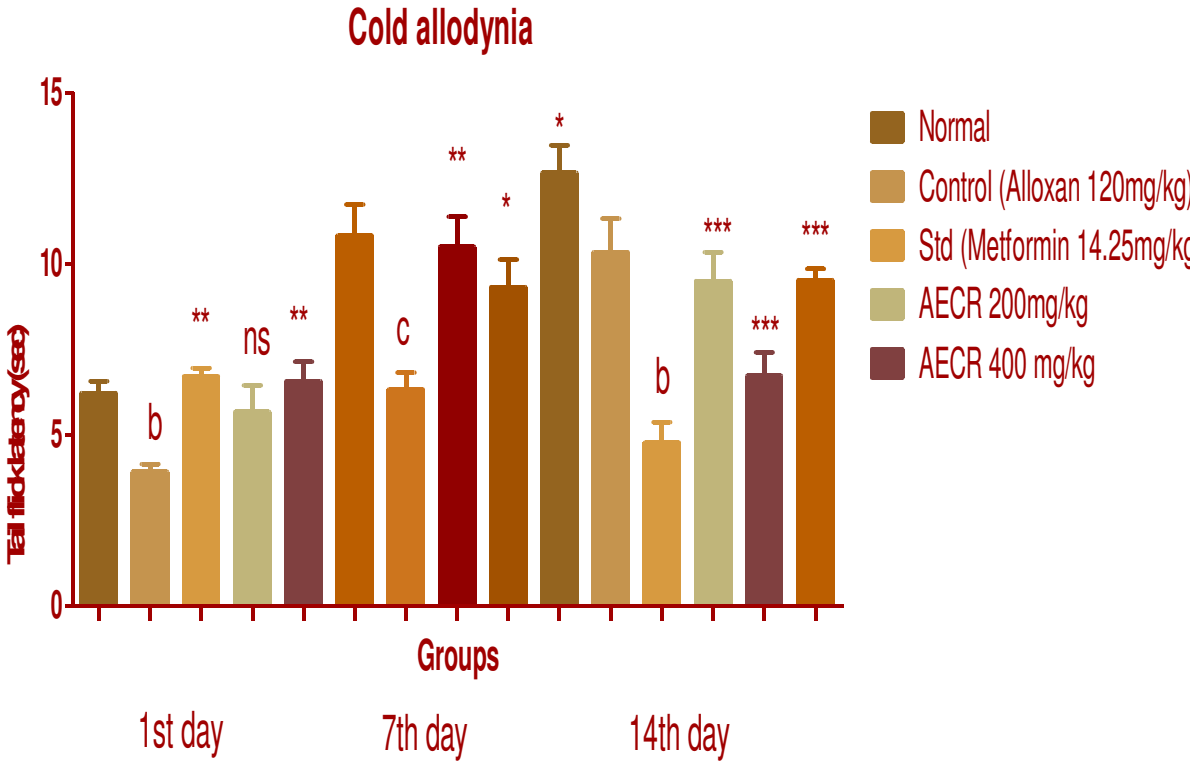
Administration of Metformin 14.25 mg/kg (13.77±0.48) orally for 14 days treatment were reduced significantly tail flick latency ($p<0.001$) as compared to control groups (12.53±0.588).

Table.2: Effect of AECR on allodynia produced by tail immersion (cold water) in alloxan induced diabetes in rats.

Treatment Groups	Tail Flick latency in seconds		
	Day-1	**Day-7**	**Day-14**
Normal	11.23±0.971	5.252±0.537	12.53±0.588
Control (Alloxan 120mg/kg)	7.57±0.649b	2.842±0.449a	8.978±1.080b
Standard (Metformin 14.25mg/kg)	12.45±0.799***	4.972±0.311**	13.77±0.480***
AECR 200 mg/kg	8.45±0.780ns	4.84±0.189**	13.68±0.510***
AECR 400 mg/kg	8.475±0.548ns	5.123±0.273***	13.92±0.419***

Data expressed in Mean ± S.E.M. n=6, ns= not significant, ANOVA followed by Dunnett's multiple comparison test **P<0.01***p <0.001 as compared Control group. and [a]p<0.001 and [b]p<0.01 as compared Normal group.

Fig.no.2:Effect of AECR on allodynia produced by tail immersion (cold water) in alloxan induced diabetes in rats.



Data expressed in Mean ± S.E.M. n=6, ns= not significant, ANOVA followed by Dunnett's multiple comparison test *p<0.05, **p<0.01, ***p<0.001 as compared to Control group.and ^{b}p<0.01 and ^{c}p<0.05 as compared to Normal group.

Effect of AECR on hyperalgesia produced by tail immersion (hot water) in alloxan induced diabetes in rats.

There was no change in tail flick latency (sec) observed in normal group of animals throughout the experiment. A gradual decline in the latency was observed in control group of animals from day 7th (6.333±0.49 $p<0.05$) onwards which was observed minimum on day 14^{th} (4.785±0.594, $p<0.01$), indicating the presence of neuropathic pain due to diabetes.

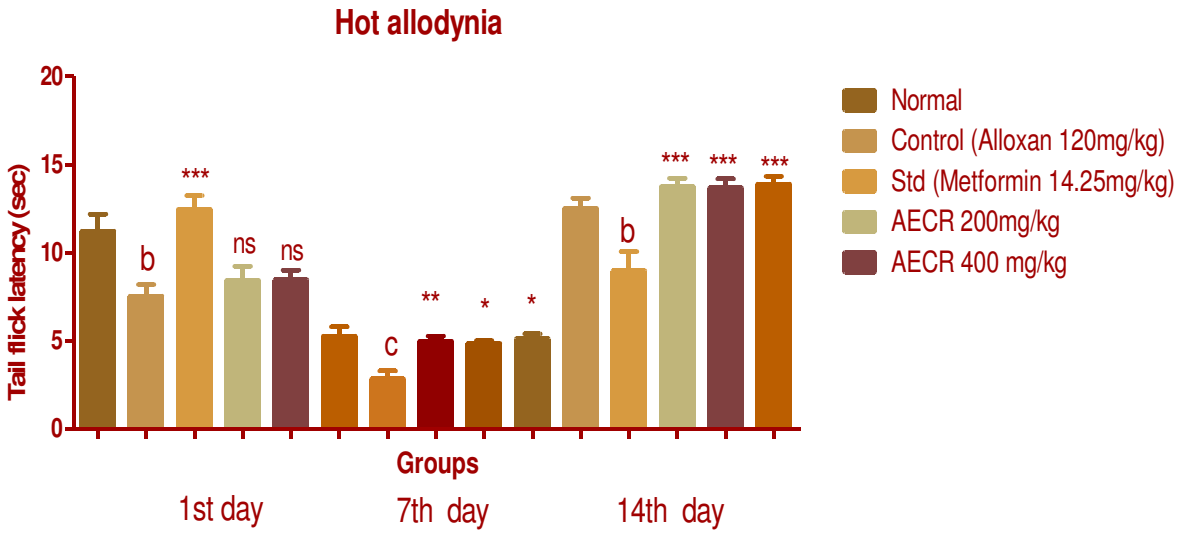
AECR 200, 400mg/kg and Metformin 14.25mg/kg group of animlas recorded a reduction in latency on day 14^{th} ($p<0.001$) which was followed by increase in pain threshold time on subsequent days, indicating absence of algesia produced by tail immersion in hot water.

Table: no.3: Effect of AECR on allodynia produced by tail immersion (hot water) in alloxan induced diabetes in rats.

Treatment group	Tail Flick Latency in seconds (hot water)		
	Day-1	Day-7	Day-14
Normal	6.233±0.357	10.83±0.909	10.35±0.986
Control (Alloxan 120mg/kg)	3.935±0.220b	6.333±0.494c	4.785±0.594b
Standard (Metformin 14.25mg/kg)	6.720±0.240**	10.50±0.885**	9.508±0.840***
AECR 200 mg/kg	5.680±0.7733ns	9.333±0.802*	6.730±0.683***
AECR 400 mg/kg	6.583±0.560**	12.67±0.802*	9.535±0.320***

Data expressed in Mean ± S.E.M. n=6, ns= not significant, ANOVA followed by Dunnett's multiple comparison test* $p<0.05$, **$p<0.01$, ***$p<0.001$ as compared to Control group. and bp<0.01 and cp<0.05 as compared to Normal group.

Fig. 3: Effect of AECR on allodynia produced by tail immersion (hot water) in alloxan induced diabetes in rats.



Data expressed in Mean ± S.E.M. n=6, ns= not significant, ANOVA followed by Dunnett's multiple comparison test* $p<0.05$, **$p<0.01$, ***$p<0.001$ as compared to Control group. and $^{b}p<0.01$ and $^{c}p<0.05$ as compared to Normal group.

Effect of AECR on thermal hyperalgesia (hot plate) in alloxan induced diabetes in rats.

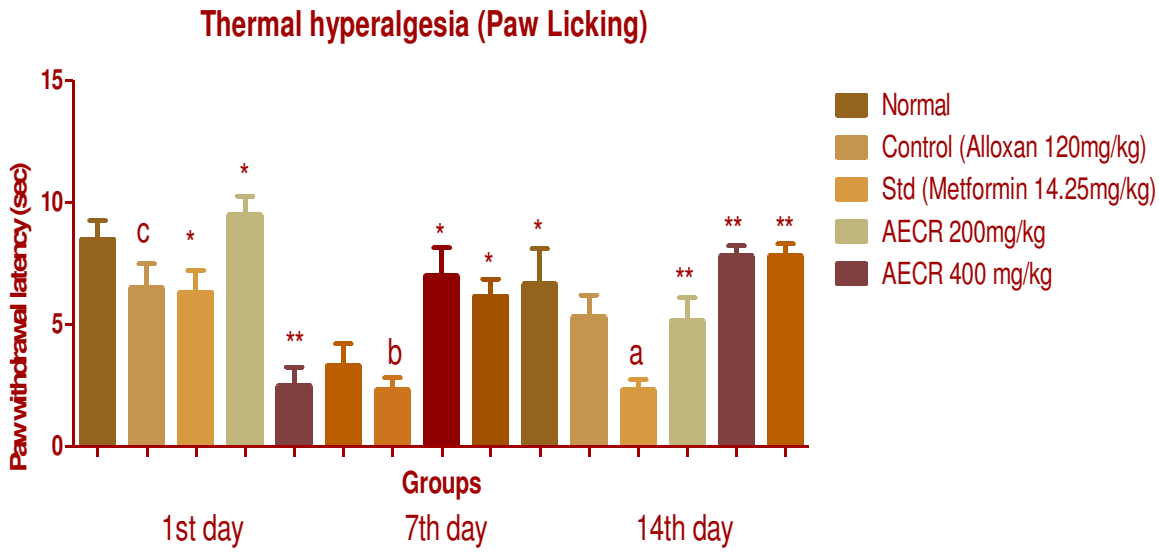
The nociceptive threshold was significantly lower ($p<0.05$) in diabetic rats as compared with the normal group. For Control group of animals there was a gradual reduction in latency (sec) observed from day 7^{th} (2.33±0.21, $p<0.05$) to till day 14^{th} (3.067±0.29 $p<0.001$) where the pain was observed to be maximum, indicating the presence of algesia by heat. In the drug treated group of animals, no significant lowering in pain latency was exhibited which implies the protective action of AECR on hyperalgesia produced in diabetic animals.

Table.no.4: Effect of AECR on thermal hyperalgesia (hot plate) in alloxan induced diabetes in rats.

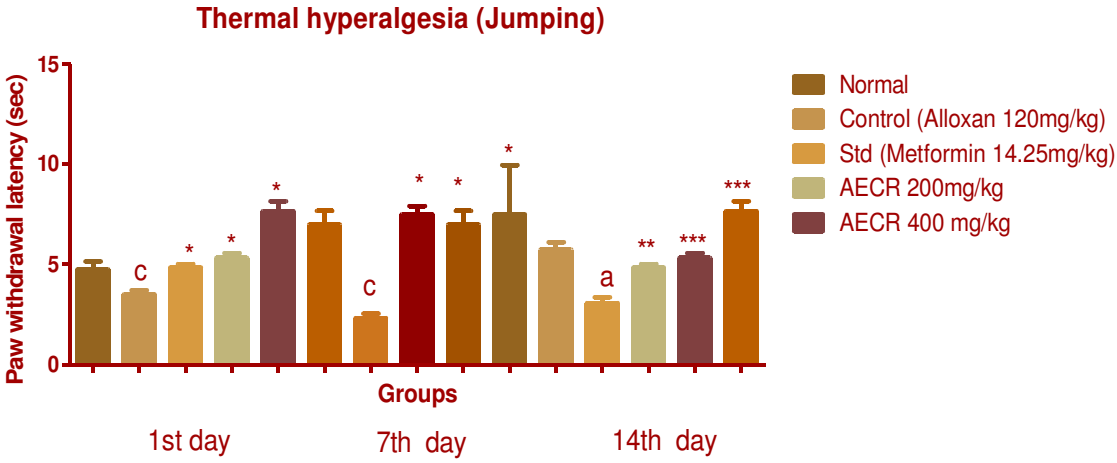
Treatment group	Thermal Hyperalgesia Latency in sec					
	Licking			Jumping		
	Day-1	Day-7	Day-14	Day-1	Day-7	Day-14
Normal	8.50±0.76	7.00±0.93	6.33±1.22	4.76±0.39	7.00±o.68	5.74±0.36
Control (Alloxan 120mg/kg)	3.96±0.53	3.33±0.33c	2.33±0.84c	3.50±0.22 c	2.33±0.21b	3.067±0.29a
Standard (Metformin 14.25mg/kg)	7.83±0.79ns	7.66±1.11**	6.33±1.11* *	4.85±0.17*	7.50±0.42*	4.85±0.17**
AECR 200 mg/kg	9.50±0.76ns	6.16±0.70*	8.33±0.49**	5.33±0.24*	7.00±0.68*	5.33±0.24***
AECR 400 mg/kg	4.50±0.76**	8.33±0.66*	9.66±1.11***	7.66±0.49**	7.50±2.47*	7.66±0.49***

Data expressed in Mean ± S.E.M. n=6, ns= not significant, ANOVA followed by Dunnett's multiple comparison test *p<0.05, **p<0.01, ***p<0.001 as compared to Control group. and ***p<0.001, cp<0.05 as compared to Normal group.

Fig.4: Effect of AECR on thermal hyperalgesia (hot plate)paw licking and jumping in alloxan induced diabetes in rats.



Data expressed in Mean ± S.E.M. One way ANOVA followed by Dunnett's multiple comparison test *p<0.05, **p<0.01, as compared to Control group. and [a]p<0.001,[b]p<0.01, [c]p<0.05 as compared to Normal group



Data expressed in Mean ± S.E.M. One way ANOVA followed by Dunnett's multiple comparison test *p<0.05, **p<0.01,***p<0.001 as compared to Control group. and [a]p<0.001, [c]p<0.05 as compared to Normal group.

Effect of AECR on Serum biochemical parameters in alloxan induced diabetes in rats

Urea

The results for the effect of the AECR in the mean values of serum urea on normal and (51.89±0.05) diabetic rats are presented in Table no 4. Our data indicated that there were the mean values of urea, ($p<0.001$) were significantly higher in control rats as compared to the normal rats (20.53±0.07). Administration of Metformin 14.25mg/kg had exhibited significantly (26.92±0.21) ($p<0.01$) lower serum urea as compared to diabetic control group (51.89±0.05).Treatment of the diabetic rats with the AECR extract 200 (34.48±0.05) & 400 mg/kg (25.64±0.04) for 14 days caused a significant decrease in urea ($p<0.05$, $p<0.01$), as compared to control group (51.89±0.05).

Creatinine

The data indicated that there were the mean values of creatinine ($p<0.001$) were significantly higher in control rats (0.88±0.02) as compared to the normal rats (0.46±0.01). Administration of Metformin 14.25mg/kg had exhibited significantly (0.47±0.02) ($p<0.001$) lower serum creatinine as compared to diabetic control group (0.88±0.02).Treatment of the diabetic rats with the AECR extract 200 (0.67±0.02) & 400 mg/kg (0.51±0.01) for 14 days caused a significant decrease in creatinine ($p<0.01$, $p<0.001$), as compared to control group (0.88±0.02).

Uric aicd

The data indicated that there were the mean values of uric acid ($p<0.001$) were significantly higher in control rats (4.74±0.06) as compared to the normal rats (3.76±0.06). Administration of Metformin 14.25mg/kg had exhibited significantly (1.64±0.05) ($p<0.001$) lower serum uric acid as compared to diabetic control group (4.74±0.06).Treatment of the diabetic rats with the AECR extract 200 (1.65±0.03) & 400 mg/kg (2.31±0.06) for 14 days caused a significant decrease in uric acid ($p<0.001$, $p<0.01$), as compared to control group (4.74±0.06).

SGOT

The data indicated that there were the mean values of SGOT ($p<0.001$) were significantly higher in control rats (45.36±2.23) as compared to the normal rats (37.31±0.88). Administration of Metformin 14.25mg/kg had exhibited significantly (39.46±0.60) ($p<0.01$) lower serum SGOT as compared to diabetic control group

(45.36±2.23). Treatment of the diabetic rats with the AECR extract 200 (40.65±0.68) & 400 mg/kg (34.00±0.80) for 14 days caused a significant decrease in SGOT ($p<0.05$, $p<0.001$), as compared to control group (45.36±2.23).

SGPT

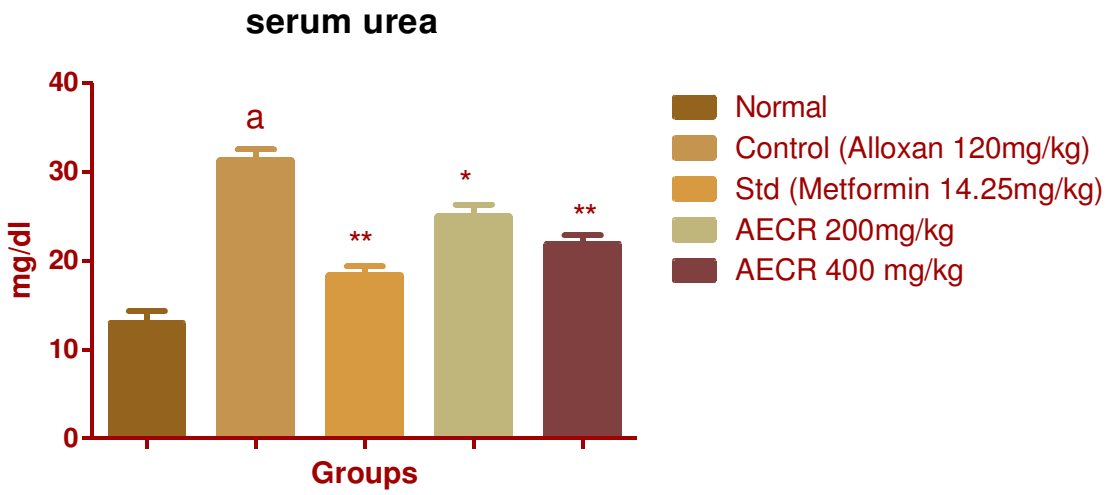
The data indicated that there were the mean values of SGPT ($p<0.001$) were significantly higher in control rats (77.90±6.07) as compared to the normal rats (51.35±0.96). Administration of Metformin 14.25mg/kg had exhibited significantly (50.48±0.62) ($p<0.001$) lower serum SGPT as compared to diabetic control group (77.90±6.07). Treatment of the diabetic rats with the AECR extract 200 (54.23±1.31) & 400 mg/kg (62.25±0.90) for 14 days caused a significant decrease in SGPT ($p<0.001$, $p<0.01$), as compared to control group (77.90±6.07).

Table.no:5: Effect of AECR on Serum biochemical parameters in alloxan induced diabetes in rats

Testing group	SERUM BIOCHEMICAL PARAMETERS				
	UREA mg/dl	**CREATININE mg/dl**	**URIC ACID mg/dl**	**SGOT mg/dl**	**SGPT mg/dl**
Normal	20.53±0.07	0.46±0.01	3.76±0.06	37.31±0.88	51.35±0
Control (Alloxan 120mg/kg)	51.89±0.05a	0.88±0.02a	4.74±0.06a	45.36±2.23a	77.90±6
Standard Metformin (4.25mg/kg)	26.92±0.21**	0.47±0.02***	1.64±0.05***	39.46±0.60**	50.48±0 **
AECR 200 mg/kg	34.48±0.05*	0.67±0.02**	1.65±0.03***	40.65±0.68*	54.23±1 **
AECR 400 mg/kg	25.64±0.04**	0.51±0.01***	2.31±0.06**	34.00±0.80***	62.25±0 *

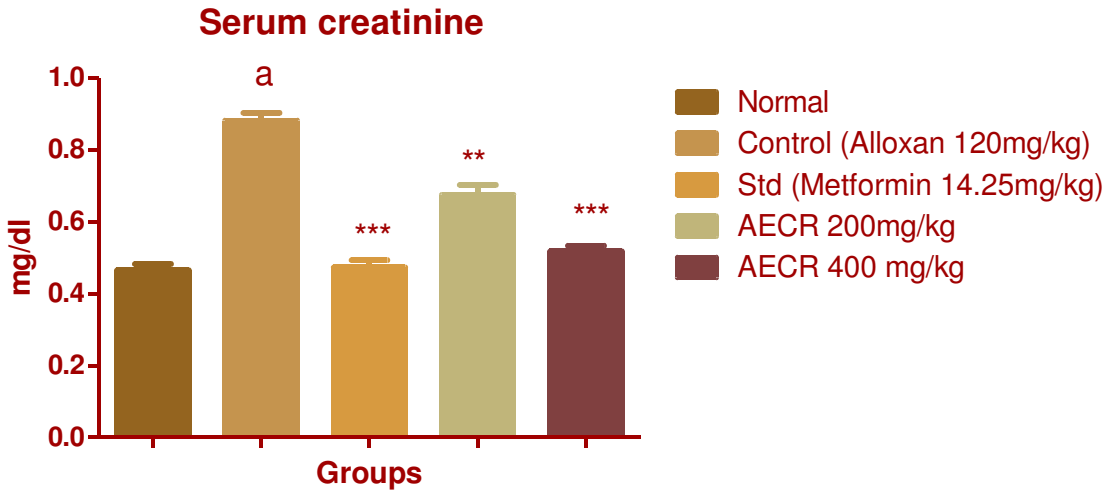
Data expressed in Mean ± S.E.M. n=6, ANOVA followed by Dunnett's multiple comparison test,*p<0.05, **p<0.01,***p<0.001 as compared to Control group.and [a]p<0.001 as compared to Normal group.

Fig.5:Effect of AECR on Serum urea in alloxan induced diabetes in rats.



Data expressed in Mean ± S.E.M. One way ANOVA followed by Dunnett's multiple comparison test *p<0.05, **p<0.01 as compared to Control group. and [a]p<0.001 as compared to Normal group.

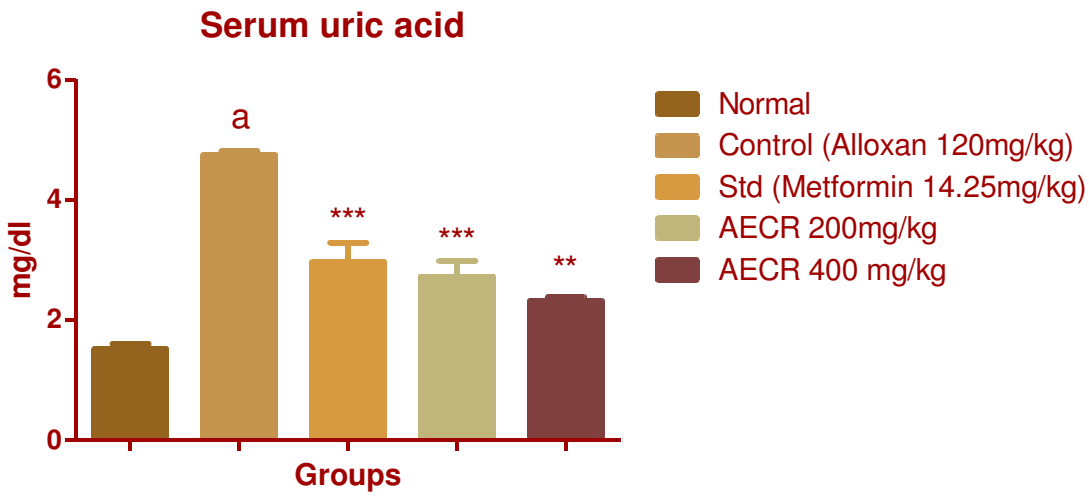
Fig.6: Effect of AECR on Serum creatinine in alloxan induced diabetes in rats.



Data expressed in Mean ± S.E.M. One way ANOVA followed by Dunnett's multiple

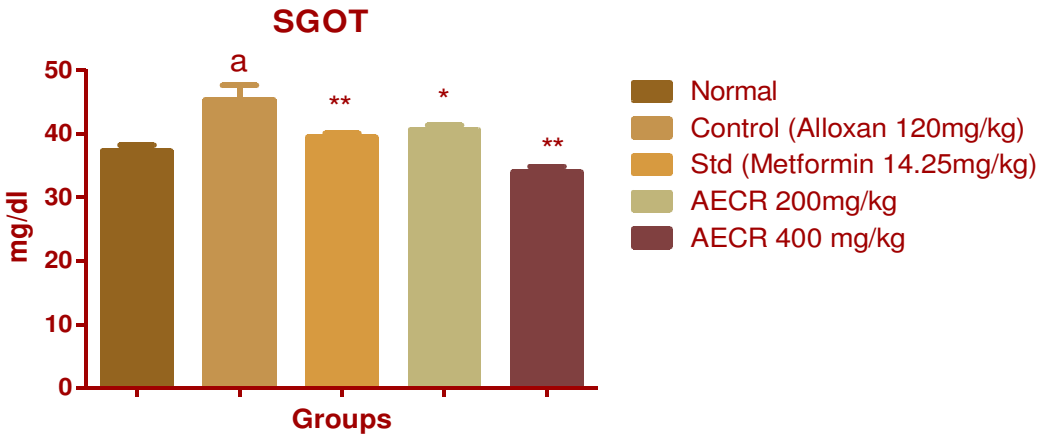
comparison test **p<0.01,***p<0.001 as compared to Control group. and [a]p<0.001 as compared to Normal group.

Fig.7: Effect of AECR on Serum uric acid in alloxan induced diabetes in rats.



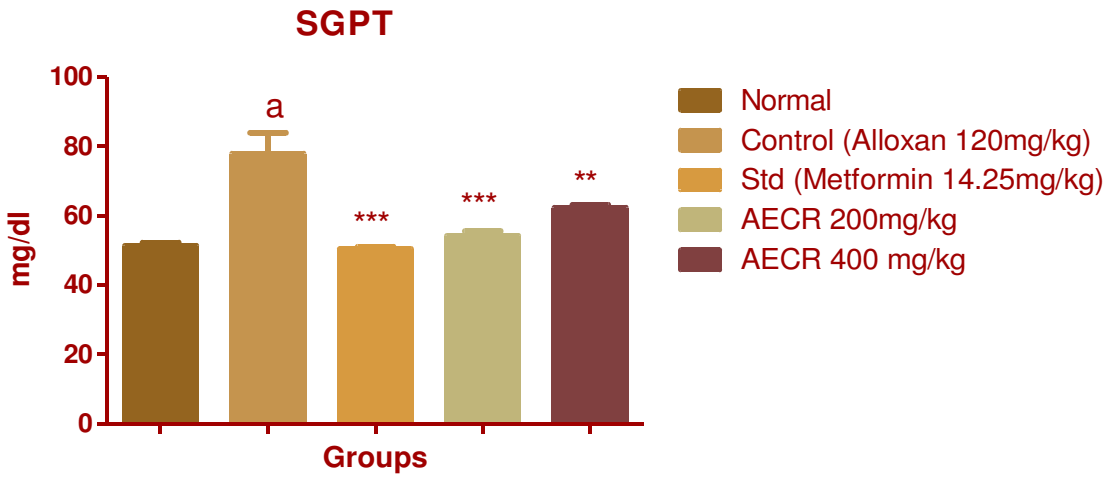
Data expressed in Mean ± S.E.M. One way ANOVA followed by Dunnett's multiple comparison test **p<0.01,***p<0.001 as compared to Control group. and [a]p<0.001 as compared to Normal group.

Fig.8: Effect of AECR on Serum SGOT in alloxan induced diabetes in rats.



Data expressed in Mean ± S.E.M. One way ANOVA followed by Dunnett's multiple comparison test *p<0.05, **p<0.01 as compared to Control group. and [a]p<0.001 as compared to Normal group.

Fig.9: Effect of AECR on Serum SGPT in alloxan induced diabetes in rats.



Data expressed in Mean ± S.E.M. One way ANOVA followed by Dunnett's multiple comparison test **p<0.01,***p<0.001 as compared to Control group. and [a]p<0.001 as compared to Normal group.

Histopathology of Pancrease

The histopathological changes were observed in control and experimental group rats. The control rat's pancreas showed that the normal appearance of islet cells. The alloxan treated rats showed vacuolization, necrotic changes and reduced islet cells of pancreas damage were observed. Oral administration of aqueous extract of *Cyperus rotundus* at the dose of 200 and 400 mg/kg body weight to alloxan treated rats showed markedly reduced the extent of necrosis, vacuolization and reduced islet cells of pancreas. In the reference group, i.e., alloxan with metformin, pancreas architecture was similar to that observed in

the control rats. The maximum curative effect against alloxan induced diabetic aberrations was achieved with the 400 mg/kg body weight.

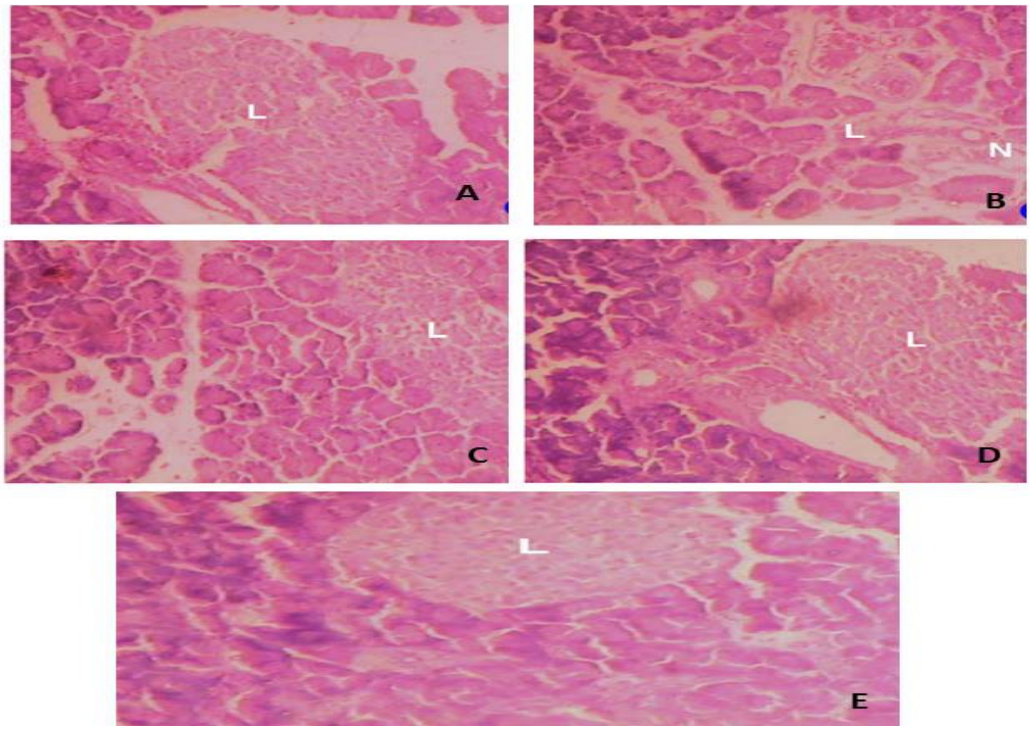


Fig. Microphotographs of pancreas tissue examined by routine hematoxylin-eosin of alloxan treated animals. **A: Normal group, B: Control group (alloxan 120mg/kg), C: Metformin 14.25mg/kg, D: AECR 200mg/kg, E: AECR 400mg/kg.**

Chapter: VII

Discussion

VII.DISCUSSION

The acute toxicity test of AECR in mice produced no death or signs of toxicity even at the dose of 2000 mg/kg which shows that the extract was well tolerated and the test doses safe in the animals.

The antidiabetic activity of AECR was evaluated in alloxan-induced diabetic rats by testing its effect on fasting blood glucose level using auto analyzer (Accu Check Active®) glucose kit. The fasting blood sugar test is a carbohydrate metabolic test which measures plasma or blood glucose levels after a fast (usually 8–12 h). During fasting the body stimulates the release of the hormone glucagon, which in turn releases glucose into the blood through catabolic processes. Normally, the body produces and processes insulin to counteract the rise in glucose levels but in diabetes, this process does not occur and tested glucose levels normally remain high[63]. Alloxan is one of the usual substances used for induction of diabetes mellitus apart from streptozotocin and has a destructive effect on the beta (β) cells of the pancreas as previously reported by[64]. Pancreas is the primary organ involved in sensing the organism's dietary and energetic states via glucose concentration in the blood and in response to elevated blood glucose, insulin is secreted[65]. However, alloxan causes diabetes through its ability to destroy the insulin-producing-cells of the pancreas[66]. When there are not enough available beta-cells to supply sufficient insulin to meet the needs of the body, insulin-dependent diabetes results[67]

A strong relationship involves between glycemia and diabetic micro vascular complications in both type 1 and type 2 diabetes production of superoxide due to oxidative stress in diabetes may be cause for vascular and neuronal complications of painful neuropathy [68]. In diabetes, intracellular hyperglycemia causes abnormalities in

blood flow and increased vascular permeability. Quantative and qualitative abnormalities of extracellular matrix contribute to an irreversible increase invascular permeability. With microvascular cell loss occurs in part as a result of programmed cell death. Hyperglycemia may also decrease production of trophic factors for endothelial and neuronal cells. Together, these changes lead to odema, ischaemia and hypoxia induced neovascularization in the retina, proteinurea, messengial matrix expansion, glomerulosclerosis in the kidney and multifocal axonal degeneration in peripheral nerves[69]. Impaired blood flow also seems to contribute to noxious stimulus hypersensitivity. Oxidative stress related reduction in perfusion is thought to play a part in cardiac autonomic dysfunction and also in small fiber sensory neuropathy[70]. Alloxan and the products of its reduction, dialuric acid, establish a redox cycle with formation of superoxide radicals. These radicals undergo dismutation to hydrogen peroxide. Thereafter highly reactive hydroxyl radicals are formed by the Fenton reaction. The action of reactive oxidant species (ROS) with a simultaneous massive increase in cytosolic calcium concentration cause rapid Beta cell destruction[71]. Early pharmaceutical intervention against the long-term consequences of hyperglycemia induced cross-linking prevents the development of severe late complications of diabetes.

Hyperglycemia has also been recently implicated in initiation and development of various types of diabetic complications. Nephropathy is one of these serious micro vascular complications that has been observed in diabetic individuals[72]. In addition, blood urea, uric acid and creatinine concentrations were increased among uncontrolled diabetic individuals and this increase could be a result of impaired renal function due to an increased blood glucose level. Our results revealed for the first time that the mean values of these end products in the serum increased in untreated diabetic rats, while they significantly decreased after the administration of AECR. Thus, this extract might improve renal function which, in turn, leads to reduction in these end products. It was reported that diabetic individuals had lower serum creatinine concentrations as well as higher serum uric acid and urea levels than nondiabetic individuals [73,74]. Thus, the reduction in urea and creatinine levels probably can be explained by a reduction in blood glucose level.

Moreover, high levels of serum uric acid, urea and creatine and SGOT and SGPT may act as a marker of kidney and liver problems. Thus, it is possible to suggest that this extract might play an important role in reducing risk of kidney and liver problems as well

as neuroprotective by lowering both hyperalgesia and allodynia as well as serum urea, uric acid, creatinine SGOT and SGPT The hyperalgesic response in tail-withdrawal test is generally attributed to central mechanisms whereas the hyperalgesic response on hot plate is attributed to the combination of both central and peripheral mechanisms [75,76]. The beneficial effects that have been seen for the first time in our study are indications of safety of AECR extract.

Chapter: VIII

Conclusion

VIII. CONCLUSION

The conclusion, reveals that oral administration of aqueous extract of *cyperus rotundus*. exhibit, Neuroprotective, nephroprotective and hepatoprotective activities via enhancing insulin production and decreasing glucogan production and decreasing a SGOT snd SGPT level in alloxan induced diabetic rats. Thus, oral use of this extract might positively affect the functional capacities of various rat tissues, particularly blood, kidney , liver and nerves against toxic action of alloxan compound (dose of 150 mg/Kg bw). These findings clearly support the traditional use of this medicinal plant in treatment of diabetes mellitus and complications shed more light in the efficacy of this plant. Thus, *Cyperus rotundus* appears to a valuable plant and ideally suited to be used in treatment of DM and prevent or delay the onset of its complications in humans, since this is a non-toxic plant.

Chapter:IX

SUMMARY

IX. SUMMARY

- Diabetes is a group of metabolic disorder of carbohydrate, fat, and protein metabolism resulted from destruction of insulin secreting pancreatic beta-cells, defects in insulin production, insulin action, or both, characterized by hyperglycemia.
- The chronic hyperglycemia results in long-term complications of diabetes include peripheral neuropathy, nephropathy and hepatic damage .
- In this study the plant root *Cyperus rotundus* had selected for the evaluation of diabetic complication. This plant has been claimed to cure nervine tonic, diuretic, antiperiodic, and traditionally used to treat diarrhoea, dysentery, leprosy, bronchitis, amenorohoea, dysmenorrhoea, renal and vesical calculi, ophthalmic disorders, blood disorders and general debility.
- Preliminary phytochemical analysis revealed that the plant root possessed phyto constituents alkaloids, steroids, saponins, tannins, Phytosterols, flavonoids, glycosides and carbohydrates.
- Type II diabetic mellitus was induced in male wistar albino rats body weight of animals 180-220 selected for the study.
- Evaluation of diabetic complications activity was assessed by using alloxan experimental model by administration of alloxan 120mg/kg i.p. for 14 days.
- Body weight and serum blood glucose were recorded before administration of AECR and metformin to the animals.
- Selected animals were then administered the alloxan 120mg/kg significantly increased the blood glucose levels in all animals.
- Administration of AECR and standard metformin were significantly reduced elevated level of serum glucose level.
- Animals treated with alloxan had significant decreased in tail immersion (cold water test) tail flick latency was observed after 14 days of diabetic complication induction in cold immersion test as compared normal group.
- AECR 200, 400mg/kg and Metformin 14.25mg/kg group of animlas recorded a reduction in latency on day 14^{th} which was followed by increase in pain threshold time on subsequent days, indicating absence of algesia produced by tail immersion in hot water.
- Diabetic control rats had exhibited the elevated serum urea, creatinine, uric acid SGOT and SGPT liver enzyme.
- Animals were sacrificed after 14 days and blood was withdrawn from retro-orbital puncture for various biochemical estimations and pancreas collected for histopathological study

- Administration of AECR 200, 400 mg/kg and Metformin 14.25mg/kg had significantly decreased the elevated level of biochemical markers such as serum urea, uric acid, creatinine SGOT, and SGPT as compared to diabetic control rats.

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PLANT AUTHENTIFICATION CERTIFICATE

SRI VENKATESWARA UNIVERSITY
TIRUPATI-517 502, A.P., INDIA

Dr. K. MADHAVA CHETTY
M.Sc., M.Ed., M.Phil., Ph.D., PG DPD
Assistant Professor
Department of Botany

Phone: +91-877-2233685 (R)
Cell: +91-9490486654
E-mail: madhavachetty@gmail.com

Date: 01-01-2013

AUTHENTICATION CERTIFICATE

I hereby certify that the following plant species for Pharmacognostical / Pharmaceutical / Pharmacological / Phytochemical / Biotechnological investigation research work is identified and their botanical name and family name is given.

Botanical Name	Family
Cyperus rotandus L.	Cyperaceae

Authenticated by

K. Madhava Chetty

(Dr. K. MADHAVA CHETTY)

DR. K. MADHAVA CHETTY
M.Sc., M.Ed., M.Phil., Ph.D., PG DPD.
ASSISTANT PROFESSOR
DEPARTMENT OF BOTANY
SRI VENKATESWARA UNIVERSITY
TIRUPATI-517 502, A.P. India

ERRATA

CPCSEA Approval Certificate

APPROVAL CERTIFICATE

This is to certify that the project title **"*Effect of Aqueous Extract of Cyperus rotundus roots on Diabetic Complications in Alloxan Induced Diabetic in Rats*"** has been approved (Approval No: 179/SICRA/IAEC) by the IAEC.

Chairman IAEC
(Dr.C.RAMESH)

Sigma Institute of Clinical Research & Administration Pvt. Ltd. HYDERABAD

CPCSEA Nominee
(Dr.A.V.SIVA KUMAR)
BIO LABS
UNI SANKYO LTD., HYDERABAD

(*NOTE: Make sure that minutes of the meeting duly signed by all the IAEC members are maintained by the Office*)