Protective Effect of Fagonia Arabica (L.) against Alloxan Monohydrate-Induced Diabetes in Albino Wistar Rats

Dissertation submitted

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OSMANIA UNIVERSITY, HYDERABAD

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In Partial Fulfillment of the Award of the Degree Of

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By

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CERTIFICATE

This is to certify that the dissertation entitled:

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(Roll no: 1007-13-514-002) during (Semester IV, 2015), MSc. Biochemistry under the guidance of (Professor. Dr. Ch. Venkata Ramana Devi), faculty member, Department of Biochemistry, University College of Science, Osmania University, Hyderabad and has been satisfactory in quality and is recommended for consideration towards partial fulfillment for the award of the degree of Master of Science in Biochemistry from Osmania University, year (2013-2014), Hyderabad.

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DECLARATION

I **Ahmed Ghdhban Theiban**, hereby declare that the project entitled **"Protective effect of Fagonia arabica (L). Against Alloxan monohydrate induced Diabetes in albino wistar rats"** submitted to OSMANIA UNIVERSITY, Hyderabad, University College of Science, Department of Biochemistry as a part of my curriculum study for the degree of **M.Sc. in Biochemistry** is the result of original work done by me. I further declare that this project or any part of this has not been published earlier.

Ahmed Ghdhban Theiban

Class: MSc. Biochemistry

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/June/2015

DEDICATED

I dedicate this humble work,….To

My beloved Father….

My beloved Mother….

My lovely wife…

My dear kids…

My dear brothers and sisters….

My beloved Father and Mother in law….

My dear teachers…

ACKNOWLEDGEMENT

((Thanks to God for His entire blessing during

the pursuit of my academic and career goals))

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Ahmed Ghdhban Theiban

Class: MSc. Biochemistry

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ABBREVIATIONS

AM	Alloxan monohydrate
%	Percentage
°C	Degree Celsius
ALP	Alkaline phosphatase
ALT	Alanine transaminase
AST	Aspartate transaminase
DM	Diabetes mellitus
FAEXT	Fagonia arabicaethanolic (70%) extract
FPG	Fasting Plasma Glucose
g	Gram
GAD	Glutamic acid decarboxylase
GDM	Gestational diabetes mellitus
HDL	High density lipoproteins
hr	Hour
IDDM	Insulin dependent diabetes mellitus
IGT	Impaired glucose tolerance
kg	Kilogram
kgbw	Kilo gram Body weight
LDL	Low density lipoproteins
mg	Milligram
min	Minute
ml	Milliliter
MRDM	Malnutrition-related diabetes mellitus
NIDDM	Non-insulin dependent diabetes mellitus
OGTT	Oral glucose tolerance test
TG	Triglycerides
VLDL	Very low density lipoproteins
GGT	Gama glutamyl transferase
I.P	Intra peritoneal

CONTENTS

Protective effect of *Fagonia arabica* (L.) against Alloxan monohydrate-induced Diabetes in albino wistar rats

ABSTRACT

Diabetes mellitus is a most common endocrine disorder, affecting more than 300 million people worldwide. For this, therapies developed along the principles of western medicine (allopathic) are often limited in efficacy, carry the risk of adverse effects, and are often too costly, especially for the developing world. In order to identify complementary or alternative approaches to existing medications, we studied the anti-diabetic potential of *Fagonia arabica* (L.). α-glucose inhibitors (AGI) are a group of compounds which inhibit the rate of breakdown of dietary oligosaccharides, polysaccharides.

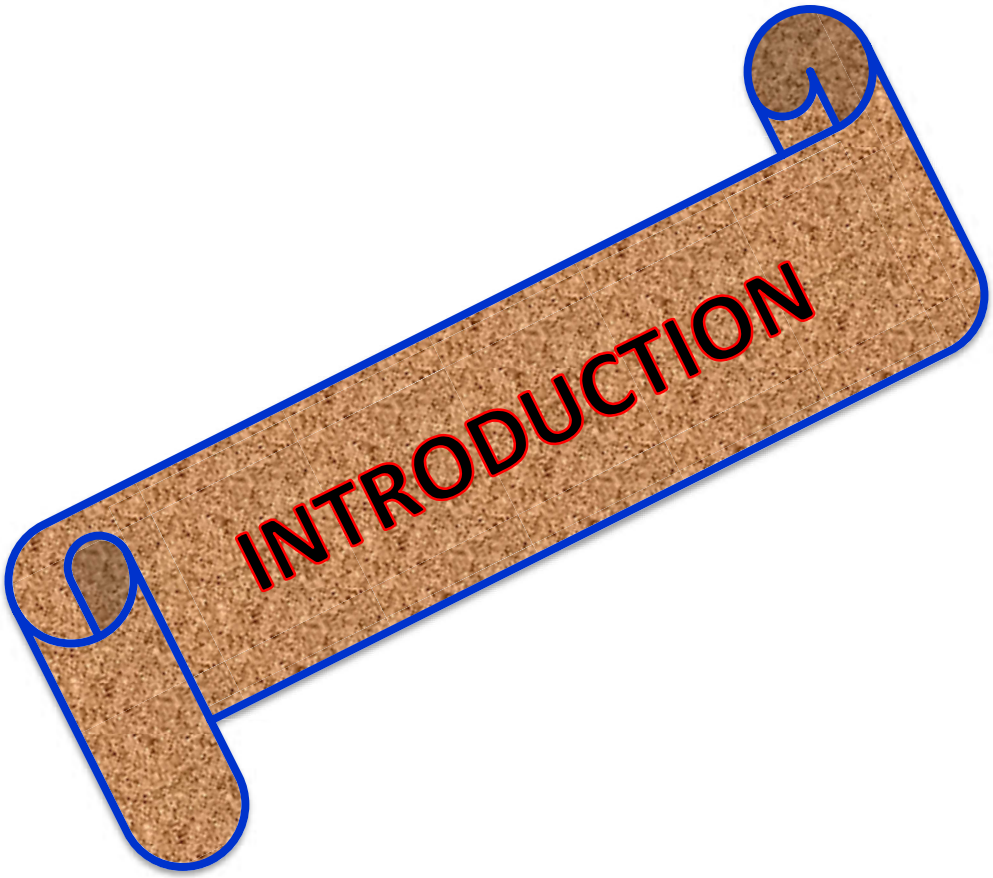
This delays the glucose absorption. Acarbose, miglitol and voglibose are different AGIs, but only acarbose is available for clinical use while miglotol and voglibose are under clinical investigation. Based on previous literature, we have selected *F.arabica (*L.*)*. Ethanolic extract of the plant was prepared by using Soxhlet apparatus with 70% ethanol. And the extract was subjected to lyophilizer to get dried ethanolic extract powder.

This extract was used to treat the diabetic rats through oral ingestion by rat oral gavage. Alloxan monohydrate is one of the chemical agents used to induce diabetes mellitus in animals. It induces diabetes by dose dependent destruction of β -cells of islets of langerhans. Diabetes was induced by a single I.P. dose Alloxan monohydrate (150 mg/kg body weight). It was observed that single intravenous dose of alloxan exhibited significant hyperglycemia.

Prior to this study rats were housed for 7 days to acclimatize to the environment (totally 52 days). The study was carried out on a 45 day protocol and the body weights, blood glucose levels were measured on Day 1, Day 7, Day 14, Day 21, Day 28, Day 35, Day 42 and Day 45 of the treatment, along with assays of AST, ALT, ALP, Lipid profile studies and histopathological examination of liver on day 45. Maximum activity was shown by the active compound with a percent variation in blood glucose levels. Glibenclamide (10mg/kg body weight) was taken as the standard and the results were quite comparable with it.

The histopathological studies also indicated that *F.arabica* is effective in regeneration of insulin secreting β-cells and thus possesses anti-hyperglycaemic activity. The results also showed that *F.arabica* protects significantly from other physiological aberrations i.e., polydypsia, polyphagia, weight loss and metabolic aberrations i.e., increase in AST, ALT, ALP, cholesterol and triglyceride levels caused by diabetes, in dose dependent manner. The compound also showed significant effect in increasing the oral glucose tolerance of rats and it also showed good hypoglycaemic activity in normoglycaemic rats. In this study the *F.arabica* ethanolic extract for anti-diabetic property was evaluated.

CHAPTER-ONE



CHAPTER-I INTRODUCTION

Diabetes mellitus can be defined as a metabolic disorder with impaired glucose utilization, characterized by hyperglycemia and insufficiency of secretion or action of endogenous insulin. Diabetes is a severe health problem and its incidence is increasing at alarming proportions. Recent estimates indicate that around 200 million people suffer from diabetes mellitus, making it the most common serious metabolic disorder worldwide. Diabetes is a major cause of mortality.

Type 2 diabetes results from the interaction between a genetic predisposition, behavioural and environmental risk factors (Neel, 1962). Although the genetic basis of type 2 diabetes has yet to be identified, there is strong evidence that modifiable risk factors such as obesity and physical inactivity are the main nongenetic determinants of the disease (Manson et al., 1991; Hamman, 1992). Altered dietary pattern also participates in the increased incidence of diabetes.

Diabetes is characterized by hyperglycemia with slowly progressive end organ damage in the eyes, kidneys, blood vessels, heart, peripheral nerves and brain (Gispen & Biessels, 2000; Northam et al., 2006). In poorly controlled diabetic animals or humans increased cell death occurs in different tissues and organs and it is involved in secondary complications of diabetes (Barber et al., 1998; Cai et al., 2002; Li et al., 2002; Pesce et al., 2002; Arroba et al., 2003; García-Cáceres et al., 2008). Chronic hyperglycemia during diabetes mellitus is a major initiator of diabetic micro-vascular complications like retinopathy, neuropathy and nephropathy (Sheetz & King, 2002).

This illness affects approximately 185 millions of people worldwide and its incidence rate is expected to double during the next 20 years (Cohen & Goedert, 2004). The prevalence of diabetes mellitus (DM) is growing rapidly worldwide and is reaching epidemic proportions (King & Rewers, 1991; Bjork, 2003). It is estimated that there are currently 285 million people with diabetes worldwide and this number is set to increase to 438 million by the year 2030. There is also consensus that the South Asia region will include three of the top ten countries in the world (India, Pakistan and Bangladesh) in terms of the estimated absolute numbers of people with diabetes (Sicree, 2009).

Although the exact reasons why Asian Indians are more prone to type 2 diabetes at a younger age and premature cardiovascular disease (CVD) remain speculative, there is a growing body of evidence to support the concept of the "Asian Indian Phenotype"5.This term refers to the peculiar metabolic features of Asian Indians characterized by a propensity to excess visceral adiposity, dyslipidaemia with low HDL cholesterol, elevated serum triglycerides and increased small, dense LDL cholesterol, and an increased ethnic (possibly genetic) susceptibility to diabetes and premature coronary artery disease (Deepa et al., 2006; Joshi, 2003).

Type 2 diabetes is often associated with obesity and hypertension. The concomitant occurrence of these disorders is commonly referred to as 'metabolic syndrome'. There are two main forms of diabetes (Zimmet et al., 2001). Type 1 diabetes is due primarily to autoimmune mediated destruction of pancreatic β-cells, resulting in dramatic insulin deficiency. Its frequency (~10%) is low relative to type 2 diabetes, which accounts for over 90% of cases. Type 2 diabetes is characterized by abnormal insulin secretion, associated with varying degrees of insulin resistance. The diabetes epidemic relates particularly to type 2 diabetes and is taking place both in developed and developing nations (Zimmet et al., 2001)

The diabetes epidemic relates particularly to type 2 diabetes and is taking place both in developed and developing nations (Zimmet et al., 2001)Ancient Indian texts make mention of the disease "Madhumeha" which would correspond to the modern term "Diabetes mellitus", suggesting that diabetes must have been present in India even before 2500 BC. Although, there is no evidence as to how prevalent the condition was, a recent article hypothesizes that it could have been quite common in India, even in ancient times (Weaver & Narayan, 2008).

1.1. Regulation of Glucose Homeostasis

Blood sugar regulation is the process by which the levels of blood sugar, primarily glucose, are maintained by the body. Glucose regulation in the body is a process of keeping the body in homeostasis. Blood sugar levels are regulated by negative feedback in order to keep the body in homeostasis. The levels of glucose in the blood are monitored by the cells in the pancreas's Islets of Langerhans. Glucose is the major source of cellular energy and its concentrations are controlled by a number of hormones, the most important being insulin and glucagon. Insulin is secreted by β-cells when blood glucose concentration rises and reduces

glucose levels by two general mechanisms; (1) inhibition of hepatic glucose production (glycogenolysis and gluconeogenesis) and (2) increasing glucose uptake into muscle and fat tissue. Glucagon is a hormone secreted by pancreatic β-cells in response to low concentrations of glucose and is responsible for elevating blood glucose levels. It acts principally at the liver and antagonizes the effects of insulin by increasing glycogenolysis and gluconeogenesis and also inhibiting glycogenesis and glycolysis. Other hormones also function in maintaining normal glucose levels.

These include amylin, glucagon-like peptide-1 (GLP-1) and glucose-dependent insulinotropic polypeptide (GIP). Amylin is actually secreted with insulin from β-cells and functions in decreasing gastric emptying, which limits glucose excursions following a meal. GLP-1 and GIP are incretins, or gut derived hormones, which have a multitude of effects, two of which are to promote the synthesis and secretion of insulin from β-cells. A decrease in the effect of these incretins contributes to the progression of diabetes.

1.1.1. Pancreas:

The pancreas is a mixed gland with a large exocrine and a much smaller endocrine gland. The endocrine cells are arranged into small islands of cells called the islets of Langerhans. Islets are generally oval in shape and their core consists primarily of b cells, while non-b- cells are located mostly in the surrounding mantle. They represent approximately 2% of the total pancreatic mass. The interactive function of both the exocrine and the endocrine parts are particularly important for the normal functioning of the body. The pancreas serves two major functions:

(i) The production of digestive enzymes which are secreted by exocrine acinar cells and routed to the intestine by a branched ductal network.
(ii) The regulation of blood sugar which is achieved by endocrine cells of the islets of Langerhans. Like all endocrine glands, islets secrete their hormones into the bloodstream and are highly vascularised. Because of its obvious medical importance, the pancreas has been subject to decades of close study.

The pancreatic islets of Langerhans are made by the aggregation of 4 major secretory cell types. Each islet is composed of 2000-4000 cells, ~60%-80% of which secrete insulin and are referred to as b cells. Glucagon secreting a cells account for ~15-20% of the islet cells, while somatostatin-secreting delta-cells and pancreatic polypeptide-secreting cells (PP) account ~ 5-10% and < 2% of the total islet cell number respectively.

Hormones that influence blood glucose level:

Hormone	Tissue of Origin	Metabolic Effect	Effect on Blood Glucose
Insulin	Pancreatic-β Cells	1) Enhances entry of glucose into cells; 2) Enhances storage of glucose as glycogen, or conversion to fatty acids; 3) Enhances synthesis of fatty acids and proteins; 4) Suppresses breakdown of proteins into amino acids, of adipose tissue into free fatty acids.	Lowers
Somatostatin	Pancreatic-δ Cells	1) Suppresses glucagon release from α cells (acts locally); 2) Suppresses release of Insulin, Pituitary tropic hormones, gastrin and secretin.	Lowers
Glucagon	Pancreatic-α Cells	1) Enhances release of glucose from glycogen; 2) Enhances synthesis of glucose from amino acids or fatty acids.	Raises
Epinephrine	Adrenal medulla	1) Enhances release of glucose from glycogen; 2) Enhances release of fatty acids from adipose tissue.	Raises
Cortisol	Adrenal cortex	1) Enhances gluconeogenesis; 2) Antagonizes Insulin.	Raises
ACTH	Anterior pituitary	1) Enhances release of cortisol; 2) Enhances release of fatty acids from adipose tissue.	Raises
Growth Hormone	Anterior pituitary	Antagonizes Insulin	Raises
Thyroxine	Thyroid	1) Enhances release of glucose from glycogen; 2) Enhances absorption of sugars from intestine	Raises

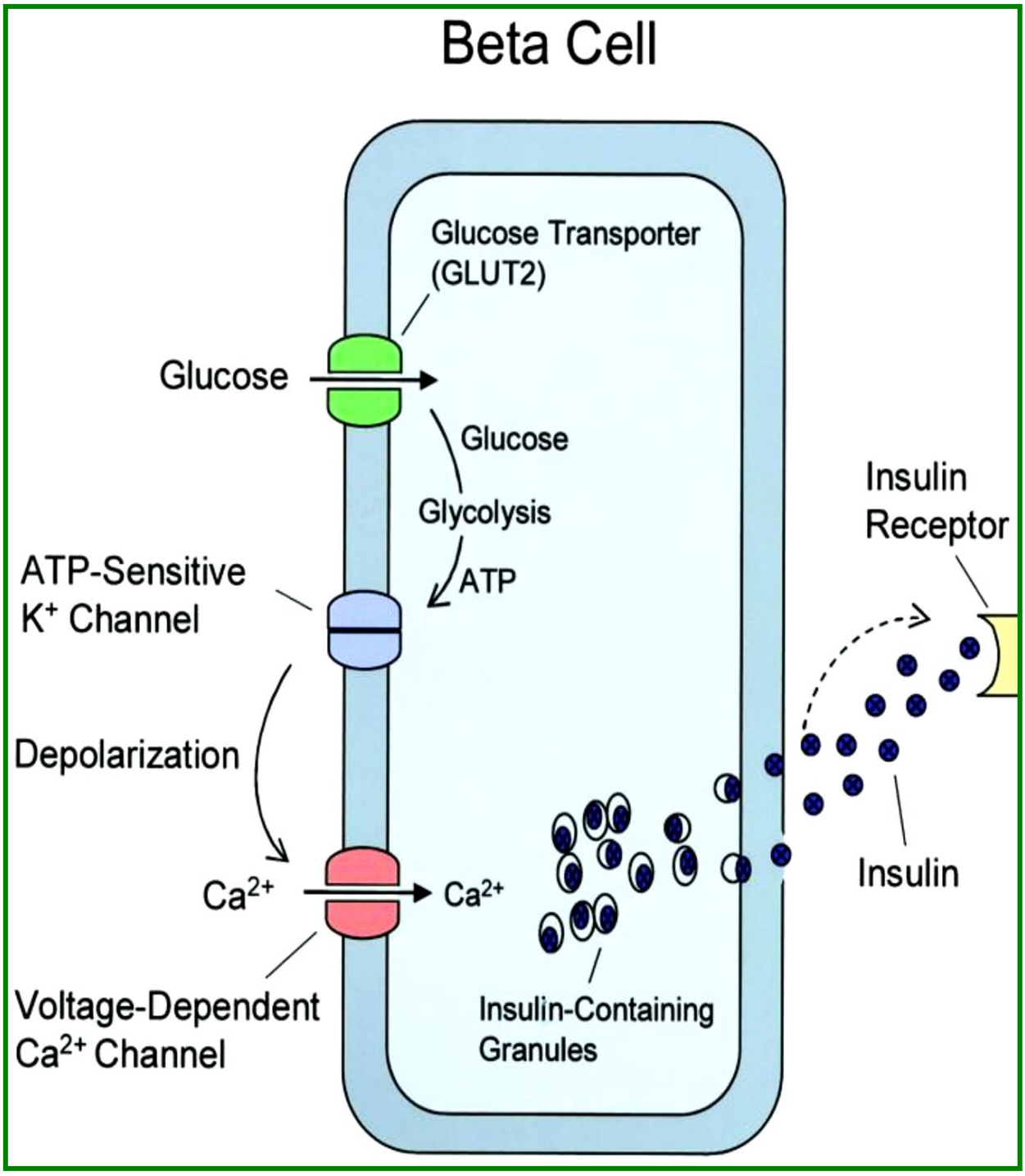


Fig. 1. Insulin release and action (Notkins, 2002).

1.1.2. Glucagon

Glucagon is the hormone secreted by pancreatic -cells. Glucagon also plays a central role in the regulation of glucose homeostasis (Baron et al., 1987; Cherrington, 1990). Glucagon stimulates glycogenolysis in the liver to increase blood glucose levels. Under postabsorptive conditions, approximately half of total hepatic glucose output is dependent on the maintenance of normal basal glucagon levels and inhibition of basal glucagon secretion with somatostatin causes a reduction in hepatic glucose production and plasma glucose concentration. After a glucose-containing meal, glucagon secretion is inhibited by hyperinsulinemia and the resultant hypoglucagonemia contributes to the suppression of hepatic glucose production and maintenance of normal postprandial glucose tolerance.

It has been shown that glucagon has a striking stimulatory effect on insulin release in the absence of glucose (Sevi, 1966). Glucagon stimulates glycogenolysis in the liver to increase blood glucose levels. The presence of specific glucagon receptors on isolated rat pancreatic β -cells as well as a subpopulation of α- and δ-cells shows the relevance of glucagon on regulation of insulin secretion. Intra-islet glucagon appears to be a paracrine regulator of cAMP in vitro (Schuit, 1996). Glucagon stimulates insulin release by elevating cAMP. cAMP through activation of protein kinase A, increases Ca^{2+} influx through voltage dependent L-type Ca^{2+} channels, thereby elevating Ca^{2+} and accelerating exocytosis (Carina, 1993). Protein phosphorylation by Ca^{2+}/Calmodulin and cAMP dependent protein kinase play a positive role in insulin granule movement which results in potentiation of insulin release from the pancreatic β-cell (Hisatomi,1996).

1.1.3. Insulin

The biological effects of insulin in classical insulin target tissues, such as skeletal muscle, fat and liver are glucose uptake, regulation of cell proliferation, gene expression and the suppression of hepatic glucose production. Insulin decreases hepatic glucose production and increases glucose entry into muscle and fat cells. Following glucose ingestion, the increase in plasma glucose concentration stimulates insulin release and the combination of hyperinsulinemia and hyperglycemia.

(1) Stimulates glucose uptake by splanchnic (liver and gut) and peripheral (primarily muscle) tissues.
(2) Suppresses endogenous (primarily hepatic) glucose production (DeFronzo, 1997, 1988; DeFronzo et al., 1981; DeFronzo & Ferrannini, 1987; Mari et al., 1994; Mandarino et al., 2001; Mitrakou et al., 1990; Cherrington, 1990). Insulin is a potent anti-lipolytic hormone and even small increments in the plasma insulin concentration markedly inhibit lipolysis, leading to a decline in the plasma level of free fatty acid (Groop et al., 1989).

In the postabsorptive state the majority of total body glucose disposal takes place in insulin independent tissues. Approximately 50% of all glucose use occurs in the brain, which is insulin-independent (Grill, 1990). Another 25% of glucose disposal occurs in the splanchnic area (liver plus gastrointestinal tissues) which is also insulin independent The remaining 25% of glucose use in the postabsorptive state takes place in insulin-dependent tissues, primarily muscle and to a lesser extent adipose tissue.

Approximately 85% of endogenous glucose production is derived from the liver and the remaining 15% is produced by the kidney. Glycogenolysis and gluconeougenesis contribute equally to the basal rate of hepatic glucose production. During glucose ingestion, insulin is secreted into the portal vein where it is taken up by the liver and suppresses hepatic glucose output. If the liver does not perceive this insulin signal and continues to produce glucose, there will be two inputs of glucose into the body, one from the liver and a second from the gastrointestinal tract and marked hyperglycemia will ensue.

The actions of insulin are mediated via the insulin receptor (IR) which belongs to the family of tyrosine kinase receptors. Binding by insulin leads to rapid autophosphorylation of the receptor, followed by tyrosine phosphorylation of IRS proteins, which induce the activation of downstream pathways such as the PI3K and the mitogen-activated protein kinase (MAPK) cascades. IR is a glycoprotein that consists of two a-subunits and two b-subunits linked by disulfide bonds (DeFronzo, 1988; Pessin & Saltiel, 2000; Whitehead et al., 2000; Saltiel & Kahn, 2001). The two a subunits of the IR are entirely extracellular and contain the insulin-binding domain.

The b-subunits have an extracellular domain, a transmembrane domain and an intracellular domain that expresses insulin-stimulated kinase activity directed toward its own tyrosine residues. Phosphorylation of the b-subunit, with subsequent activation of IR tyrosine kinase, represents the first step in the action of insulin on glucose metabolism. Mutagenesis of any of the three major phosphorylation sites (at residues 1158, 1163, and 1162) impairs IR kinase activity, leading to a decrease in the metabolic and growth-promoting effects of insulin (Ellis et al., 1986; Chou et al., 1987). Therefore IR activation and subsequent stimulation of IR second messenger cascades, including the translocation of insulin sensitive GLUTs participate in

Enhancing properties of insulin. Activation of the insulin signal transduction system in insulin target tissues stimulates glucose transport through a mechanism that involves translocation of a large intracellular pool of glucose transporters (associated with low density microsomes) to the plasma membrane and their subsequent activation after insertion into the cell membrane.

Type 2 diabetes is characterized by a progressive decline in pancreatic b cell function and chronic insulin resistance (DeFronzo, 1987; Kudva & Butler, 1997). Pancreatic β-cell dysfunction is central to the development of diabetes, possibly due to a combination of decreased β -cell mass and insulin secretion defects. The number of β -cells within the pancreas is an important determinant of the amount of insulin that is secreted. Most studies have demonstrated a modest reduction (20%–40%) in β-cell mass in patients with long-standing type 2 diabetes (Gepts & Lecompte, 1981; Kloppel et al., 1985; Clark et al., 1988; Butler et al., 2003). A reduction in pancreatic b-cell function and mass leads to hyperglycemia (elevated blood sugar) in both type 1 and type 2 diabetes. In type 1diabetes, autoimmune destruction of the b-cell itself severely reduces b-cell mass, resulting in marked hypoinsulinemia and potentially life threatening ketoacidosis.

In contrast, during the progression to type 2 diabetes, impaired b-cell compensation in the setting of insulin resistance (impaired insulin action) eventually leads to b-cell failure and a modest but significant reduction in b-cell mass (Butler et al., 2003; Yoon et al., 2003). Regulation of the b-cell mass appears to involve a balance of b-cell replication and apoptosis as well as development of new islets from exocrine pancreatic ducts (Finegood et al., 1995; Bonner-Weir,

2000). Disruption of any of these pathways of b-cell formation or increased rates of b-cell death could cause a decrease in b-cell mass. Diabetes results when insulin production by the pancreatic islet b-cell is unable to meet the metabolic demand of peripheral tissues such as liver, fat and muscle.

The therapy for people with b cell loss is insulin administration. This exogenous insulin is deposited subcutaneously, regardless of whether it is injected or infused and has different kinetics from endogenous insulin released from b cells. Normally, insulin is released from a b cell within a minute of the cell being exposed to glucose, with equally rapid turn-off once the blood glucose level has returned to normal. This fine-tuning is not possible with subcutaneously administered insulin. As a result blood glucose levels in those with insulin-dependent diabetes often fluctuate widely.

Aggressive attempts to prevent hyperglycemia result in an increased incidence of hypoglycemia. Hypoglycaemic episodes have detrimental effects on the brain and in addition, it is associated with impairment in learning and memory (Flood et al., 1990). Being a major problem in clinical practice, hypoglycemia unawareness is associated with an increased risk of coma. By contrast, prolonged hyperglycemia will lead to an increased incidence of microvascular complications such as retinopathy and nephropathy. To avoid the variations in blood glucose levels in those with insulin-dependent diabetes and to reduce the chance of long-term complications, it would be helpful for new b cells to be produced.

1.2. Causes of T2DM

T2DM as a common and complex disease has been characterized by the following causes:

Obesity: obesity is also considered a key risk factor for T2DM. The association between increasing body mass index (BMI) and greater weight gain and risk of diabetes is most pronounced among Asians, suggesting that lower cut off BMI values are needed to identify Asians at a higher risk of diabetes (Shai et al., 2006). BMI cut point for Indians for any cardiometabolic risk factors is 23 kg/m2 in both sexes, whereas that of waist circumference (WC) is 87cm for men and 82cm for women (Mohan et al. 2007).

Abdominal adiposity: there is also a probable indication that there is a preferential abdominal adiposity in Indians irrespective of the degree of general adiposity (Ramachandran et al., 2002).

Imbalance of human metabolism is associated with T2DM: Changes in work patterns from heavy labour to sedentary, the increase in computerization and mechanization, and improved transport are just a few of the changes that have had an impact on human metabolism (Zimmet et al., 2001). Genes: since 2007, genome-wide association studies has catalogued around 20 genes (like TCF7L2, HHEX, CDKAL1, SLC30A8 etc.) showing a strong association (with modest odds ratio ranges between 1.2 to 1.5) with T2DM (Sladek et al., 2007, WTCCC 2007, Scott et al., 2007).

Ethnicity: the interethnic differences (like differences in prevalence of T2DM among Europeans, Americans, Chinese, and Asian Indians) in insulin resistance may have an environmental or genetic explanation. The main acquired factors that seemingly increase insulin resistance in all ethnic groups include obesity, sedentary lifestyle, diet rich in animal products, and aging (Abate & Chandalia, 2001).

1.3. Complications of diabetes

The effects of unregulated glucose control can lead to severe macro and microvascular complications. In fact, the correlation of these complications with glucose levels have been used to derive the cut offs for diagnosis of diabetes mentioned above (WHO., 2006). Diabetes primarily affects the heart, blood vessels, eyes, kidney and nerves (World Health Organization Fact Sheet N° 312., 2007). It is a leading cause of blindness and renal failure. Microvascular complications refer to those affecting small blood vessels in the retina, kidney, and peripheral nerves and can lead to retinopathy, nephropathy and neuropathy, respectively. Diabetic retinopathy occurs as a result of long-term damage to blood vessels in the retina and can lead to blindness or severe visual impairment.

Diabetes can also cause the development of cataract through the formation of sorbitol deposits on the lens of the eye. Sorbitol is a product of the polyol pathway formed by the action of aldose reductase, which becomes over expressed in type 2 diabetes, and is believed to be intimately involved with organ damage. Diabetes is among the leading causes of kidney failure

and 10-20% of diabetics die from this (World Health Organization Fact Sheet N° 312., 2007). Diabetic neuropathy occurs as a result of damage to the nerves and results in tingling, pain, numbness and weakness in the extremities, which left untreated, can lead to infection, ulceration and possibly amputation. Macrovascular complications refer to diseases affecting large blood vessels in the heart, brain and peripheral circulation leading to cardiovascular diseases such as atherosclerosis, heart attack and stroke, which are responsible for 50% of deaths of diabetics (World Health Organization Fact Sheet N° 312., 2007).

It is hypothesized that there are four main mechanisms by which hyperglycemia induces microvascular and macrovascular complications (Brownlee, 2001);

(1) increased polyol pathway flux
(2) increased glycation end-product formation;
(3) activation of protein kinase C and
(4) increased hexosamine pathway flux.

A common effect of each is that they increase the production of superoxide by the mitochondrial electron-transport chain. Superoxide is a reactive oxygen species that leads to oxidative stress and can subsequently cause the tissue damage that is observed in diabetes. This suggests that antioxidants, as free-radical scavengers, may be used therapeutically in the future to prevent the complications associated with diabetes (Brownlee, 2001).

1.4. Epidemiology of diabetes

The press release on 14 NOVEMBER - World Diabetes Day 2011 pointed out that, the new figures indicate the number of people living with DM is expected to rise from 366 million in 2011 to 552 million by 2030, if no urgent action is taken. This equates to approximately three new cases every ten seconds or almost ten million per year. In addition, report said, in some of the poorest regions in the world such as Africa, where infectious diseases have traditionally been the focus of health care systems, diabetes cases are expected to increase by 90% by 2030. At least 78% of people in Africa are undiagnosed and do not know they are living with diabetes. In addition, few clinical reports released by International Diabetes Federation are as follows (2011) .

- 80% of people with diabetes live in low and middle income countries.
- 78,000 children develop type 1 diabetes every year
- The greatest number of people with diabetes are between 40-59 years of age

DM is recognized by the World Health Organization (WHO) as a growing worldwide epidemic with more than 171 million people worldwide (2.8%) afflicted in 2000 and it is conservatively estimated that the number will more than double to 366 million (4.4%) by 2030 (Wild et al., 2004). The WHO predicts that diabetes mellitus will become one of the world's leading causes of death and disability within the next quarter century (World Health Organization Fact Sheet N° 236., 2006). In 2005, it was estimated that between 1.1-2.9 million people died from diabetes and its complications, making it the fifth leading cause of death in the world (Roglic et al., 2005).

Type 2 DM accounts for 90-95% of all cases of diabetes and is largely associated with obesity and physical inactivity, which have been shown to lead to insulin resistance. In fact, obesity is the greatest risk factor and it is estimated that 80% of diabetics are overweight (Triplitt et al., 2006). The increase in this global phenomenon has been largely attributed to the spread of the "western lifestyle", which refers to the combined detrimental effects of decreased exercise and unhealthy diet. In terms of the total number of people afflicted, the top three countries are India, China and the United States (US) (Wild et al., 2004). In India, 31.7 million people had diabetes in 2000 but this number is expected to skyrocket to 79.4 million by 2030. In China, 20.8 million had diabetes in 2000 increasing to 42.3 million by 2030. 17.7 million people had diabetes in the US in 2000 and it is predicted to rise to 30.3 million by 2030.

Type 2 DM is part of the "metabolic syndrome", also referred to as syndrome X, which includes a set of disorders characterized by obesity, insulin resistance, hypertension and dyslipidemia. It is a chronic metabolic disorder that even with current therapies progressively worsens with time and some of its complications include retinopathy, nephropathy, neuropathy and atherosclerotic cardiovascular disease (i.e. stroke, heart attack and foot ulcers).

Insulin resistance and β-cell failure underlie the disease. In the initial stages of insulin resistance, glucose homeostasis can be maintained through hyperinsulin secretion by β -cells.

Overt diabetes only occurs when β -cells can no longer compensate for insulin resistance. It is reported that newly diagnosed patients with type 2 diabetes mellitus have approximately 50% β -cell function (UK Prospective Diabetes Study Group., 1995), due in part to a 30% reduction in β -cell mass. In this study, an increase in fasting plasma glucose levels in patients treated by diet alone or with sulfonylurea therapy after six years as associated with a decline in insulin levels due to progressive β -cell dysfunction. Therefore, amelioration of the decline in β -cell function is critical in altering the progressive nature of the disease. Unfortunately, there is no therapy aimed at preserving β -cell function.

There is no cure for diabetes, but the progression of the disease may be slowed down considerably through proper diet and regular physical activity. Present treatment is aimed at maintaining strict glycemic control and while some patients may be managed by diet and exercise, more typically, one or a combination of oral hypoglycemic agents are required for effective glycemic control. However, even with current pharmacological treatment the disease progressively worsens with time.

For these reasons, the development of new drugs is actively being pursued. The United Nations (UN) has recognized the diabetes epidemic as a threat to the entire world and in an effort to raise public awareness has declared November 14 (beginning in 2007) as World Diabetes Day (United Nations: General Assembly., 2007). Aside from the human pain and suffering, the financial burden that this disease places on economic development throughout the world is enormous. The total cost in 1997 in the US alone has been estimated at $98 billion (World Health Organization Fact Sheet N° 236., 2006).

This includes $44 billion in direct healthcare costs and another $54 billion in indirect loss of productivity. In 2002, the cost increased to $132 billion and is estimated to rise to $192 billion in 2020 (American Diabetes Association., 2003). Health-care costs for nations range from 2.5-15% of annual health care budgets (World Health Organization Fact Sheet N° 236., 2006).

Because of the enormous scale of the disease, cost-effective therapies will be required to treat people, especially those from poorer developing parts of the world that cannot afford expensive medications. The solution to this problem is certainly complex and will require a novel

and concerted global effort that combines modern "western" medicine with alternative traditional systems used throughout many parts of the world.

1.5. Overview of interrelationship between oxidative stress and Diabetes

Oxidative stress corresponds to an imbalance between the rate of oxidant production and that of their degradation. Aerobic organisms such as vertebrates and man in particular produce their energy from the oxidation of organic substrates by molecular oxygen. The complete four-electron reduction of molecular oxygen occurs within mitochondria and produces water, at the end of the respiratory chain. Sometimes molecular oxygen is partly reduced instead of the proteins of the respiratory chain, and superoxide and various reactive oxidant intermediates are produced, leading to secondary oxidations and tissue insults.

Excessively high levels of these free radicals cause damage to cellular proteins, membrane lipids and nucleic acids, and eventually cell death. Various mechanisms have been suggested to contribute to the formation of these reactive oxygen-free radicals. Glucose oxidation is believed to be the main source of free radicals. In its enediol form, glucose is oxidized in a transition-metal dependent reaction to an enediol radical anion that is converted into reactive ketoaldehydes and to superoxide anion radicals.

The superoxide anion radicals undergo dismutation to hydrogen peroxide, which if not degraded by catalase or glutathione peroxidase, and in the presence of transition metals, can lead to production of extremely reactive hydroxyl radicals (Jiang, 1990; Wolff & Dean, 1987). Superoxide anion radicals can also react with nitric oxide to form reactive peroxynitrite radicals (Halliwell & Gutteridge, 1990; Hogg, 1993). Hyperglycemia is also found to promote lipid peroxidation of low density lipoprotein (LDL) by a superoxide-dependent pathway resulting in the generation of free radicals (Tsai et al., 1994; Kawamura et al., 1994).

Another important source of free radicals in diabetes is the interaction of glucose with proteins leading to the formation of an Amadori product and then advanced glycation endproducts (AGEs) (Hori et al., 1996; Mullarkey et al., 1990).

These AGEs, via their receptors (RAGEs), inactivate enzymes and alter their structures and functions (McCarthy et al., 2001), promote free radical formation (Baynes, 1991 & 1999), and quench and block antiproliferative effects of nitric oxide (Vlassara,1997; Wautier et al., 1994). By increasing intracellular oxidative stress, AGEs activate the transcription factor NF-kB, thus promoting up-regulation of various NFKB controlled target genes (Mohamed et al., 1999).

NF-kB enhances production of nitric oxide, which is believed to be a mediator of islet β-cell damage. The pathogenesis of type 2 diabetes is complex but typically begins with insulin resistance at target organs such as liver, muscle and adipose. In order to compensate for this, there is initially an increase in insulin production.

This hyperinsulinemic state is only temporary and over time insulin secretion diminishes due to progressive β-cell deterioration. The combined effects of insulin resistance and α-cell dysfunction results in a diminished capacity to limit hepatic glucose production as well as to decrease uptake and utilization of glucose in muscle and adipose tissue (i.e. insulin resistance).

Insulin resistance is a complex disease that typifies the metabolic syndrome and is the result of a number of defects along the insulin signaling cascade (Moneva & Dagogo-Jack, 2002). Other likely factors include increased concentrations of free-fatty acids (FFA's), tumor necrosis factor-α (TNF-α) and the hormone resistin (Moneva & Dagogo-Jack, 2002). Elevated FFA's produce insulin resistance by inhibiting glucose uptake and its oxidation (i.e. glycolysis) in skeletal muscle. FFA's also increase hepatic gluconeogenesis.

Both TNF-α and resistin are produced by adipose tissue in greater amounts in obese diabetic individuals. TNF- α impairs insulin action while resistin is known to antagonize the effects of insulin (Moneva & Dagogo-Jack, 2002).

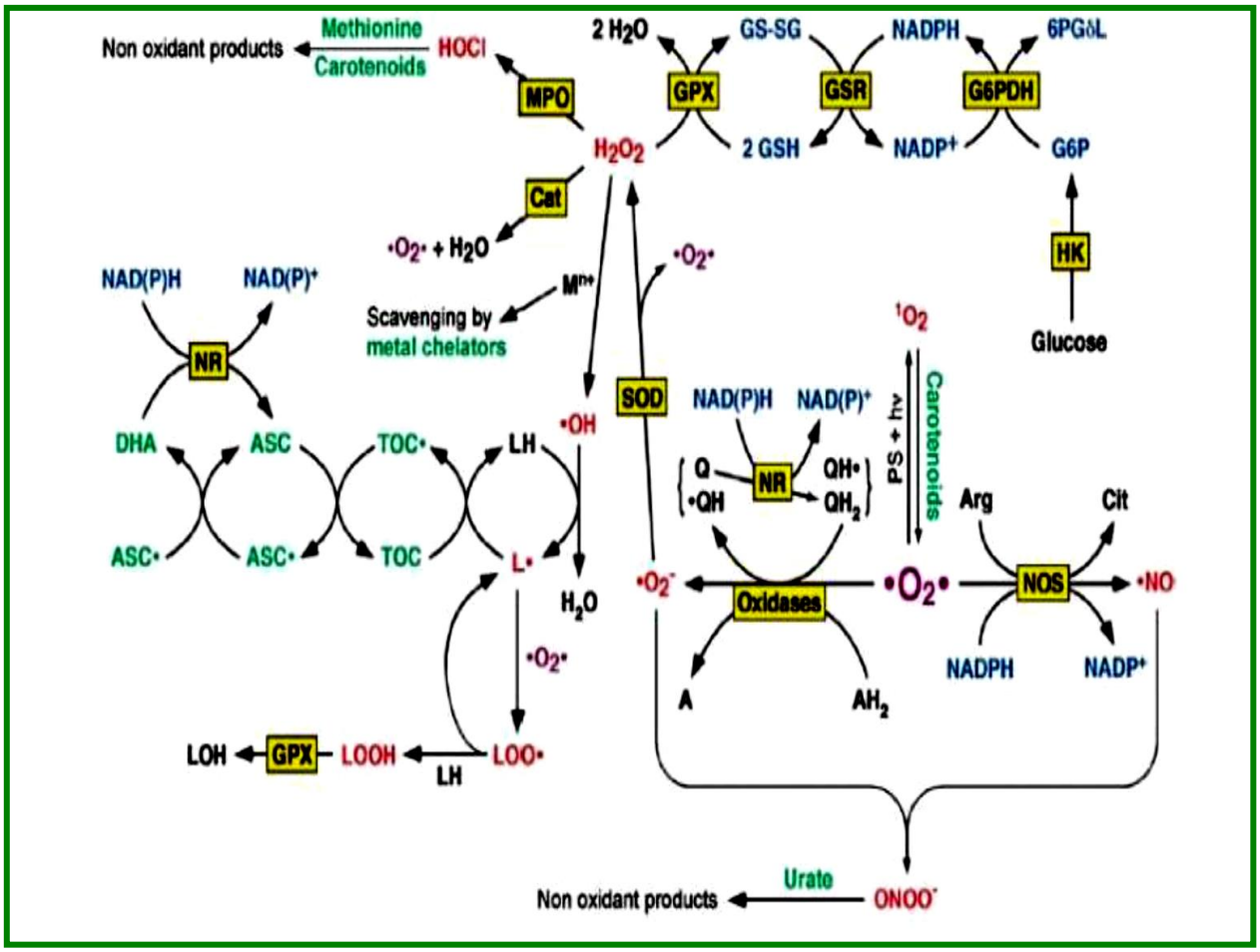


Fig. 2. Overview of free radical and ROS formation and elimination.

Abbreviations: 6PGL- 6 phosphogluconolactone; A, oxidised substrate; AH2, reduced substrate; Arg, arginine; ASC, Ascorbate; ASC*, semidehydroscrbate; Cat, catalase; Cit, Citrulline; DHA, dehydroascorbate; G6-P, glucose 6 phosphate; GSH, reduced glutathione; GSSG, oxidised glutathione; GSR, glutathione reductase; HK, hexokinase; L*, Lipid; LOH, an hydroxylated lipid; LOO*, A lipid peroxy radical; LOOH, A lipid hydroperoxide; MPO, Myeloperoxidase; Ps, Photosensitiser, Q, Ubiquonone; QH*, Ubisemiquonone; Q

The main pathway of free radical and ROS formation starts with the partial reduction of molecular oxygen to superoxide by oxidases or the reduced or semiquonone forms of ubiquonole (coenzyme Q). the hydrogen peroxide produced by dismutation of superoxide can decompose in the presence of transition metals to give rise to hydroxyl radicals, an extremely instable oxidant. The latter can initiate lipid peroxidation in the presence of oxygen. Another strong oxidant

peroxynitrite is formed by reaction between superoxide and nitric oxide, an intracellular messanger formed from arginine, NADPH and oxygen.

Increased hepatic glucose production in type 2 diabetes is attributed to both hepatic insulin resistance and increased glucagon levels (Triplitt et al., 2006). β-cells can compensate for resistance by secreting more insulin. This hyperinsulinemic state is only temporary, as β-cells cannot maintain insulin levels required to maintain euglycemia. This is referred to as the "petering out" effect and occurs due to apoptosis of β-cells. High glucose and free fatty acids contribute to β-cell malfunction, in a condition called glucolipotoxicity. When insulin resistance can no longer be overcome, transition to type 2 DM occurs.

1.6. Biological relevance of markers of oxidative stress and Diabetes

Possible sources of oxidative stress in diabetes include decreased level of antioxidant enzyme activities and increased generation of reactive oxygen species (ROS), which leads to lipid peroxidation and glycation. While, the biochemical significance of oxidative stress has been understood for some time, the estimation of oxidative stress in vivo is quite difficult (Favier, 1997) with no standardization available. The measurement of antioxidant enzymes activities namely superoxide dismutase (SOD), glutathione peroxidase (GPx), and catalase (CAT) and malondialdehyde (MDA), a parameter of lipid peroxidation have been acknowledged as tools for the assessment of oxidative damage in vivo (Westie, 2000; Favier, 1997).

The primary ROS produced in the course of oxygen metabolism is superoxide, which is a highly reactive, cytotoxic ROS. Superoxide is dismutated to a far less reactive product, hydrogen peroxide (H2O2), by a family of metalloenzymes known as SOD (Vaziri et al, 2003). The ubiquitous SODs catalyze the disproportionation of superoxide to molecular oxygen and peroxide and thus are critical for protecting the cell against the toxic products of aerobic respiration.

Thus, SOD is the front line of defence against ROS-mediated injury. GSH is by far the most important antioxidant in most mammalian cells. This ubiquitous tripeptide, γ-Glu-CysGly, performs many cellular functions. In particular, the thiol containing moiety is a potent reducing agent. (Apel & Hirt, 2004) Intracellular GSH is converted to GSSG by selenium-containing GSH peroxidase, which catalyzes the reduction of H2O2 in the presence of GSH and GSH peroxidase

is coupled with oxidation of glucose-6-phosphate and of 6-phosphogluconate, which provides NADPH for reduction of GSSG by GSSG reductase.

This is a major pathway of H2O2 metabolism in many cells. It is thus important for the protection of membrane lipids against oxidation. Intermediates such as O2 and H2O2 are formed extensively in biological systems, and these produce reactive oxygen species that can lead to organic peroxide formation. GSH has the important function of destroying reactive oxygen intermediates and free radicals that are constantly formed in metabolism (Meister, & Anderson, 1983).

Catalase (CAT, H2O2:H2O2 oxidoreductase) is an enzyme that decompose hydrogen peroxide (H2O2) to molecular oxygen (O2) and water (H2O). This activity of catalase is known as catalytic activity. It also exhibits peroxidatic activity and catalyses the oxidation of various hydrogen donors in the presence of relatively lower concentrations of hydrogen peroxide (Cetin et al., 1997).

$2O^-_2+2H^+ \longrightarrow H_2O_2+O_2$

$H_2O_2+GSH \longrightarrow H_2O+GSSG$

GSSG + NAD (P)H $\longrightarrow$ GSH + NAD $(P)^+$

CAT+ H_2O_2 $\longrightarrow$ (CAT- H2O2) complex-1

(CAT+ H_2O_2)+ H_2O_2 $\longrightarrow$ CAT+ 2H2O + O2 (catalytic activity)

(CAT+H_2O_2)+AH_2 $\longrightarrow$ CAT+ $2H_2O$+ A (peroxidative activity)

Cascade of reaction for breakdown of free radical in living organism

1.7. Role of inflammatory cytokine and adiponectin in type-2 diabetes

Insulin affects cells through binding to its receptor on the surface of insulin-responsive cells. The stimulated insulin receptor phosphorylates itself and several substrates, including members of the insulin receptor substrate (IRS) family, thus initiating downstream signaling events (White, 1997; Saltiel & Pessin, 2002). The inhibition of signaling downstream of the insulin receptor is a primary mechanism through which inflammatory signalling leads to insulin resistance. Exposure of cells to TNF-α or elevated levels of free fatty acids stimulates inhibitory phosphorylation of serine residues of IRS-1 (Yin et al., 1998).

This phosphorylation reduces both tyrosine phosphorylation of IRS-1 in response to insulin and the ability of IRS-1 to associate with the insulin receptor and thereby inhibits downstream signalling and insulin action (Hotamisligil et al., 1996). Insulin has a regulatory effect on FFA metabolism. A defect in the ability of insulin to regulate the FFA metabolism could contribute to increase FFA levels (Fig. 3).

The adiponectin secretes a number of peptides named adipocytokines or adipokines. The most abundantly secreted adipokine is adiponectin and its action leads to activation of AMP-activated protein kinase (AMPK) and peroxisome proliferator-activated receptor. These intracellular pathways are involved in fatty-acid oxidation and glucose uptake and suggest a role of adiponectin as an endogenous insulin sensitizer (Yamauchi et al., 2003).

A huge number of studies have appeared on the correlation between adiponectin, type 2 diabetes, coronary artery disease and diet-induced obesity, with conclusion that down-regulation of adiponectin in all these conditions, has been suggested to contribute to the pathogenesis of these diseases. Furthermore, insulin-sensitizing action of adiponectin is through activation of AMPK in the peripheral tissues, which include stimulation of fatty acid oxidation and glucose uptake in skeletal muscle and suppression of glucose production in the liver (Fang & Sweeney, 2006).

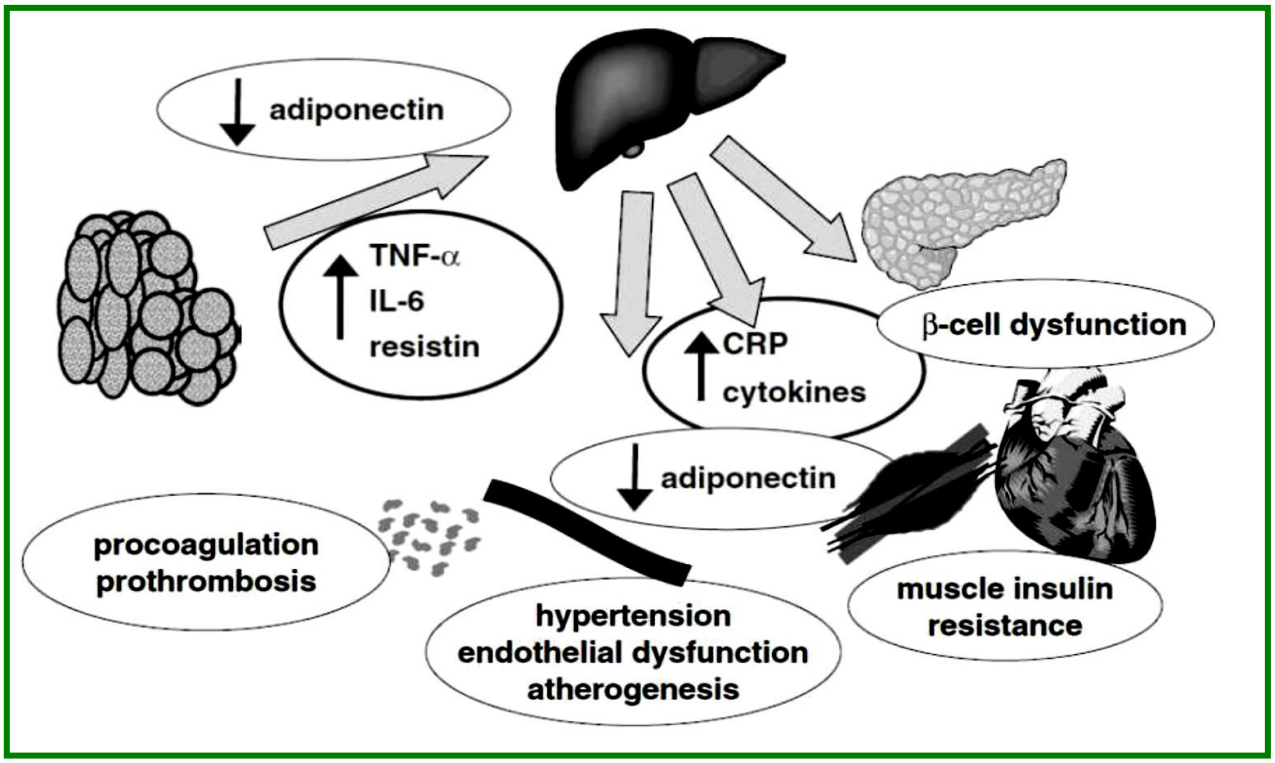


Fig.3. Mechanism for adipocytokine effects on liver, pancreas, heart, muscle arteries and platelets (Fang & Sweeney, 2006)

1.8. Diabetes and glucose transporters

Glucose derived from the diet is transferred from the lumen of the small intestine, and both dietary glucose and glucose synthesised within the body have to be transported from the circulation into target cells. These processes involve the transfer of glucose across plasma membranes and this occurs via integral transport proteins.

- The Na+-dependent glucose co-transporters (SGLT, members of a larger family of Na-dependent transporters, gene name SLC5A) (Wright, 2001).
- The facilitative Na+-independent sugar transporters (GLUT family, gene name SLC2A) (Mueckler, 1994).

The facilitative transporters (GLUT) utilise the diffusion gradient of glucose (and other sugars) across plasma membranes and exhibit different substrate specificities, kinetic properties and tissue expression profiles.

GLUT-1 is expressed particularly in the brain (including the blood–brain barrier) and erthyrocytes. Moderate levels of expression are also observed in adipose tissue, muscle and the liver. GLUT-2 is expressed primarily in pancreatic β-cells, the liver and the kidneys. In the β-cells, GLUT-2 is thought to play a role in the glucose-sensing mechanism, while in the liver it is expressed on the sinusoidal membrane of hepatocytes and allows for the bi-directional transport of glucose under hormonal control.

GLUT-2 is also found on the basolateral surface of proximal renal tubules and enterocytes, where it forms part of the transcellular pathway for glucose and fructose transport. GLUT-3 has a high affinity for glucose and this is consistent with its presence in tissues where the demand for glucose as a fuel is considerable, in particular the brain. The insulin-responsive glucose transporter, GLUT-4, is found in heart, skeletal muscle and adipose tissue, where it is responsible for the reduction in the postprandial rise in plasma glucose levels; it is also found in the brain (Rayner et al., 1994).

Insulin-resistant glucose utilization in peripheral tissues such as muscle and adipose tissues is a universal feature of both insulin-dependent (IDDM) and non-insulin-dependent (NIDDM) diabetes mellitus.

GLUT expression is down regulated when there is relative insulin deficiency, such as in STZ induced diabetes (Charron, 1999).In this process, GLUT, SREBP-1c along with other components plays crucial role. (Charron et al., 1989). SREBP-1c regulates the transcription of genes involved in cholesterol and fatty acid metabolism (Ayala et al., 2009). SREBP-1c expression and nuclear abundance were low in the liver of STZ-induced diabetic rats, and markedly increased after insulin treatment (Shimomura et al., 1999).

1.9. Incretin and incretin effect

Eating provokes the secretion of multiple gastrointestinal hormones involved in the regulation of gut motility, secretion of gastric acid and pancreatic enzymes, gall bladder contraction, and nutrient absorption. Gut hormones also facilitate the disposal of absorbed glucose through the stimulation of insulin secretion from the endocrine pancreas.

The observation that enteral nutrition provided a more potent insulinotropic stimulus compared with isoglycaemic intravenous challenge led to the development of the incretin concept (Elrick et al., 1964). The first incretin to be identified, glucose-dependent insulinotropic polypeptide (GIP), was purified from porcine intestinal extracts and had weak effects on gastric acid secretion. But more potent insulinotropic actions in human beings (Dupré et al., 1973). GIP is a 42-amino acid hormone synthesised in duodenal and jejunal enteroendocrine K cells in the proximal small bowel.

A second incretin hormone, glucagon-like peptide-1 (GLP-1) was identified after the cloning of the cDNAs and genes encoding proglucagon (Fig. 5). GLP-1 exists in two circulating equipotent molecular forms, GLP-1(7-37) and GLP-1(7-36) amide, although GLP-1(7- 36) amide is more abundant in the circulation after eating. Most GLP-1 is made in enteroendocrine L cells in the distal ileum and colon, but plasma levels of GLP-1, like GIP, also increase within minutes of eating. Hence a combination of endocrine and neural signals probably promote the rapid stimulation of GLP-1 secretion well before digested food transits through the gut to directly engage the L cell in the small bowel and colon.

Plasma levels of GLP-1 are low in the fasted state, in the range of 5–10 pmol/L and increase rapidly after eating, reaching 15–50 pmol/L. The circulating levels of intact GLP-1 and GIP decrease rapidly because of enzymatic inactivation, mainly dipeptidyl peptidase-4 (DPP-4), and renal clearance (Orskov et al., 1993). Whether additional proteases, such as human neutral endopeptidase, are also essential determinants of GLP-1 inactivation is being investigated. Both GIP and GLP-1 contain alanine at position 2, and hence are excellent substrates for DPP-4. Indeed, DPP-4 is essential for incretin inactivation, and mice with targeted inactivation of the DPP-4 gene have raised levels of plasma GIP and GLP-1, increased insulin secretion, and reduced glucose excursion after glycaemic challenge (Marguet et al., 2000). As a result of DPP-4 activity, intact, biologically active GLP-1 represents only 10–20% of total plasma GLP-1 (Deacon et al., 1995).

Both GIP and GLP-1 exert their actions by the engagement of structurally distinct G-protein-coupled receptors (GPCRs). The GIP receptor is predominantly expressed on islet β cells, and to a lesser extent, in adipose tissue and in the central nervous system. By contrast, the GLP-1

receptor (GLP-1R) is expressed in islet α and ß cells and in peripheral tissues, including the central and peripheral nervous systems, heart, kidney, lung, gastrointestinal tract (Fig. 5).

Activation of both incretin receptors on β cells leads to rapid increases in levels of cAMP and intracellular calcium, followed by insulin exocytosis, in a glucose-dependent manner (Drucker et al., 1987). More sustained incretin receptor signalling is associated with activation of protein kinase A, induction of gene transcription, enhanced levels of insulin biosynthesis, and stimulation of β-cell proliferation (Drucker., 2006). Both GLP-1R and GIP receptor activation also promote resistance to apoptosis and enhanced β-cell survival, in both rodent (Li et al., 2003) and human islets (Farilla et al., 2003).

Consistent with the distribution of GLP-1R expression, GLP-1 also inhibits glucagons secretion, gastric emptying, and food ingestion, and promotes enhanced glucose disposal through neural mechanisms (Burcelin et al., 2001), actions that also contribute to the control of glucoregulation. Notably, effects on glucagon secretion like those on insulin secretory responses, are glucose- dependent, whereas counter-regulatory release of glucagons in response to hypoglycaemia is fully preserved even in the presence of pharmacological concentrations of GLP-1 (Nauck et al., 2002).

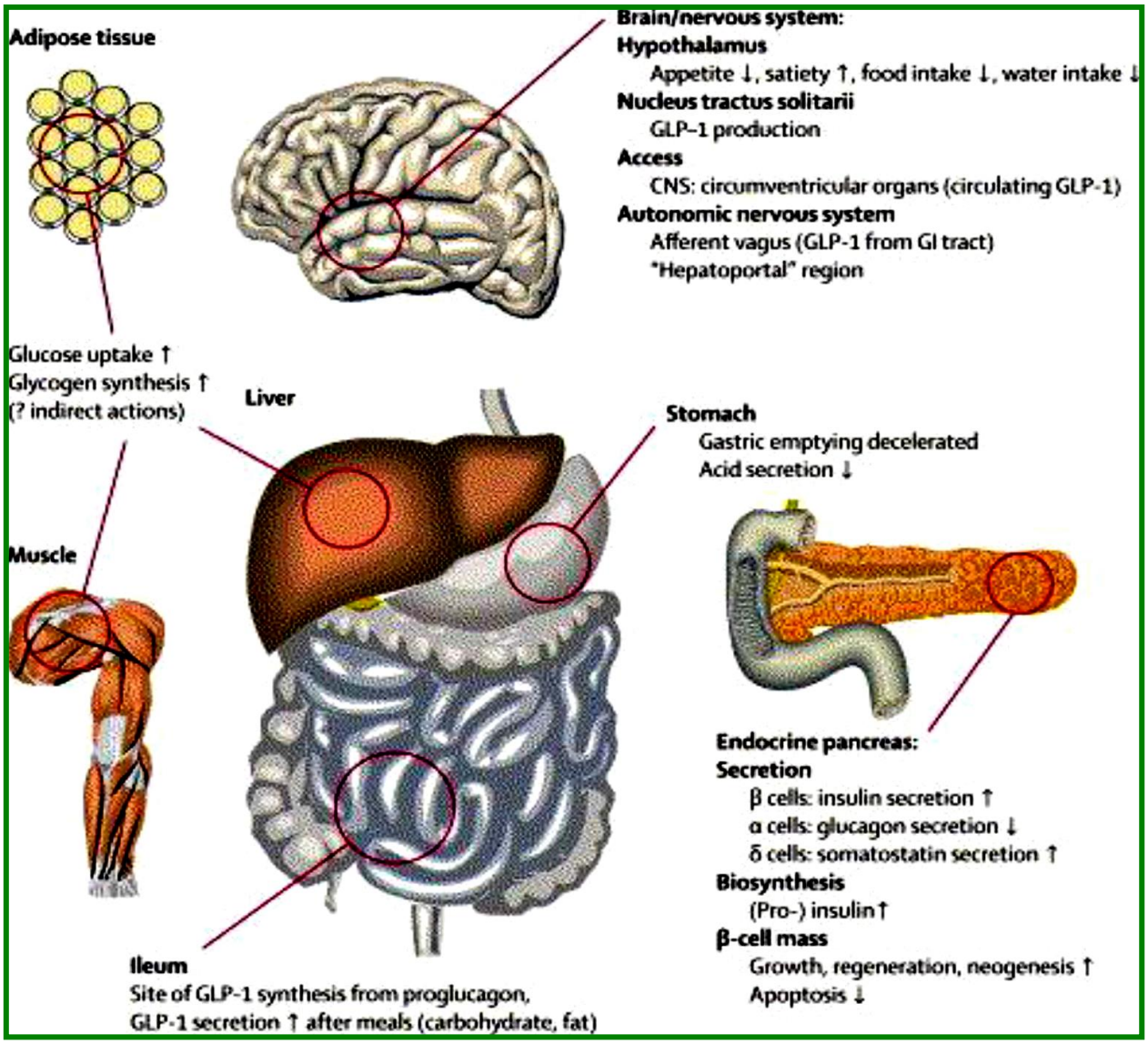


Fig. 4. Physiology of GLP-1 secretion and action on GLP-1 receptors in different organs and tissues (Drucker, 2006)

1.10. Current Oral Hypoglycaemic Agents

The current therapeutic strategies to treat diabetes are aimed at maintaining glycemic control (FPG between 80-120 mg/dl) and glycated haemoglobin (HbA1c) levels at or below 7%. Maintenance of HbA1c levels at or below 7% has been shown to decrease the risk of developing microvascular complications (UK Prospective Diabetes Study Group., 1995 & 1998). When proper diet and exercise fail to attain glycemic control the use of anti-diabetic agents becomes necessary. A variety of oral hypoglycemic agents are currently available and these can be

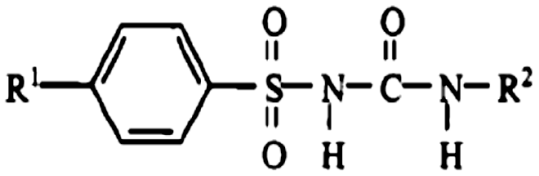
generally classified as (1) insulin secretagogues (2) biguanides (3) insulin sensitizers (4) α-glucosidase inhibitors or (5) dipeptidyl peptidase-IV (DPP-IV) inhibitors.

The insulin secretagogues include the sulfonylureas and meglitinides and both stimulate insulin release from the pancreas by a common mechanism. Sulfonylureas and meglitinides stimulate insulin secretion by binding to the sulfonylurea receptor of ATP -sensitive K+ channel on β-cells. Meglitinides bind to the sulfonylurea receptor, but also bind to an additional site on the β-cell to induce insulin secretion. Because they secrete insulin independent of glucose concentration, hypoglycemia is a serious side effect of sulfonylureas and meglitinides. Another side effect is their tendency to cause weight gain.

This is undesirable especially considering that 80% of diabetics are already overweight. Despite these problems, sulfonylureas are considered a frontline treatment regimen. Meglitinides have similar side effects but they are less pronounced. Some patients do not respond to sulfonylureas while others who have responded may fail to do so after several years. After 10 years of monotherapy with a sulfonylurea, they generally become ineffective and most patients require a second agent to maintain glucose control (Turner et al., 1999). Some examples of sulfonylureas are given in Fig. 6, along with the meglitinide, repaglinide.

Biguanides include metformin and phenformin (Fig. 7). Their mechanism of action is not completely clear, but it is generally believed that they inhibit hepatic glucose production by decreasing gluconeogenesis, stimulating glycolysis and resenzitizing the liver to insulin. They may also resensitize muscle tissue to insulin and decrease intestinal absorption of glucose.

Recently, metformin has been shown to increase levels of GLP-1 (Mannucci et al., 2001), a potent endogenous insulinotropic hormone, in obese non-diabetic patients, but its mechanism of action remains controversial. Metformin is presently a frontline treatment option that may be used alone or in combinations with other agents. A beneficial side effect is that it is associated with weight loss, and this makes it preferable to sulfonylureas to treat severly obese diabetics.



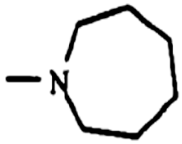
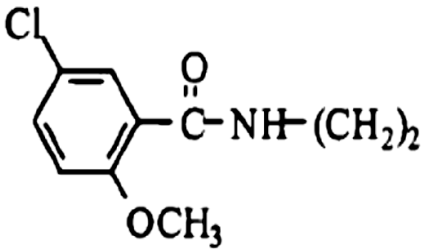
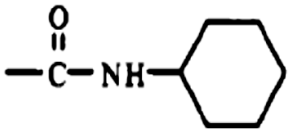
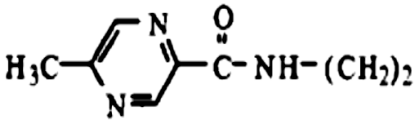
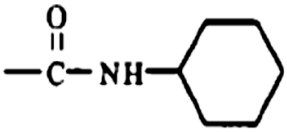
	R^1	R^2
Tolbutamide	CH_3	$CH_3(CH_2)_3$
Tolazamide	CH_3	
Chlorpropamide	Cl	$CH_3(CH_2)_2$
Glybenclamide		
Glipizide		

Fig. 5. Structure of sulfonylureas and a meglitinide.

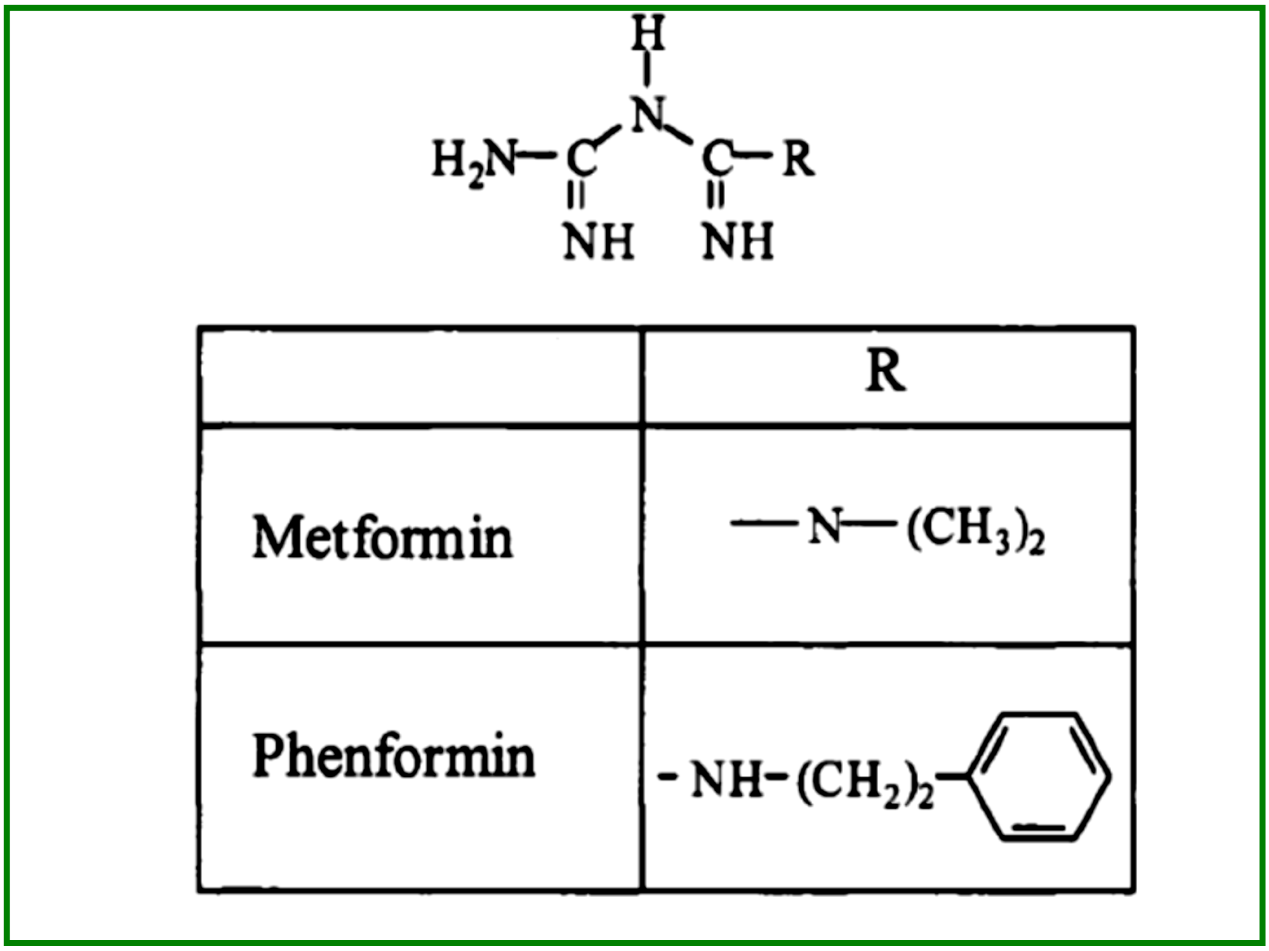


Fig.6. Structure of Biguanides

Insulin sensitizers include ligands for the peroxisome-proliferator activated receptor ɤ (PPAR- ɤ) such as thiazolidinediones (TZD). These drugs enhance insulin sensitivity in adipose, muscle and liver by stimulating the nuclear PPAR-ɤ receptor, which (1) upregulates proteins required for metabolism of glucose and lipids and (2) activates the glucose transporter gene (GLUT-4) in muscle and adipose tissue. Thiazolidinediones reduce hyperglycemia by increasing cellular glucose consumption, glucose uptake and sensitivity in muscle and adipose tissue.

They do not affect insulin levels. PPAR-ɤ agonists also promote adipocyte differentiation and as a result can cause weight gain as a side effect. To counteract this, dual PPAR-α/ɤ agonists are being sought. PPAR-ɤ agonists such as fibrates lower lipid triglycerides and raise high density lipoprotein cholesterol (HDLc). They are used to treat hyperlipidemia and are capable of

inducing weight loss. The structure of two thiazolidinediones, pioglitazone and rosiglitazone, are shown in Fig. 8.

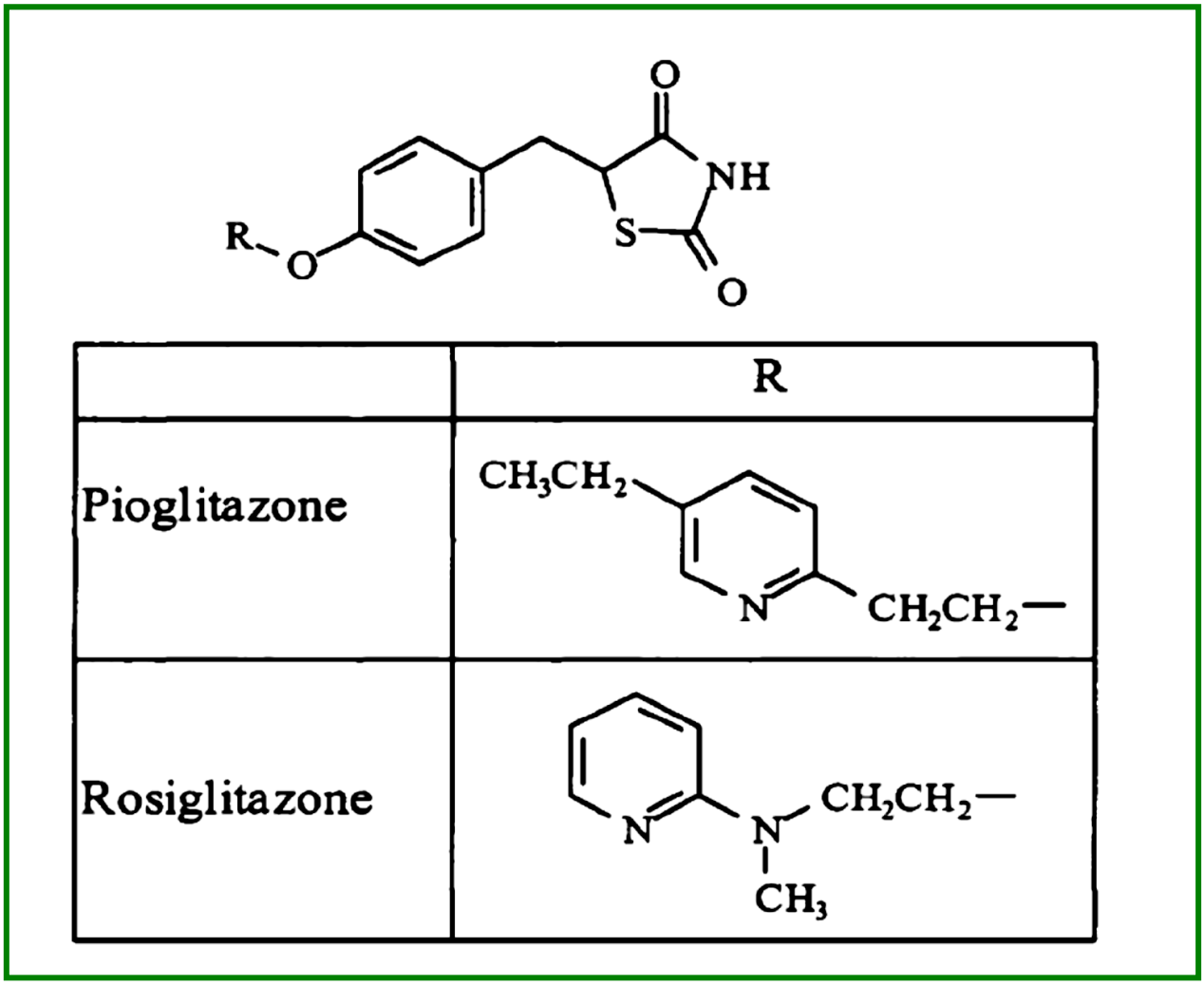


Fig. 7. Structure of thiazolidinediones, rosiglitazone and pioglitazone

One of the therapeutic approaches for reducing postprandial hyperglycemia in patients with DM is to prevent absorption of carbohydrates after food uptake. Only monosaccharides, such as glucose and fructose, can be transported out of the intestinal lumen into the bloodstream. Complex starches, oligosaccharides, and disaccharides must be broken down into individual monosaccharides before being absorbed in the duodenum and upper jejunum. This digestion is facilitated by enteric enzymes, including pancreatic α-amylase, and α-glucosidases that are attached to the brush border of the intestinal cells. α-glucosidase inhibitors delay absorption of

complex carbohydrates and thus inhibit postprandial glucose peaks thereby leading to decreased postprandial insulin levels.

Currently, four α-glucosidase inhibitors exist: acarbose, miglitol, voglibose and emiglitate (Fig. 9). Of these, acarbose is by far the most prescribed drug. In most guidelines it is not a drug of first choice but used as an addition to other drugs for type 2 diabetes when treatment goals are not met, or in case of contra-indications for other medications (EDPG, 1999). They have only modest antidiabetic activity by themselves and are usually used in combination therapy. Side effects include GI disturbances such as flatulence, diarrhea and abdominal pain.

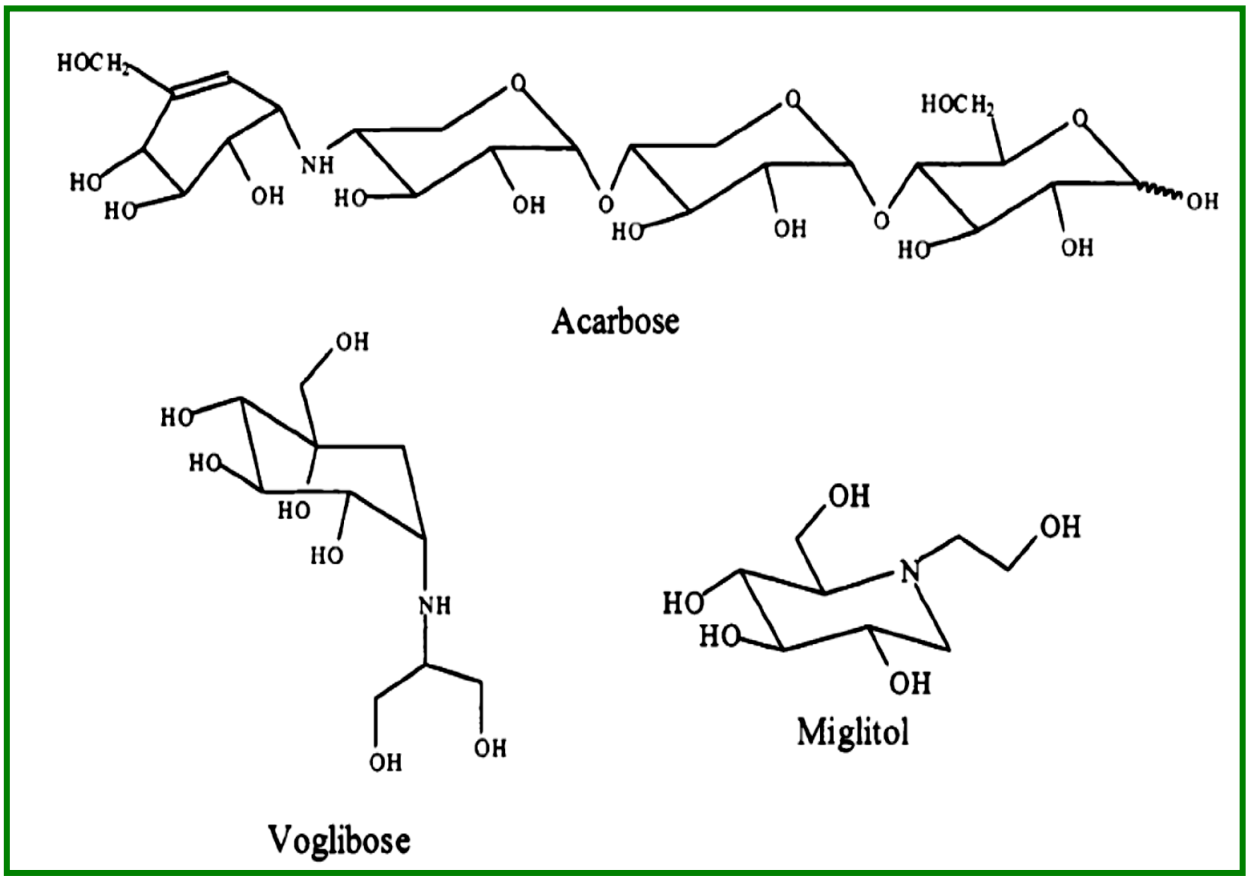


Fig.8. Structures of α-glucosidase inhibitors

Dipeptidyl-peptidase IV (DPP-4) is a ubiquitous enzyme that can be detected in the endothelium of different organs and that is measurable as circulating enzymatic activity in plasma. The incretins, namely glucagon-like peptide-1 (GLP-1) and glucose-dependent insulinotropic peptide (GIP), are the only substrates of DPP-4 that have been well validated in

humans. DPP-4 has also been implicated in the regulation of several additional peptides, such as pituitary adenylate cyclase-activating polypeptide (PACAP) and gastrin-releasing peptide (GRP); however, in humans, these peptides have not been definitively shown to be relevant in vivo substrates for this enzyme (Mest & Mentlein, 2005) DPP-4 cleaves and inactivates GLP-1 within a few minutes (Mentlein, 1999).

Combination therapy is an option when one drug is no longer particularly effective. After 3 years monotherapy with either a sulfonylurea or metformin, approximately 50% of patients have HbA1c above 7% and after 9 years this number increases to approximately 75% (Turner et al., 1999). In this case, a second agent of a different class is usually added to the regimen to restore glycemic control through an additive or synergistic effect.

The most common combination is metformin with a sulfonylurea. Other useful combinations include metformin and a TZD, metformin with a meglitinide, or an α-glucosidase inhibitor with either metformin or a sulfonylurea. In the case when two agents are no longer effective a third agent of another class might also be added (i.e. TZD to a combination of metformin and a sulfonylurea).

Finally, when oral hypoglycemic therapy has failed to achieve therapeutic goals in type 2 diabetes, subcutaneous insulin injections are required to prevent hyperglycemia. These hypoglycemic agents are useful in limiting hyperglycemia, but they do not address the associated dyslipidemia and atherosclerotic cardiovascular disease, nor do they alter the natural progression of the disease. Therapies which can increase or even preserve β-cell mass would represent a major advance. While a cure is not currently available, research has led to a greater understanding of the etiology of the disease and has resulted in the emergence of novel targets that are being exploited for possible use. GLP-1 based therapy represents such a target and has already been successful.

As per ancient literature, more than 800 plants are reported to have antidiabetic properties (Eddouks et al. 2004). Ethnopharmacological surveys indicate that more than 1200 plants are used in traditional medicine for their alleged hypoglycemic activity (Kesari et al. 2007). Medicinal plants, since times immemorial, have been used in virtually all cultures as a source of medicine. A study of ancient literature indicates that diabetes was fairly well known and well

conceived as an entity in ancient India. The knowledge of the system of diabetes mellitus, as the history reveals, existed with the Indians since prehistoric age.

Its earliest reference (1000 BC in the Ayurvedic literature) is found in mythological form where it is said to have originated by eating Havisha, (Latha & Pari, 2003) a special food, which used to be offered at the times of yagna organized by Dakshaprajapati. Ayurvedic antidiabetic herbs improve digestive power, increase one of the Rasas (gastric secretions); being Laghu, get easily digested in the body; and being Ruksha, decrease output of overall body fluids e.g. urine, sweat etc. Food items, which are 'Madhumehaghna' (antidote), are an important underlying principle of therapy for the prameha (diabetes) patient.

Food items which correct the metabolic imbalance by their action e.g. foods exhibiting 'rasa', 'katu', 'laghu', 'medaghna', properties are old cereals, roasted cereals, barley, jawar, ragi, mung dal, horsegram, tur dal, drumstick leaves, bittergourd, jamun, amla, fig, raw papaya, milk, meat of animals that live in dry region, etc. The indigenous diet may not be useful in lowering the blood sugar to the same extent as insulin and other hypoglycaemic agents do. But it has some other influences, which may be useful for the management of the disease and its complications (Subbulakshmi & Naik 2001). Indian materia medica has mentioned numerous dravyas, which have been reported effective in 'Madhumeha' (Sabu & Subburaju 2002).

Plants-based products have been popular all over the world for the centuries. In diabetes, some herbal alternatives are proven to provide symptomatic relief and assist in the prevention of the secondary complications of the disease. Some herbs have also been proven to help in the regeneration of ß-cells and in overcoming resistance.

In addition to maintaining normal blood sugar level, some herbs are also reported to possess antioxidant activity and cholesterol-lowering action. The management of type 2 diabetes mellitus (NIDDM) is possible with the drugs that can lower the blood sugar level in one hand and restore the liver glycogen level on the other. In modern system of medicine, there is no drug, which is reported to posses both of these properties (Shrabana et al. 2003).

However, the hypoglycemic effect of some herbal extracts have been confirmed in human and animal models of type 2 diabetes and conventional drugs have been derived from the active

molecules of these medicinal plants. Metformin, a less toxic biguanides and potent oral glucose-lowering agent, was developed from Galega officianalis and used to treat diabetes (Daniel & Norman, 2001). Out of dozens of oral medications for diabetes, only one medication (metformin) is approved for use in children and it has been originated from herbs (Michael et al. 2005).

Regardless of the type of diabetes, patients are required to control their blood glucose with medications and/or by adhering to an exercise program and a dietary plan. Insulin therapy by injection is given to those with type 1 DM and also to some patients with type 2 DM when oral hypoglycaemic drugs fail to lower blood glucose (Alam & Mahpara 2003). Due to modernization of lifestyle, non-insulin dependent diabetes mellitus is becoming a major health problem in developing countries.

Patients with type 2 DM are usually placed on a restricted diet and are instructed to exercise, the purpose of which primarily is weight control. If diet and exercise fail to control blood glucose at the desired level, oral antidiabetic medication is prescribed (Derek 2001). Oral antidiabetic agents exert their effects by various mechanisms:

(1) Stimulation of β-cells in the pancreas to produce more insulin (sulfonylureas, and meglitinides).
(2) Increasing the sensitivity of muscles and other tissues to insulin (thiazolidinediones).
(3) Decreasing gluconeogenesis by the liver (biguanides).
(4) Delaying the absorption of carbohydrates from the gastrointestinal tract (α-glucosidase inhibitors). These treatments have their own drawbacks, ranging from the developing of resistance and adverse effects to lack of responsiveness in large segment of patients population.

Sulfonylureas lose effectiveness for 44% of patients within six years. Also, these treatments are associated with side effects or even toxic effects (e.g., thiazolidinediones may cause liver toxicity; sulphonylureas might worsen heart disease, lower the glucose below the normal range and increase the body weight gain; bloating, flatulence, diarrhea and abdominal discomfort and pain are the major complaints with glucosidase inhibitors) (Dey et al. 2002 & Michael et al. 2005). According to literature, two-thirds of medications prescribed for use in children have not been proven safe or effective for this patient population (Michael et al. 2005). Moreover, none of

these glucose-lowering agents adequately controls the hyperlipidemia that frequently met with the disease.

The limitations of currently-available oral antidiabetic agents either in terms of efficacy/safety coupled with the emergence of the disease into a global epidemy have encouraged a concerted effort to discover drugs that can manage type 2 diabetes more efficiently (Ranjan & Ramanujam, 2002). Also, with increasing incidence of diabetes mellitus in rural population throughout the world and due to adverse effects of synthetic medicine, there is a clear need for development of indigenous, inexpensive botanical sources for anti-diabetic crude or purified drugs.

1.11. Mechanism of action of herbal antidiabetics

The antidiabetic activity of herbs depends upon variety of mechanisms. The mechanism of action of herbal anti-diabetic could be grouped as:

1. Adrenomimeticism, pancreatic β-cell potassium channel blocking, cAMP (2nd messenger) stimulation (Marles & Farnsworth 1996)
2. Inhibition in renal glucose reabsorption (Eddouks et al. 2002)
3. Stimulation of insulin secretion from β-cells of islets or/and inhibition of insulin degradative processes (Pulok et al. 2006)
4. Reduction in insulin resistance (Pulok et al. 2006)
5. Providing certain necessary elements like calcium, zinc, magnesium, manganese and copper for the β-cells (Mohamed et al. 2006)
6. Regenerating and/or repairing pancreatic β-cells (Mohamed et al. 2006)
7. Increasing the size and number of cells in the islets of Langerhans (Mohamed et al. 2006)
8. Stimulation of insulin secretion (Esmaeili & Yazdanparast 2004)
9. Stimulation of glycogenesis and hepatic glycolysis (Miura et al. 2001)
10. Protective effect on the destruction of the β-cells (Kim et al. 2003)
11. Improvement in digestion along with reduction in blood sugar and urea (Krishnan 1968)
12. Prevention of pathological conversion of starch to glucose (Sepha & Bose 1956)
13. Inhibition of β -galactocidase and α–glucocidase (Sharma & Mujumdar 1990)
14. Cortisol lowering activities (Gholap & Kar 2004)

15. Inhibition of α-amylase (Heidari et al. 2005)

16. Preventing oxidative stress that is possibly involved in pancreatic ß-cell dysfunction found in diabetes (Hideaki et al. 2005).

Herbal products are often perceived as safe because they are "natural". In India, in recent years, there is increased research on traditional ayurvedic herbal medicines on the basis of their known effectiveness in the treatment of ailments for which they have been traditionally applied. Hence, the wide range of plant constituents could have different sites of action within the body, herbs exerts different mechanism of actions including the mechanism of actions of synthetic oral hypoglycaemic drugs. Fagonia is a genus of flowering plants in the caltrop family, Zygophyllaceae. The roughly 18 species it contains are commonly known as fagonbushes. Their distribution includes parts of Africa, the Mediterranean Basin, and parts of the Americas.

1.12. Classification of *Fagonia Arabica*(L.)*:*

Kingdom	:	Plantae
Order	:	Zygophyllales
Family	:	Zygophyllaceae
Subfamily	:	Zygophylloideae
Genus	:	Fagonia L.
Species	:	Arabica

Fig.9. Surface view of *Fagonia Arabica* (L.)

Morphological characters of *Fagonia arabica* (Veena S. Kasture et al, 2014):

S. No	Morphological Characters	Plant name: *Fagonia arabica L.*
1	Color	Dark green
2	Length of the plant as whole	20-55cm
3	Surface	Glandular, pubescent, often with adhering sand grains
4	Stems	Erect, branched, woody, solid, glandular, striate; nodes swollen, whitish green; internodes long (1.6 – 3 cm)
5	Leaflets margin	Entire
6	Flowers	1.3- 1.5 cm diam. At anthesis, eracteate, pedicellate (0.5-0.6 cm long), complete,regular, perfect, actinomorphic, bisexual, hypogenous, pentamerous
7	Color of Fruits	green
8	Seeds	Ovate, compressed flat, with mucilaginous coat

Vernacular names of *F. arabica*

Sanskrit	:	Dhhamaasaa, Dhanvayasaa, Duraalabhaa, Samudraantaa, Gandhaari, Kachhuraa, Anantaa
Arabic name	:	Shokat-Ah-E-Albeefa
Hindi	:	Ustarkhar
Marathi	:	Dhamaasaa
Oriya	:	Dusparsa
Persian	:	Badavard
Telugu	:	Chitikara, Cittigara
Urdu	:	Badawarde, Badawand, Badaward
Unani	:	Dhamaasaa

1.13. Phyto-constituents of *F. arabica*: (Veena S. Kasture et al, 2014)

Phytochemical investigation of different extracts of *F. arabica* revealed the presence of carbohydrates, flavonoid, glycosides, steroids, saponins, alkaloids, triterpenoidal glycosides, amino acids, Chlorides, Sulphates, Anthraquinones, Irodoids, Cyanogenic glycosides and Coumarin.

a. Stigmasterol

PubChem CID : 5280794

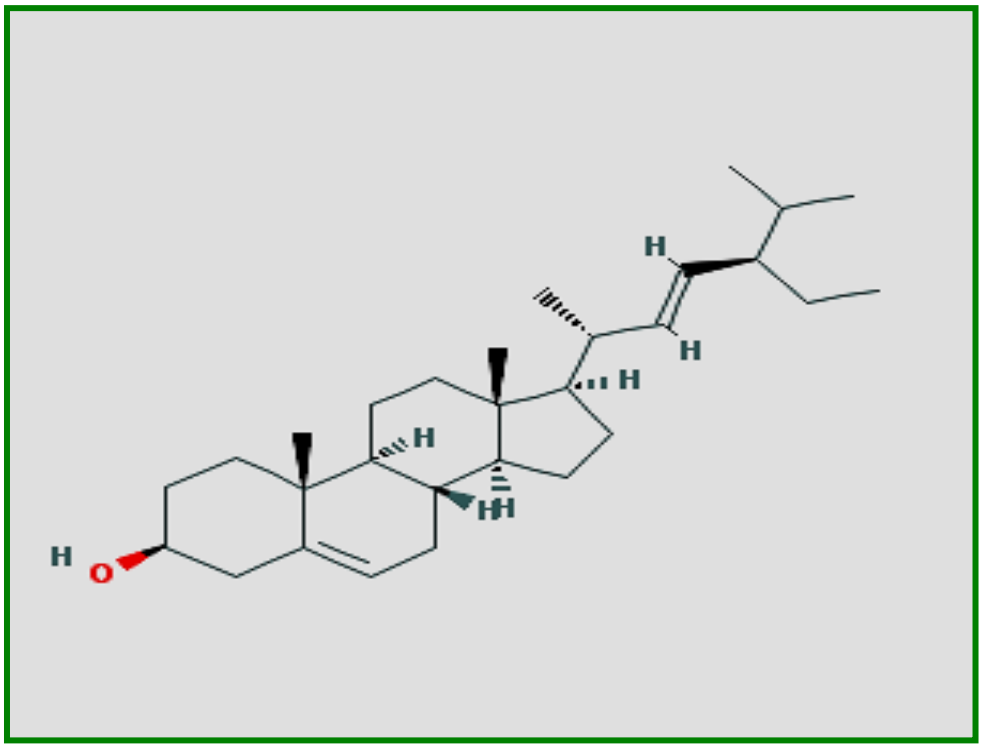
Chemical Names : Stigmasterin; STIGMASTEROL; beta-Stigmasterol; (24S)- 5,22-Stigmastadien-3beta-ol; Stigmasta-5, 22-dien-3-beta-ol; Stigmasta-5, 22-dien-3-ol, etc.

Molecular Formula : C29H48O

Molecular Weight : 412.69082 g/mol

InChI Key : HCXVJBMSMIARIN-PHZDYDNGSA-N

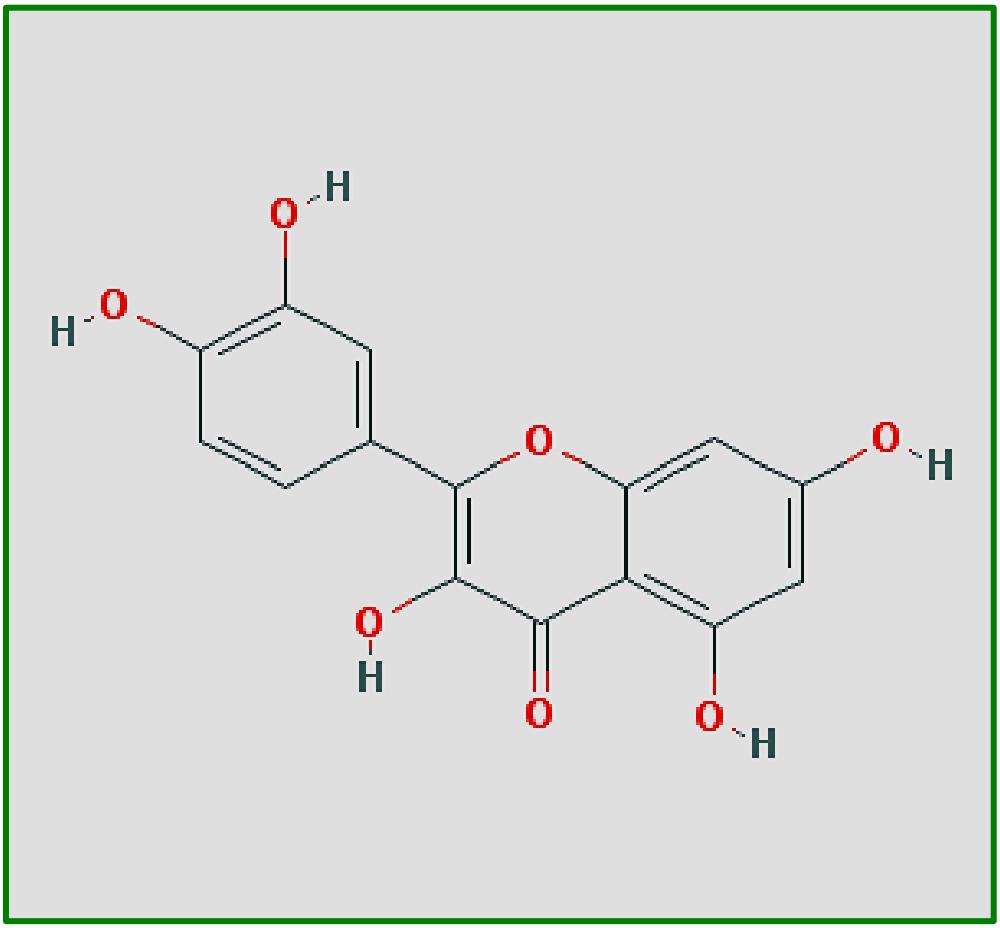
UNII : 99WUK5D0Y8



Stigmasterol

b. Quercetin:

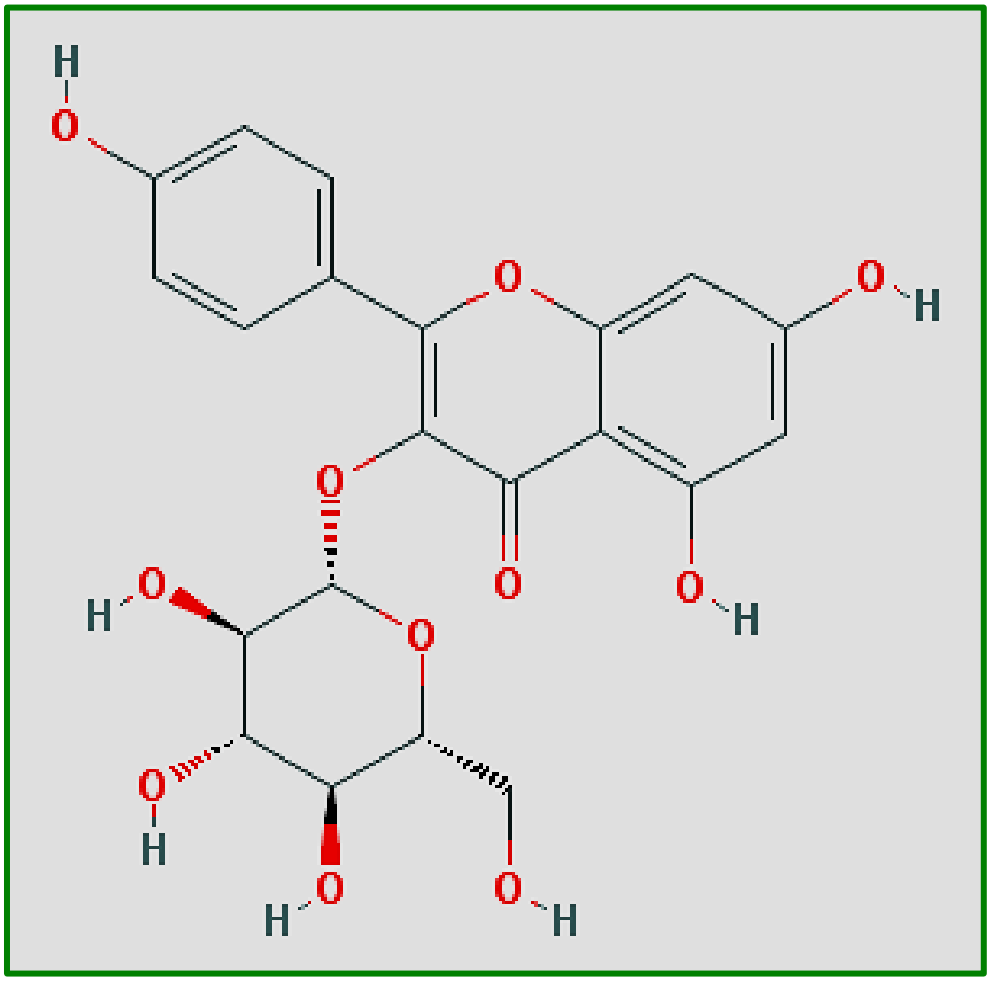
PubChem CID	:	5280343
Chemical Names	:	quercetin; Sophoretin; Xanthaurine; Meletin; 117-39-5; Quercetine; etc.
Molecular Formula	:	$C_{15}H_{10}O_7$
Molecular Weight	:	302.2357 g/mol
InChI Key	:	REFJWTPEDVJJIY-UHFFFAOYSA-N
UNII	:	9IKM0I5T1E



Quercetin

c. Kaempferol

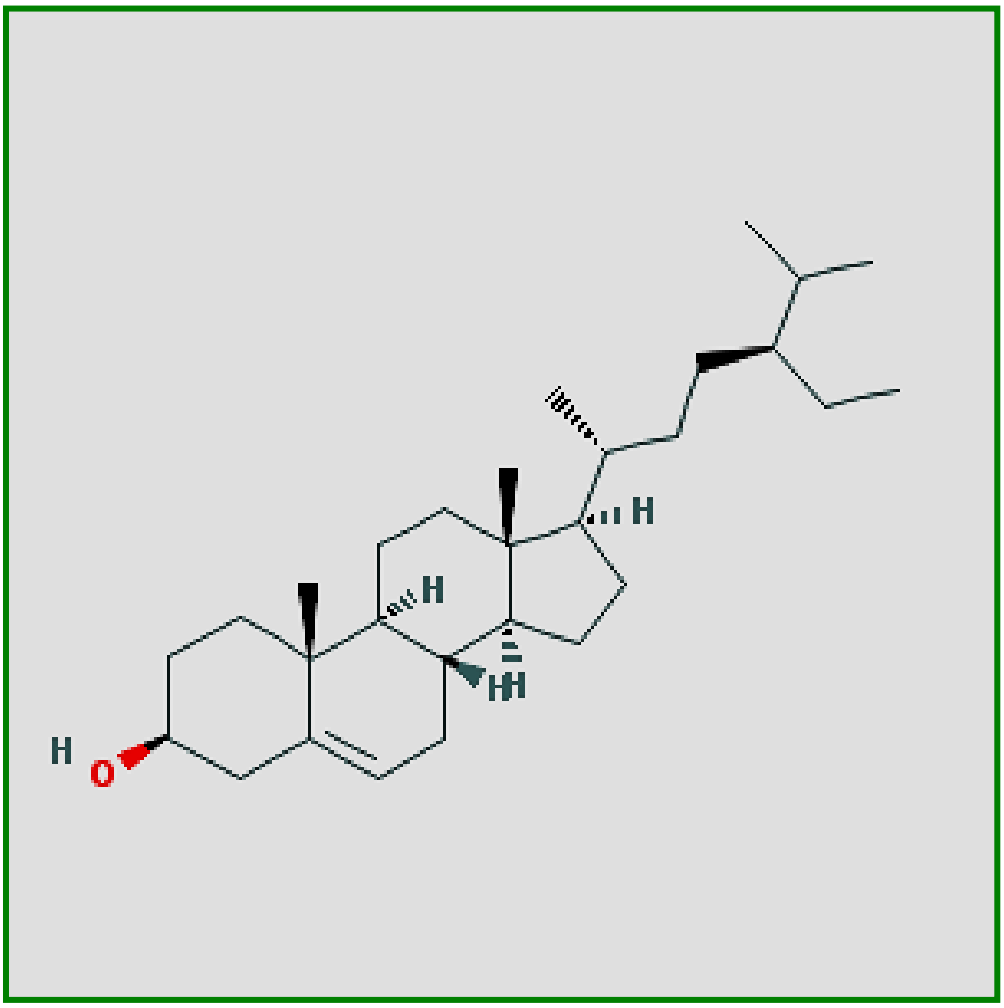
PubChem CID	:	5282102
Chemical Names	:	Astragalin; Astragaline; Kaempferol 3-O-glucoside; asragalin; kaempferol-3-glucoside; 480-10-4; etc.
Molecular Formula	:	C21H20O11
Molecular Weight	:	448.3769 g/mol
InChI Key	:	JPUKWEQWGBDDQB-QSOFNFLRSA-N
UNII	:	APM8UQ3Z9O



Kaempferol

d. β-Sitosterol

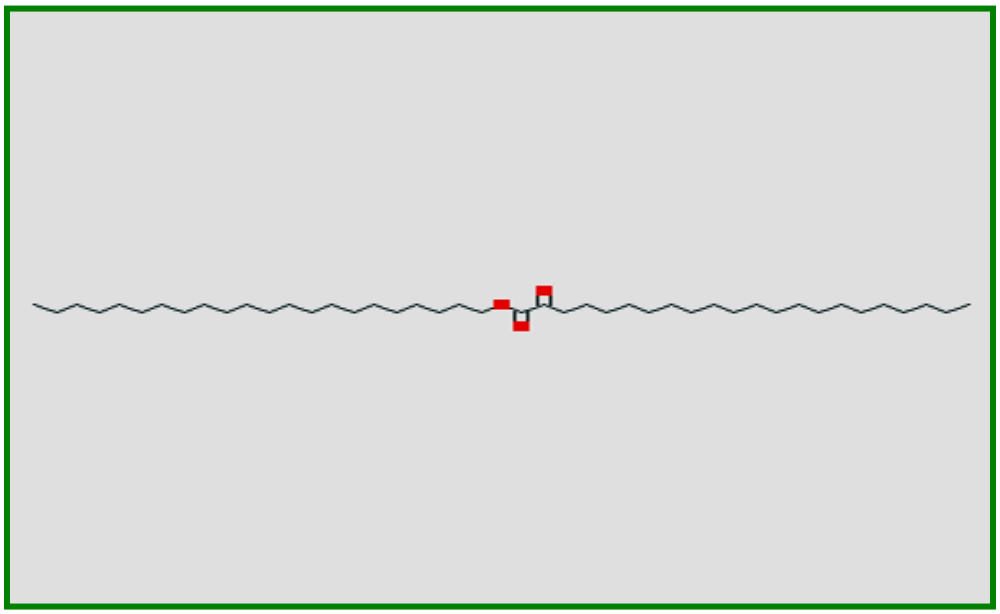
PubChem CID	:	222284
Chemical Names	:	BETA-SITOSTEROL; Sitosterol; 22,23-Dihydrostigmasterol; Quebrachol; Cinchol; Cupreol; etc.
Molecular Formula	:	C29H50O
Molecular Weight	:	414.7067 g/mol
InChI Key	:	KZJWDPNRJALLNS-VJSFXXLFSA-N
UNII	:	S347WMO6M4



β-Sitosterol

e. Docosyl Docosonate

PubChem CID	:	91574620
Molecular Formula	:	$C_{44}H_{86}O_3$
Molecular Weight	:	663.15184 g/mol
InChI Key	:	QTTGBVCDUJWMJD-UHFFFAOYSA-N



Docosyl Docosonate

f. Ascorbic acid:

PubChem CID : 54670067

Chemical Names : ascorbic acid; vitamin C; l-ascorbic acid; ascorbate; Ascoltin;

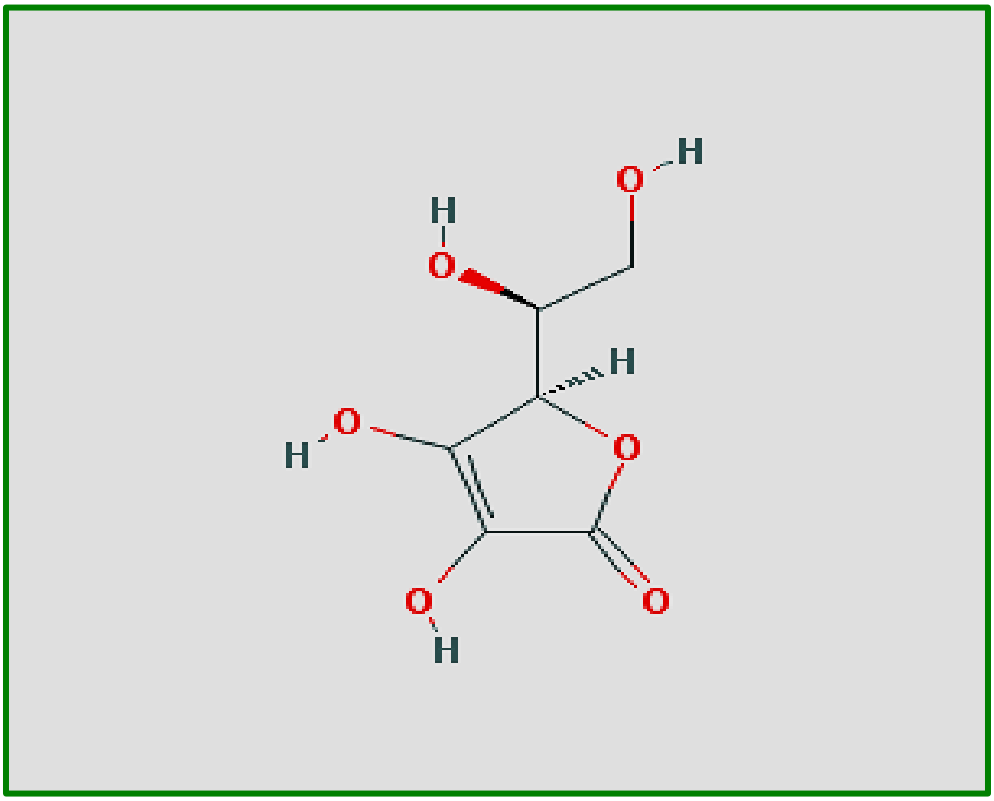
L(+)-Ascorbic acid; etc.

Molecular Formula : C6H8O6

Molecular Weight : 176.12412 g/mol

InChI Key : CIWBSHSKHKDKBQ-JLAZNSOCSA-N

UNII : PQ6CK8PD0R



Ascorbic acid

Because of the anti-oxidant and hypo lipidemic activity of the *F.arabica*, we have selected to know the anti-diabetic property of the plant. There is no information available on the anti-diabetic activity of *F.arabica.* Hence the present study is designed to evaluate the anti-diabetic property of the *F.arabica* in Alloxan monohydrate induced diabetes in albino wistar rats.

CHAPTER-I AIM and OBJECTIVES

Diabetes is the leading cause of mortality in many parts of the world. Although modern drugs are effective in preventing diabetes, their use is often limited because of their side effects. The present work is designed to find out phyto-medicines which are useful in the treatment of diabetes. This is an attempt to investigate the anti-diabetic effect of *Fagonia arabica* (L.) using rat models, in which diabetes was induced by using Alloxan monohydrate (10mg/kgbw).

- To prepare aqueous extract of *Fagonia arabica* (L.).
- To screen the extract for anti-diabet0ic activity and dose fixation.
- To induce diabetes by Alloxan monohydrate 150 mg/kgbw by I.P. Injection.
- Analysis of Liver marker enzymes ALT, AST and ALP in serum.
- Analysis of serum lipid profile such as TC, TG, LDL and HDL.
- Histopathological studies of Liver tissues.

CHAPTER-TWO

CHAPTER-II REVIEW OF LITERATURE

The term diabetes mellitus describes a metabolic disorder of multiple aetiology characterized by chronic hyperglycaemia with disturbances of carbohydrate, fat and protein metabolism resulting from defects in insulin secretion, insulin action, or both. The effects of diabetes mellitus include long-term damage, dysfunction and failure of various organs. Diabetes mellitus may present with characteristic symptoms such as thirst, polyuria, blurring of vision, and weight loss. In its most severe forms, ketoacidosis or a non-ketotic hyperosmolar state may develop and lead to stupor, coma and, in absence of effective treatment, death.

Often symptoms are not severe, or may be absent, and consequently hyperglycaemia sufficient to cause pathological and functional changes may be present for a long time before the diagnosis is made. The long-term effects of diabetes mellitus include progressive development of the specific complications of retinopathy with potential blindness, nephropathy that may lead to renal failure, and/or neuropathy with risk of foot ulcers, amputation, Charcot joints, and features of autonomic dysfunction, including sexual dysfunction. People with diabetes are at increased risk of cardiovascular, peripheral vascular and cerebrovascular disease. Several pathogenetic processes are involved in the development of diabetes. These include processes which destroy the beta cells of the pancreas with consequent insulin deficiency, and others that result in resistance to insulin action. The abnormalities of carbohydrate, fat and protein metabolism are due to deficient action of insulin on target tissues resulting from insensitivity or lack of insulin. (W.H.O., Diabetes online, 2015)

Diabetes-specific microvascular disease is a leading cause of blindness, renal failure and nerve damage, and diabetes-accelerated atherosclerosis leads to increased risk of myocardial infarction, stroke and limb amputation. Four main molecular mechanisms have been implicated in glucose-mediated vascular damage. All seem to reflect a single hyperglycaemia-induced process of overproduction of superoxide by the mitochondrial electron-transport chain. This integrating paradigm provides a new conceptual framework for future research and drug discovery.(Michael Brownlee, 2001).

Intensive glycemic control could decrease the frequency or severity of diabetic microvascular complications, we performed a prospective study of Japanese patients with non-insulin-dependent diabetes mellitus (NIDDM) treated with multiple insulin injection treatment. A total of 110 patients with NIDDM was randomly assigned to multiple insulin injection treatment group (MIT group) or to conventional insulin injection treatment group (CIT group). Fifty-five NIDDM patients who showed no retinopathy and urinary albumin excretions <30 mg/24 h at the baseline were evaluated in the primary-prevention cohort, and the other 55 NIDDM patients who showed simple retinopathy and urinary albumin excretions <300 mg/24 h were evaluated in the secondary-intervention cohort.

The appearance and the progression of retinopathy, nephropathy and neuropathy were evaluated every 6 months over a 6-year period. The worsening of complications in this study was defined as an increase of 2 or more steps in the 19 stages of the modified ETDRS interim scale for retinopathy and an increase of one or more steps in 3 stages (normoalbuminuria, microalbuminuria and albuminuria) for nephropathy. The cumulative percentages of the development and the progression in retinopathy after 6 years were 7.7% for the MIT group and 32.0% for the CIT group in the primary-prevention cohort (P = 0.039), and 19.2% for MIT group and 44.0% for CIT group in the secondary-intervention cohort (P = 0.049).

The cumulative percentages of the development and the progression in nephropathy after 6 years were 7.7% for the MIT group and 28.0% for the CIT group in the primary-prevention cohort (P = 0.032), and 11.5% and 32.0%, respectively, for the MIT and CIT groups in the secondary-intervention cohort (P = 0.044). In neurological tests after 6 years, MIT group showed significant improvement in the nerve conduction velocities, while the CIT group showed significant deterioration in the median nerve conduction velocities and vibration threshold. Although both postural hypotension and the coefficient of variation of R-R interval tended to improve in the MIT group, they deteriorated in the CIT group.

In conclusion, intensive glycemic control by multiple insulin injection therapy can delay the onset and the progression of diabetic retinopathy, nephropathy and neuropathy in Japanese patients with NIDDM. From this study, the glycemic threshold to prevent the onset and the

progression of diabetic microangiopathy is indicated as follows; HbA1c < 6.5%, FBG < 110 mg/dl, and 2-h post-prandial blood glucose concentration <180 mg/dl. (Yasuo Ohkubo, 1995).

Type 2 diabetes is characterized by impaired insulin secretion. Some but not all studies suggest that a decrease in β-cell mass contributes to this. We examined pancreatic tissue from 124 autopsies: 91 obese cases (BMI >27 kg/m2; 41 with type 2 diabetes, 15 with impaired fasting glucose [IFG], and 35 nondiabetic subjects) and 33 lean cases (BMI <25 kg/m2; 16 type 2 diabetic and 17 nondiabetic subjects). We measured relative β-cell volume, frequency of β-cell apoptosis and replication, and new islet formation from exocrine ducts (neogenesis).

Relative β-cell volume was increased in obese versus lean nondiabetic cases ($P = 0.05$) through the mechanism of increased neogenesis ($P < 0.05$). Obese humans with IFG and type 2 diabetes had a 40% ($P < 0.05$) and 63% ($P < 0.01$) deficit and lean cases of type 2 diabetes had a 41% deficit ($P < 0.05$) in relative β-cell volume compared with nondiabetic obese and lean cases, respectively. The frequency of β-cell replication was very low in all cases and no different among groups.

Neogenesis, while increased with obesity, was comparable in obese type 2 diabetic, IFG, or nondiabetic subjects and in lean type 2 diabetic or nondiabetic subjects. However, the frequency of β-cell apoptosis was increased 10-fold in lean and 3-fold in obese cases of type 2 diabetes compared with their respective nondiabetic control group ($P < 0.05$). We conclude that β-cell mass is decreased in type 2 diabetes and that the mechanism underlying this is increased β-cell apoptosis. Since the major defect leading to a decrease in β-cell mass in type 2 diabetes is increased apoptosis, while new islet formation and β-cell replication are normal, therapeutic approaches designed to arrest apoptosis could be a significant new development in the management of type 2 diabetes, because this approach might actually reverse the disease to a degree rather than just palliate glycemia. (Alexandra E. Butler et al, 2003)

Quantitative study of insular tissue has revealed that the number of B cells is greatly diminished in Patients with acute juvenile diabetes from the time of clinical onset of the disease. The number of these cells is as a rule less than 10 per cent of normal. Such B cells as are still

present show the cytological signs of marked activity. The normal or supranormal insular activity that is usually found in juvenile diabetics in this stage of the disease cannot therefore be due to the presence of a normal insular tissue, but is produced by a small number of hyperactive B cells. On the basis of histological findings (presence of islets of large size, signs of new islet formation), it may be assumed that during the preclinical phase of juvenile diabetes, an extra pancreatic factor has exerted a strong stimulant action on the insular tissue. In the long run this must lead to exhaustion of the islet-forming capacity on the pancreatic parenchyma and to a decrease in the number of the B cells.

By the time the disease becomes clinically manifest only the latter stage of this process can be observed and the majority of islets consist of A cells or of atrophic tissue devoid of B cells. Peri- and intra-insular inflammatory infiltrates have been found in 68 per cent of those patients with juvenile diabetes who died soon after the clinical onset of their disease.

In other words, and contrary to the generally held view, this lesion is not uncommon. It is specific for diabetes and has never been observed in the chronic cases. In patients with chronic juvenile diabetes, the B cells are completely absent, except in occasional cases. The islets consist of small, atrophic cells. A valid assessment of the functional capacity of insular tissue can only be achieved if as much use as possible is made of quantitative techniques and of cytological examination. (Willy Gepts et al, 1965).

Nϵ-(carboxymethyl)lysine, Nϵ-(carboxymethyl)hydroxylysine, and the fluorescent cross-link pentosidine are formed by sequential glycation and oxidation reactions between reducing sugars and proteins. These compounds, termed glycoxidation products, accumulate in tissue collagen with age and at an accelerated rate in diabetes. Although glycoxidation products are present in only trace concentrations, even in diabetic collagen, studies on glycation and oxidation of model proteins in vitro suggest that these products are biomarkers of more extensive underlying glycative and oxidative damage to the protein. Possible sources of oxidative stress and damage to proteins in diabetes include free radicals generated by autoxidation reactions of sugars and sugar adducts to protein and by autoxidation of unsaturated lipids in plasma and membrane proteins. The oxidative stress may be amplified by a continuing cycle of metabolic

stress, tissue damage, and cell death, leading to increased free radical production and compromised free radical inhibitory and scavenger systems, which further exacerbate the oxidative stress. Structural characterization of the cross-links and other products accumulating in collagen in diabetes is needed to gain a better understanding of the relationship between oxidative stress and the development of complications in diabetes. Such studies may lead to therapeutic approaches for limiting the damage from glycation and oxidation reactions and for complementing existing therapy for treatment of the complications of diabetes. (John W Baynes, 1991). Type 2 diabetes mellitus is increasingly common, primarily because of increases in the prevalence of a sedentary lifestyle and obesity.

Whether type 2 diabetes can be prevented by interventions that affect the lifestyles of subjects at high risk for the disease is not known. Randomly assigned 522 middle-aged, overweight subjects (172 men and 350 women; mean age, 55 years; mean body-mass index [weight in kilograms divided by the square of the height in meters], 31) with impaired glucose tolerance to either the intervention group or the control group.

Each subject in the intervention group received individualized counseling aimed at reducing weight, total intake of fat, and intake of saturated fat and increasing intake of fiber and physical activity. An oral glucose-tolerance test was performed annually; the diagnosis of diabetes was confirmed by a second test. The mean duration of follow-up was 3.2 years. The mean (±SD) amount of weight lost between base line and the end of year 1 was 4.2±5.1 kg in the intervention group and 0.8±3.7 kg in the control group; the net loss by the end of year 2 was 3.5±5.5 kg in the intervention group and 0.8±4.4 kg in the control group ($P<0.001$ for both comparisons between the groups).

The cumulative incidence of diabetes after four years was 11 percent (95 percent confidence interval, 6 to 15 percent) in the intervention group and 23 percent (95 percent confidence interval, 17 to 29 percent) in the control group. During the trial, the risk of diabetes was reduced by 58 percent ($P<0.001$) in the intervention group. The reduction in the incidence of diabetes was directly associated with changes in lifestyle. (Jaakko Tuomilehto, 2001).

Type 2 diabetes mellitus is a chronic metabolic disorder that results from defects in both insulin secretion and insulin action. An elevated rate of basal hepatic glucose production in the presence of hyperinsulinemia is the primary cause of fasting hyperglycemia; after a meal, impaired suppression of hepatic glucose production by insulin and decreased insulin-mediated glucose uptake by muscle contribute almost equally to postprandial hyperglycemia. In the United States, five classes of oral agents, each of which works through a different mechanism of action, are currently available to improve glycemic control in patients with type 2 diabetes.

The recently completed United Kingdom Prospective Diabetes Study (UKPDS) has shown that type 2 diabetes mellitus is a progressive disorder that can be treated initially with oral agent monotherapy but will eventually require the addition of other oral agents, and that in many patients, insulin therapy will be needed to achieve targeted glycemic levels. In the UKPDS, improved glycemic control, irrespective of the agent used (sulfonylureas, metformin, or insulin), decreased the incidence of microvascular complications (retinopathy, neuropathy, and nephropathy). This review examines the goals of antihyperglycemic therapy and reviews the mechanism of action, efficacy, nonglycemic benefits, cost, and safety profile of each of the five approved classes of oral agents. A rationale for the use of these oral agents as monotherapy, in combination with each other, and in combination with insulin is provided. (Ralph A. DeFronzo, 1999).

Diabetes mellitus is a strong risk factor for cardiovascular and renal disease. We investigated whether the angiotensin-converting-enzyme (ACE) inhibitor ramipril can lower these risks in patients with diabetes. 3577 people with diabetes included in the Heart Outcomes Prevention Evaluation study, aged 55 years or older, who had a previous cardiovascular event or at least one other cardiovascular risk factor, no clinical proteinuria, heart failure, or low ejection fraction, and who were not taking ACE inhibitors, were randomly assigned ramipril (10 mg/day) or placebo, and vitamin E or placebo, according to a two-by-two factorial design.

The combined primary outcome was myocardial infarction, stroke, or cardiovascular death. Overt nephropathy was a main outcome in a substudy. The study was stopped 6 months early (after 4·5 years) by the independent data safety and monitoring board because of a consistent

benefit of ramipril compared with placebo. Ramipril lowered the risk of the combined primary outcome by 25% (95% Cl 12–36, p=0·0004), myocardial infarction by 22% (6–36), stroke by 33% (10–50), cardiovascular death by 37% (21–51), total mortality by 24% (8–37), revascularisation by 17% (2–30), and overt nephropathy by 24% (3–40, p=0·027). After adjustment for the changes in systolic (2·4 mm Hg) and diastolic (1·0 mm Hg) blood pressures, ramipril still lowered the risk of the combined primary outcome by 25% (12–36, p=0·0004). Ramipril was beneficial for cardiovascular events and overt nephropathy in people with diabetes. The cardiovascular benefit was greater than that attributable to the decrease in blood pressure. This treatment represents a vasculoprotective and renoprotective effect for people with diabetes. (Hertzel C Gerstein, 2000).

A population association has consistently been observed between insulin-dependent diabetes mellitus (IDDM) and the "class 1" alleles of the region of tandem-repeat DNA (5' flanking polymorphism [5'FP]) adjacent to the insulin gene on chromosome 11p. This finding suggests that the insulin gene region contains a gene or genes contributing to IDDM susceptibility. However, several studies that have sought to show linkage with IDDM by testing for cosegregation in affected sib pairs have failed to find evidence for linkage. As means for identifying genes for complex diseases, both the association and the affected-sib-pairs approaches have limitations. It is well known that population association between a disease and a genetic marker can arise as an artifact of population structure, even in the absence of linkage. On the other hand, linkage studies with modest numbers of affected sib pairs may fail to detect linkage, especially if there is linkage heterogeneity.

We consider an alternative method to test for linkage with a genetic marker when population association has been found. Using data from families with at least one affected child, we evaluate the transmission of the associated marker allele from a heterozygous parent to an affected offspring. This approach has been used by several investigators, but the statistical properties of the method as a test for linkage have not been investigated. In the present paper we describe the statistical basis for this "transmission test for linkage disequilibrium" (transmission/disequilibrium test [TDT]).

We then show the relationship of this test to tests of cosegregation that are based on the proportion of haplotypes or genes identical by descent in affected sibs. The TDT provides strong evidence for linkage between the 5'FP and susceptibility to IDDM. The conclusions from this analysis apply in general to the study of disease associations, where genetic markers are usually closely linked to candidate genes. When a disease is found to be associated with such a marker, the TDT may detect linkage even when haplotype-sharing tests do not. (R S Spielman, 1993)

The diagnosis of non-insulin-dependent diabetes mellitus (NIDDM) has accompanied the rise in obesity in the pediatric population, as it has among adults. Medical records of 1027 consecutive patients from birth to age 19 years with a diagnosis of diabetes from 1982 to 1995 at a regional, university-affiliated pediatric diabetes referral center were reviewed and classified according to criteria of the National Diabetes Data Group. The number of patients with a diagnosis of NIDDM rose from approximately 4% of new diagnoses of diabetes in patients from birth to age 19 years before 1992, to 16% in 1994.

Among patients 10 to 19 years of age, NIDDM accounted for 33% of diagnoses of diabetes in 1994. The incidence of adolescent NIDDM in Greater Cincinnati increased tenfold, from 0.7/100,000 per year in 1982 to 7.2/100.000 per year in 1994. The mean (±SD) age and body mass index at presentation were 13.8 ± 1.9 years and 37.7 ± 9.6 kg/m2, respectively. The overall female/male ratio was 1.7:1, and female patients were seen 1 year earlier than male patients ($p < 0.01$). Male subjects had a higher body mass index than female subjects ($p < 0.05$). A first-degree relative with NIDDM was identified for 65% of patients. At presentation, 21% of the patients had had a diagnosis of at least one other condition associated with obesity.

There is an increasing incidence of NIDDM among adolescents in Greater Cincinnati, accompanying the national rise in adolescent obesity. Obesity and strong family histories of NIDDM are important risk factors. Because NIDDM leads to long-term morbidity, the prevention of obesity, as well as early identification of overt disease, is critical. (Orit Pinhas-Hamiel, 1996). The worldwide increase in type 2 diabetes mellitus is becoming a major health concern. We aimed to assess the effect of acarbose in preventing or delaying conversion of impaired glucose tolerance to type 2 diabetes. In a multicentre, placebo-controlled randomised

trial, we randomly allocated patients with impaired glucose tolerance to 100 mg acarbose or placebo three times daily. The primary endpoint was development of diabetes on the basis of a yearly oral glucose tolerance test (OGTT). Analyses were by intention to treat. Randomly allocated 714 patients with impaired glucose tolerance to acarbose and 715 to placebo.

We excluded 61 (4%) patients because they did not have impaired glucose tolerance or had no postrandomisation data. 211 (31%) of 682 patients in the acarbose group and 130 (19%) of 686 on placebo discontinued treatment early. 221 (32%) patients randomised to acarbose and 285 (42%) randomised to placebo developed diabetes (relative hazard 0·75 [95% CI 0·63–0·90]; p=0·0015). Furthermore, acarbose significantly increased reversion of impaired glucose tolerance to normal glucose tolerance (p<0·0001). At the end of the study, treatment with placebo for 3 months was associated with an increase in conversion of impaired glucose tolerance to diabetes. The most frequent side-effects to acarbose treatment were flatulence and diarrhoea. Acarbose could be used, either as an alternative or in addition to changes in lifestyle, to delay development of type 2 diabetes in patients with impaired glucose tolerance. (Jean-Louis Chiasson ET AL, 2002).

A moderate increase in cardiovascular risk with increasing levels of glycosylated hemoglobin in persons with diabetes mellitus. This association seems to be similar in persons with type 1 and type 2 diabetes and is present across diverse geographic populations. In some studies, this association seems to be independent of other known risk factors for cardiovascular disease. The magnitude of the effect for total cardiovascular disease, fatal and nonfatal coronary heart disease, and stroke was similar.

Compared with coronary heart disease and stroke, the pooled results of the few studies on glycosylated hemoglobin and peripheral arterial disease in persons with type 1 and type 2 diabetes suggest the possibility of a stronger association between glycosylated hemoglobin levels and this outcome (pooled risk estimates were 1.32 and 1.28 in persons with type 1 and type 2 diabetes, respectively). However, all pooled relative risk estimates reported here are based on small numbers of studies. (Elizabeth Selvin ET AL, 2004). The potential role of physical activity in the primary prevention of non-insulin-dependent diabetes mellitus (N IDDM) is largely

unknown. We examined the association between regular vigorous exercise and the subsequent incidence of NIDDM in a prospective cohort of 87 253 US women aged 34-59 years and free of diagnosed diabetes, cardiovascular disease, and cancer in 1980. During 8 years of follow-up, we confirmed 1303 cases of NIDDM. Women who engaged in vigorous exercise at least once per week had an age-adjusted relative risk (RR) of NIDDM of 0·67 ($p<0·0001$) compared with women who did not exercise weekly.

After adjustment for body-mass index, the reduction in risk was attenuated but remained statistically significant (RR=0·84, p=0·005). When analysis was restricted to the first 2 years after ascertainment of physical activity level and to symptomatic NIDDM as the outcome, age-adjusted RR of those who exercised was 0·5, and age and body-mass index adjusted RR was 0·69. Among women who exercised at least once per week, there was no clear dose-response gradient according to frequency of exercise. Family history of diabetes did not modify the effect of exercise, and risk reduction with exercise was evident among both obese and nonobese women. Multivariate adjustments for age, body-mass index, family history of diabetes, and other variables did not alter the reduced risk found with exercise. Our results indicate that physical activity may be a promising approach to the primary prevention of NIDDM. (J.E. Manson, 1991).

Risk factors for coronary artery disease in patients with type 2 diabetes mellitus. A stepwise selection procedure, adjusting for age and sex, was used in 2693 subjects with complete data to determine which risk factors for coronary artery disease should be included in a Cox proportional hazards model. 3055 white patients (mean age 52) with recently diagnosed type 2 diabetes mellitus and without evidence of disease related to atheroma. Median duration of follow up was 7.9 years. 335 patients developed coronary artery disease within 10 years. Angina with confirmatory abnormal electrocardiogram; non-fatal and fatal myocardial infarction. Coronary artery disease was significantly associated with increased concentrations of low density lipoprotein cholesterol, decreased concentrations of high density lipoprotein cholesterol, and increased triglyceride concentration, haemoglobin A1c, systolic blood pressure, fasting plasma glucose concentration, and a history of smoking.

The estimated hazard ratios for the upper third relative to the lower third were 2.26 (95% confidence interval 1.70 to 3.00) for low density lipoprotein cholesterol, 0.55 (0.41 to 0.73) for high density lipoprotein cholesterol, 1.52 (1.15 to 2.01) for haemoglobin A1c, and 1.82 (1.34 to 2.47) for systolic blood pressure. The estimated hazard ratio for smokers was 1.41(1.06 to 1.88). A quintet of potentially modifiable risk factors for coronary artery disease exists in patients with type 2 diabetes mellitus. These risk factors are increased concentrations of low density lipoprotein cholesterol, decreased concentrations of high density lipoprotein cholesterol, raised blood pressure, hyperglycaemia, and smoking. (R C Turner, 1998).

The relation between adult weight change and the risk for clinical diabetes mellitus among middle-aged women. Prospective cohort study with follow-up from 1976 to 1990. 2204 cases of diabetes were diagnosed during 1.49 million person-years of follow-up. After adjustment for age, body mass index was the dominant predictor of risk for diabetes mellitus. Risk increased with greater body mass index, and even women with average weight (body mass index, 24.0 kg/m2) had an elevated risk. Compared with women with stable weight (those who gained or lost less than 5 kg between age 18 years and 1976) and after adjustment for age and body mass index at age 18 years, the relative risk for diabetes mellitus among women who had a weight gain of 5.0 to 7.9 kg was 1.9 (95% CI, 1.5 to 2.3).

The corresponding relative risk for women who gained 8.0 to 10.9 kg was 2.7 (CI, 2.1 to 3.3). In contrast, women who lost more than 5.0 kg reduced their risk for diabetes mellitus by 50% or more. These results were independent of family history of diabetes. The excess risk for diabetes with even modest and typical adult weight gain is substantial. These findings support the importance of maintaining a constant body weight throughout adult life and suggest that the 1990 U.S. Department of Agriculture guidelines that allow a substantial weight gain after 35 years of age are misleading. (Graham A, 1995).

Type 2 diabetes mellitus is characterised by resistance of peripheral tissues to insulin and a relative deficiency of insulin secretion. To find out which is the earliest or primary determinant of disease, we used a minimum model of glucose disposal and insulin secretion based on intravenous glucose tolerance tests to estimate insulin sensitivity (SI), glucose effectiveness (ie,

insulin-independent glucose removal rate, SG), and first-phase and second-phase beta-cell responsiveness in normoglycaemic offspring of couples who both had type 2 diabetes. 155 subjects from 86 families were followed-up for 6-25 years. More than 10 years before the development of diabetes, subjects who developed the disease had lower values of both SI (mean 3.2 [SD 2.4] vs 8.1 [6.7] 10(-3) I min-1 pmol-1 insulin; $p < 0.0001$) and SG (1.6 [0.9] vs 2.3 [1.2] 10(-2) min-1, $p < 0.0001$) than did those who remained normoglycaemic).

For the subjects with both SI and SG below the group median, the cumulative incidence of type 2 diabetes during the 25 years was 76% (95% confidence interval 54-99). By contrast, no subject with both SI and SG above the median developed the disease. Subjects with low SI/high SG or high SI/low SG had intermediate risks. Insulin secretion, especially first phase, tended to be increased rather than decreased in this prediabetic phase and was appropriate for the level of insulin resistance. The development of type 2 diabetes is preceded by and predicted by defects in both insulin-dependent and insulin-independent glucose uptake; the defects are detectable when the patients are normoglycaemic and in most cases more than a decade before diagnosis of disease. (Martin BC et al, 1992)

More than 400 traditional plant treatments for diabetes mellitus have been recorded, but only a small number of these have received scientific and medical evaluation to assess their efficacy. Traditional treatments have mostly disappeared in occidental societies, but some are prescribed by practitioners of alternative medicine or taken by patients as supplements to conventional therapy. However, plant remedies are the mainstay of treatment in underdeveloped regions. A hypoglycemic action from some treatments has been confirmed in animal models and non-insulin-dependent diabetic patients, and various hypoglycemic compounds have been identified. A botanical substitute for insulin seems unlikely, but traditional treatments may provide valuable clues for the development of new oral hypoglycemic agents and simple dietary adjuncts (Clifford J Bailey et al, 1989).

Many traditional treatments have been recommended in the alternative system of medicine for treatment of diabetes mellitus; however, the mechanism of most of the herbals used has not been defined. AIMS: This study was carried out to clarify the effect of fenugreek, garlic and

onion, recommended in Persian folklore medicine as beneficial in the treatment of diabetes, on blood glucose and their possible effect on pancreatic tissue. Diabetes mellitus was induced in 20 out of 25 adult male albino rats, using intraperitoneal injection of 185 mg/kg BW alloxan. The diabetic rats were divided into four groups, three of which were fed a diet containing 12.5% BW *Allium sativum* (garlic), *Allium cepa* (onion) or *Trigonella foenum-graecum* (fenugreek) for 15 days. The fourth group (positive control) received an ordinary diet.

The remaining non-diabetic rats (negative control group) received neither alloxan nor the mentioned plants. Following consumption of plants, blood glucose was measured every day and on the last day the pancreas were removed and stained with H&E and Gomeri aldehyde fuchsin (GAF). Morphology of the pancreatic sections and the following morphometric factors were studied: volume density of B cells, volume density of islets, percent of B cells, number of islets per square millimeter, average area of islets and average volume density of B cell in whole pancreas. One-way Analysis of Variance (ANOVA) test and Duncan's multiple range tests were used to evaluate the data.

The results of this study indicate that only garlic was able to reduce blood glucose significantly compared with the control group ($P<0.05$). In the control positive group all the mentioned morphometric factors were significantly changed in comparison with the control negative (normal health) group, but the same did not show significant change between treated and untreated diabetics (Gholamali A Jelodar et al, 2005).

Diet has been recognized as a corner stone in the management of diabetes mellitus. Spices are the common dietary adjuncts that contribute to the taste and flavour of foods. Besides, spices are also known to exert several beneficial physiological effects including the antidiabetic influence. This review considers all the available information from animal experimentation as well as clinical trials where spices, their extracts or their active principles were examined for treatment of diabetes. Among the spices, fenugreek seeds, garlic (*Allium sativum*), onion (*Allium cepa*), and turmeric (*Curcuma longa*) have been experimentally documented to possess antidiabetic potential. In a limited number of studies, cumin seeds (*Cuminum cyminum*), ginger (*Zingiber officinale*), mustard (*Brassica nigra*), curry leaves (*Murraya koenigii*) and coriander

(*Coriandrum sativum*) have been reported to be hypoglycaemic (K. Srinivasan et al, 2005). Recently use of herbal medicines, have been considered as an alternative for therapeutic usage. So, this study was undertaken to evaluate the hypoglycemic and hypolipidemic effects of fenugreek seeds in type 2 diabetic patients. Methods: In a clinical trial study, 24 type 2 diabetic patients were placed on 10 grams/day powdered fenugreek seeds mixed with yoghurt or soaked in hot water for 8 weeks. Weight, FBS, HbA1C, total cholesterol, LDL, HDL and food record were measured before and after the study.

The differences observed in food records, BMI and serum variables were analyzed using paired-t-test and t-student and $P \leq 0.05$ was considered as significant. Results: After exclusion of 6 cases for changing in medication or personal problems, the results of 18 patients (11consumed fenugreek in hot water and 7 in yoghurt)were studied. Findings showed that FBS, TG and VLDL-C decreased significantly (25 %, 30 % and 30.6 % respectively) after taking fenugreek seed soaked in hot water whereas there were no significantly changes in lab parameters in cases consumed it mixed with yoghurt. BMI, Energy, Carbohydrate, Protein and fat intake remained unchanged during study. Conclusion: This study shows that fenugreek seeds can be used as an adjuvant in the control of type 2 diabetes mellitus in the form of soaked in hot water (Nazila Kassaian et al, 2013).

Diabetes mellitus is a heterogeneous metabolic disorder characterized by hyperglycaemia resulting in defective insulin secretion, resistance to insulin action or both. The use of biguanides, sulphonylurea and other drugs are valuable in the treatment of diabetes mellitus; their use, however, is restricted by their limited action, pharmaco-kinetic properties, secondary failure rates and side effects. Trigonella foenum-graecum, commonly known as fenugreek, is a plant that has been extensively used as a source of antidiabetic compounds from its seeds and leaf extracts. Preliminary human trials and animal experiments suggest possible hypoglycaemic and anti-hyperlipedemic properties of fenugreek seed powder taken orally.

The results show that the action of fenugreek in lowering blood glucose levels is almost comparable to the effect of insulin. Combination with trace metal showed that vanadium had additive effects and manganese had additive effects with insulin on in vitro system in control and

diabetic animals of young and old ages using adipose tissue. The Trigonella and vanadium effects were studied in a number of tissues including liver, kidney, brain peripheral nerve, heart, red blood cells and skeletal muscle. Addition of Trigonella to vanadium significantly removed the toxicity of vanadium when used to reduce blood glucose levels. Administration of the various combinations of the antidiabetic compounds to diabetic animals was found to reverse most of the diabetic effects studied at physiological, biochemical, histochemical and molecular levels. Results of the key enzymes of metabolic pathways have been summarized together with glucose transporter, Glut-4 and insulin levels. Our findings illustrate and elucidate the antidiabetic/insulin mimetic effects of Trigonella, manganese and vanadium (Najma Zaheer Baquer, 2011).

Type 2 diabetes has become a global epidemic. Modern medicines, despite offering a variety of effective treatment options, can have several adverse effects. Ayurveda, a science that uses herbal medicines extensively, originated in India. Of considerable interest is the adoption of Ayurveda by the mainstream medical system in some European countries (e.g., Hungary), emphasizing this modality is increasing worldwide recognition. From ancient times, some of these herbal preparations have been used in the treatment of diabetes.

This paper reviews the accumulated literature for 10 Indian herbs that have antidiabetic activity and that have been scientifically tested. Few of these herbs, such as Momordica charantia, Pterocarpus marsupium, and Trigonella foenum greacum, have been reported to be beneficial for treating type 2 diabetes. Mechanisms such as the stimulating or regenerating effect on beta cells or extrapancreatic effects are proposed for the hypoglycemic action of these herbs (Wan-Li Xue et al, 2007).

Traditional Medicines derived from medicinal plants are used by about 60% of the world's population. This review focuses on Indian Herbal drugs and plants used in the treatment of diabetes, especially in India. Diabetes is an important human ailment afflicting many from various walks of life in different countries. In India it is proving to be a major health problem, especially in the urban areas. Though there are various approaches to reduce the ill effects of diabetes and its secondary complications, herbal formulations are preferred due to lesser side effects and low cost. A list of medicinal plants with proven antidiabetic and related beneficial

effects and of herbal drugs used in treatment of diabetes is compiled. These include *Allium sativum, Eugenia jambolana, Momordica charantia Ocimum sanctum, Phyllanthus amarus, Pterocarpus marsupium, Tinospora cordifolia, Trigonella foenum graecum* and *Withania somnifera*. One of the etiologic factors implicated in the development of diabetes and its complications is the damage induced by free radicals and hence an antidiabetic compound with antioxidant properties would be more beneficial. Therefore information on antioxidant effects of these medicinal plants is also included (Manisha Modak, 2007).

Diabetes mellitus is the most common metabolic disorder worldwide. To date, there have been no reports on the frequency of use of herb medicines in the managements of diabetes mellitus in Jordan. This cross-sectional study was conducted by interviewing 310 diabetic patients visiting two medical centers in Jordan: Jordan University of Science & Technology Medical Center and Sarih Medical Center between December 2003 and August 2004. It is found that 31% of interviewed patients have used herbal products (96 patients).

The results revealed that the most commonly used herbs by diabetic patients in Jordan were *Trigonella foenumgraecum* (22.9%), *Lupinus albus* (14.6%), *Alliumm sativum* (11.5%), *Allium cepa* (5.2%), *Nigella sativa* (7.3%), *Zea mays* L. (6.3%), *Urtica dioica* L. (8.3%), *Eucalyptus globules* LA (9.4%), *Olea europea* L. (3.1%), *Cumminum cyminum* (9.4%), *Coriandrum sativum* (10.4%), *Salvia officinalis* L. (3.1%), and *Tilia cordata* (1%).

Furthermore, it is found that 47.9% of the patients used herbs according to advice from their friends on a daily basis. The side effects were reported by 36.5% of the patients and include headache, nausea, dizziness, itching, palpitation, and sweating. Among the patients, 72.9% used the herbs as adjunctive therapy along with their anti-diabetic drugs and 80.2% of the patients informed their physicians about their use.

A 79.2% of the sample confirmed their intention to re-use these herbs as 86.5% of them were satisfied with their diabetes control. There was a significant relationship between the use of herbs, the patient's place of residence and his/her level of education. The main conclusion of this study was that the use of medicinal herbs among diabetic patient in Jordan is common. Therefore,

it is essential to increase the level of awareness among diabetic patients and health care providers regarding the efficacy and toxicity of these medicinal herbs (SA Otoom et al, 2006).

Fagonia species is a tropical herb belonging to family Zygophyllaceae, found in the entire Indian subcontinent. Fagonia arabica is popular in India and commonly known as 'Dhamasa' in Marathi language. Dhamasa is one of the ingredients of ayurvedic medicine, used as antihypertensive, anti-inflammatory, analgesic, antioxidant & thrombolytic. As Dhamasa is major ingredient of many Ayurvedic medicines, its identification is more important for evaluation of antihypertensive activity for which it is mostly used in Ayurvedic medicines.

The identification of drug involves the morphology and microscopical parameters. The evaluation parameters for plant based drugs as per official guidelines are, physical, chemical and biological evaluation. The active chemical constituents of Fagonia species are carbohydrates, flavonoid, glycosides, steroids, saponins, alkaloids, triterpenoidal glycosides, amino acids, Chlorides, Sulphates, Anthraquinones, Irodoids, Cyanogenic glycosides and Coumarin.

The triterpenoidal glycosides and Flavonoids were isolated & chemical structures established from aerial parts of F. arabica and indica respectively. The reported pharmacological activity of Fagonia species are anticancer, antimicrobial, antiviral, analgesic, anti-inflammatory, antipyretic as a coolant, used for skin diseases, and urinary tract infections, as antioxidant and thrombolytic. This review gives a focus mainly on the biological activities of the Fagonia Species and some of their isolated compounds, their pharmacological actions, clinical studies and medicinal applications of Fagonia along with their safety evaluation. (Veena S. Kasture, 2014).

Atherothrombotic diseases such as myocardial or cerebral infarction are serious consequences of the thrombus formed in blood vessels. Thrombolytic agents are used to dissolve the already formed clots in the blood vessels; however, these drugs have certain limitations which cause serious and sometimes fatal consequences. Herbal preparations have been used since ancient times for the treatment of several diseases.

Herbs and their components possessing antithrombotic activity have been reported before; however, herbs that could be used for thrombolysis has not been reported so far. This study

investigated the herbal preparations (aqueous extract) possess thrombolytic activity. (Sweta Prasad et al, 2007).

The aqueous methanolic extract of the aerial parts of Fagonia arabica L. (family Zygophyllaceae) was successively fractionated using certain organic solvents. From the ethyl acetate fraction, two flavonoid glycosides were isolated and identified as kaempferol-7-O-rhamnoside and acacetin-7-O-rhamnoside. Four triterpenoidal glycosides were isolated from the butanolic layer. Their structures were elucidated on the basis of the spectral and chemical data as 3-O--d-glucopyranosyl-(153)-αÐl-arabinopyranoside oleanolic acid (1), 3-O-α-larabinopyranosyl quinovic acid 28-O--d-glucopyranoside (2), 3-O-[-d-glucopyranosyl- (152)]--d-glucopyranosyl-(153)-α-l-arabinosyl oleanolic acid (3) and 3-O--d-glucopyranosyl-(153)-α-l-arabino-pyranosyl quinovic acid 28-O--d-glucopyranoside (4).

The two monodesmosidic saponins 1 and 3 were found to possess strong molluscicidal activity against Biomphalaria alexandrina snails, the intermediate host of Schistosoma mansoni in Egypt (LC90 = 13.33 and 16.44 μm), whereas the other two bidesmosidic saponins 2 and 4 as well as the two flavonoid glycosides were inactive up to 50 μm. (Eman A. El-Wakil et al, 2006)

These findings provide evidence for the study *in vivo* anti-diabetic beneficial effects of the *Fagonia arabica* (L.).

CHAPTER-THREE

CHAPTER-III MATERIALS AND METHODS

The designing of methodology involves a series of steps taken in a systematic way in order to achieve the set goal(s) under the prescribed guidelines and recommendations. It includes in it all the steps from Extract preparation, observation, selection of dose value, standardization of protocol, usage of instruments, preparation of reagents, formation of protocols and final execution of the standardized protocol. All this requires good build of mind and a good and soft technical hand to handle the materials and procedure in a true scientific manner.

3.1. Chemicals used

Chemicals used in this study were of analytical grade and of highest purity procured from standard commercial sources in India.

Name of chemical	***Source/Make***
Alkaline phosphatase kit	Erba
Alloxan monohydrate	Sigma Aldrich
Cholestrol kit	Erba
Diethyl ether	Rankem
DMSO	Labo chemie
Eosin	MERK
Ethanol	Rankem
Ethyl acetate	Rankem
Formalin	MERK
Glibenclamide	Sigma Aldrich
Glucose kit	Erba
Glucose/Sucrose	Qualigens
Hematoxylin	MERK
Isopropyl alcohol	Rankem
Methanol	Rankem

Petroleum ether	Rankem
Picric acid	MERK
AST kit	Erba
ALT kit	Erba
Sodium chloride	Qualigens
Triglycerides kit	Erba
Xylene	MERK

3.2. Instruments

Following instruments were used for the study:

Name of the instrument	***Source***
Auto-analyzer	Robonik
Automatic knife sharpener	Yorco
Centrifuge	Remi
Cylomixer	Remi
Digital weighing balance	Shimadzu
Distillation unit	Borosil
Gluco-meter	Accu check Go
Grinding mill	Hamburg
Heating mantle	MAC
Micro centrifuge	Remi
Microtome	York Scientific Industries
Oven	Bells India
Refrigerator	LG
Rotary evaporator	Lab Sil
Tissue floatation bath	Teknik
Water bath	Techno India
Wax dispenser	Yorco

3.3. Preparation of *F.arabica* (L.) ethanolic (70%) Extract (FAEXT):

The air dried powdered plant was extracted with 70% ethanol in a Soxhlet apparatus at 60°C. The extract was concentrated to syrupy solution using rotary evaporator under reduced pressure at 40°C. The thick solution was lyophilized using freeze drying system. The yield (13. 5%) was used for the experimental studies (Hussein M. Ageely et al, 2014).

Fig.10. *Fagonia arabica (L.)* whole plant ethanolic powder

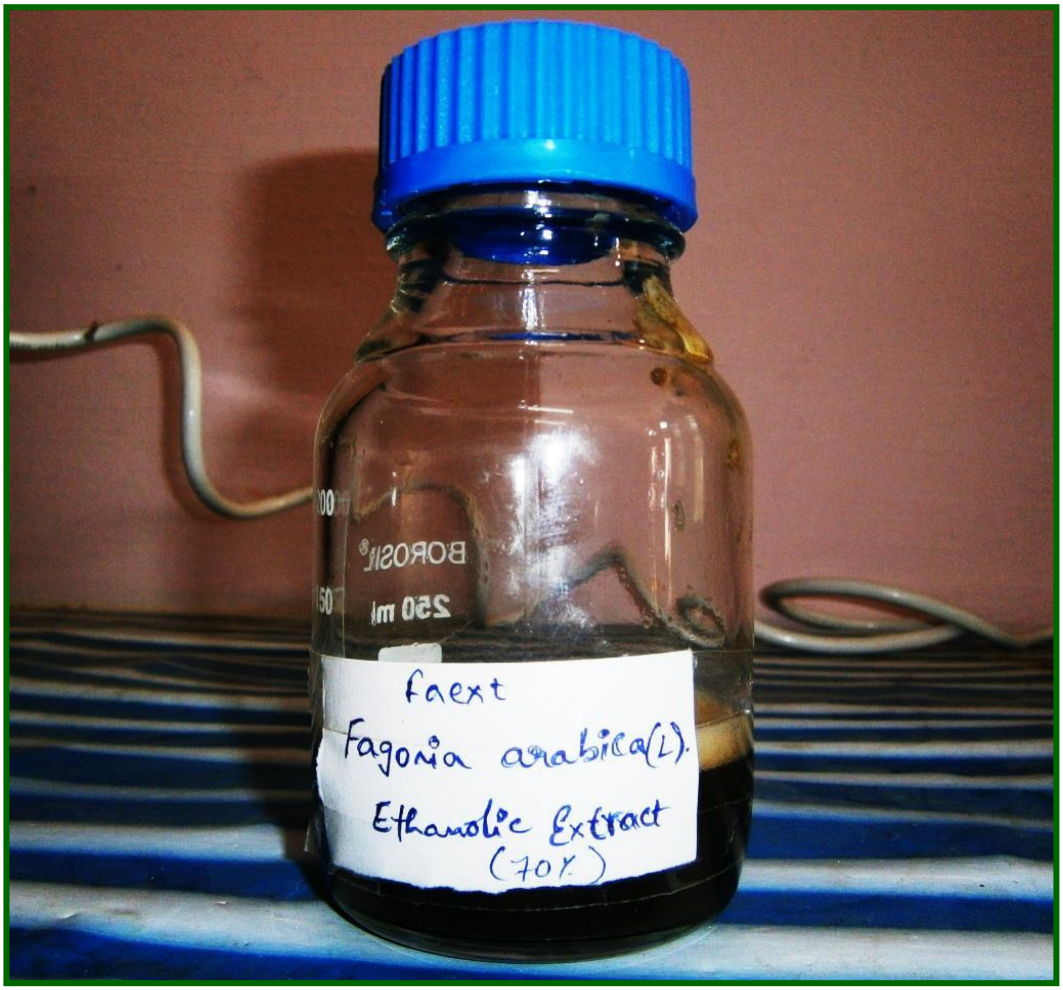


Fig.11. Fagonia arabica Ethanolic (70%) extract (FAEXT)

Fagonia arabica (*F.arabica*) is a tropical herb belonging to family Zygophyllaceae, found in the entire Indian subcontinent and is commonly known as 'Dhamasa'. It is a green shrub of 1 to 3 feet height found on calcareous rocks distributed throughout the Mediterranean region of Africa, Afghanistan, India and Pakistan (Rizvi MA et al, 1996). Different parts of this herb have been used to cure various ailments, namely hematological, neurological, endocrinological and inflammatory disorders (Chopra RN, 1956; Saeed MA, 1969; Chopra RM, 1982; Hooker JD, 1975; Saeed MA, 1999; Saeed MA, 2003).

It has also been reported to contain wide variety of antioxidants and triterpenoids saponins (Miyase T et al, 1996; Khalik SMA et al, 2000). Its infusion is effective as a cooling agent in stomatitis. It is known to purify blood and also acts as a deobstruent (Saeed MA, 2003; Said HM

et al, 1996; Hooker JD et al, 1975). It is also used for skin diseases, small pox and for endothermic reaction in the body (Watt G et al, 1972). The twigs of the plant are used as remedy for snake bite and also applied externally as paste on tumours and for the swellings of neck (Rizvi MA et al, 1996). Very recently the thrombolytic activity of *F.arabica* has been demonstrated (Rakesh Das et al, 2010). Ravindra et al investigated the effect of *F. arabica* on the cytotoxicity, cellular energy status and ROS production in the chemical ischemia/reperfusion induced rat pheochromocytoma cells (Ravindra Satputea et al, 2012).

3.4. Acute toxicity study

The acute toxicity of F. arabica alcoholic extract was determined in rats according to the previous method (Adedapo *et al*., 2009) with slight modifications. Rats that fasted for 12 h were randomly divided into four groups (n = 5). Graded doses of the extract (100, 250, 500 and 1000 mg/kg bw) were separately administered to the rats. All the animals were then observed over a period of 10 days for deaths and signs of acute toxicity.

Animal toxicity study Oral administration of graded doses (100 to 2000 mg/kg p.o.) of the alcoholic extract of F. arabica to rats, did not produce any mortality and changes in behavior, breathing, cutaneous effects, sensory nervous system responses or gastrointestinal effects during the 10 days observation period. The obtained results signify that the use of the plant for treatment is safe. So the dose of 250 mg/kgbw was selected for anti-diabetic studies.

3.5. Experimental animals

Male albino Wistar rats, weighing: 120 ± 20g were used for the present study, procured from Indian Institute of Science, Bangalore, India. All the animals were maintained under regulatory laboratory conditions (12L: 12D; Humidity: 76% and temperature: 28 ± 2^0 C) in the Department of Biochemistry, S.K. University, Anantapur. The maintenance and the handling of animals were performed according to the rules and regulations of Institutional animal Ethical Committee, Sri Krishnadevaraya University.

1- Healthy Wistar strain albino rats were selected and randomly divided into five groups with six animals in each group serving as: (feed and water every day for all groups),
2- *Fagonia arabica* extract every day for group (C and D). AM only for (1 and 7) day for group (B and D), Glibenclamide only for (35, 42, 45) for group E

a. Group 'A' = Normal rats (Only fed with normal rat feed every day)(1-45) day
b. Group 'B'= Alloxan monohydrate (AM) (150 mg/kgbw) rats (DM) (1,7) day
c. Group 'C' = *Fagonia arabica* plant aqueous extract (*Faext*) 250 mg/kgbw (1-45)
d. Group 'D' = AM group (150 mg/ kgbw) (1,7) + Faext 250 mg/kgbw (DM)(1-45)
e. Group 'E' = Reference control i.e. Standard drug (Glibenclamide, 10 mg/kgbw).

(A)

(B)

(C)

(D)

(E)

Fig: 12 Healthy Wistar strain albino rats were selected and randomly divided into five groups. (A,B.C,D.E) and I.P injection of AM and orally of FAEXT

2. An identification mark was given to the rats of each group using picric acid as dye following standard procedure of DSRU-RRIUM, Sgr. [RRISGR/SOP-AH/002 (Animal identification marking)]. Each rat of a group was marked at a specific position viz.- Head; Back; Tail; Head and Back; Head and Tail; and one was left Blank i.e. unmarked. Each rat was weighed and the doses were calculated accordingly.

3. Body weight determination: Every week during the treatment the body weight of the animals in each group was recorded.

4. Diabetes was induced in each group using freshly prepared solution of Alloxan monohydrate dissolved in normal saline (0.91% w/v of NaCl) except the group "A" which served as normal control. For inducing diabetes the rats were kept on fasting for 18 hours and diabetes was induced by giving a single IP injection of Alloxan monohydrate (150 mg/kg b. wt.) following standard methodology of DSRU-RRIUM, Sgr. [RRISGR/SOP-TX/008 (Intraperitoneal route of drug administration)]. To prevent fatal hypoglycemia due to massive pancreatic insulin release, the rats were provided with 20% glucose solution after six hours supplied in water bottles in their cages for next 24 hours.

5. The fasting blood glucose level of the rats was measured after 72 hours. The rats having fasting blood glucose level above 200mg/dl were selected for further experimentation.

6. Group 'A' was fed with simple drinking water which served as normal control; group 'B' in which diabetes was induced was also fed with simple drinking water, serving as Diabetic control; Group 'C' in FAEXT was given 250 mg/kgbw. of extract orally; Group 'D' in which diabetes was induced was given 150 mg/kgbw of AM intraperitoneally and FAEXT was given 250 mg/kgbw; group 'E' was given the standard drug glibenclamide (10 mg/kg of bw) orally by the help of 18 No. cannula following standard methodology of DSRU-RRIUM, Sgr. [RRISGR/SOP/TX/001 (Dosing of rats by oral route)]. All the groups were given respective treatments daily for 45 days.

7. Blood was collected on the 0^{th} day, means the day on which the dosing was started 1^{st} day, 7^{th} day, 14^{th} day, 21^{st} day, 28^{th} day, 35^{th} day, 42^{nd} day and 45^{th} day, through the retro orbital sinus of the rats.

8. Physiological parameters like food intake, fluid intake, urine excretion and body weight

were monitored during the experimentation as per standard methodology of DSRU-RRIUM, Sgr. In order to check the effect of the extracts on the weight of rats, weight of the rats was recorded prior to the administration of the extracts and at the end of the study i.e. on the 45th day. The urine volume may not be measured instead an overview was made by the wetting of saw (cage bedding) by urine. The initial and final amount of feed provided to rats in the cages was weighed and the average food intake value was calculated. Similarly the average fluid intake was determined by measuring the initial and final volume of water.

9. Estimation of Glucose and other clinical parameters: The serum from the blood was separated as under:

a. Sample was collected (preferably in glass tubes) and left for 1 hr at 37°C to allow it to clot.

b. Using a glass pasteur carefully the clot was loosened from the sides of the tube.

c. The serum was centrifuged at 3000 rpm for 10 min at 4°C.

d. The serum was removed from the clot by gently pipetting off into a clean tube using a glass pasteur or a micropipette.

e. The serum was labeled with the animal number and the estimations were made. The serum glucose level; the enzymes SGOT, SGPT and ALP level and the lipid profile (total cholesterol and triglyceride level) was determined enzymatically on Robonik semi auto-analyzer.

3.6. Screening of dose dependence

In order to check the dose dependence different doses of the extract(s) like 100, 250, 500, 1000 mg/kgbw, were given to the different groups of rats following the same methodology as above. Different doses of the FAEXT were given to the different groups in order to determine the dose dependence of the extracts and the results are shown.

It shows that the reduction in fasting blood glucose level by the ethyl acetate and the aqueous extracts is significant ($p<0.001$) at all the concentrations that were taken i.e., from 250mg/kg body weight up to 1000mg/kg body weight, however the percentage variation that represents the percentage increase/decrease in the blood glucose levels from Day0 to Day45 shows a systematic increase in the activity of the ethanolic extract in a dose dependent manner.

3.7. Blood Glucose test

The glucose test was performed in overnight fasted (18hr) normal rats as per Bonner, 1988. Healthy rats were randomly selected and distributed into five groups (n=6). One of those groups was administered distilled water and the rest four groups and glibenclamide (30mg/kg bw) groups. Glucose (2g/kgbw) was fed 1 hr after the administration of the active compound and glibenclamide. Blood was withdrawn from the tail vein at 0, 60, 90,120 and 150 min of glucose administration and glucose levels were estimated using Accucheck Go blood glucose monitoring kit.

The blood glucose level of the rats was measured after overnight fasting.

1- Group 'A' was fed with simple drinking water which served as normal control and rest of the groups were fed with the respective active compound, mentioned above, i.p., following standard methodology. All the groups were given respective treatments daily for 45 days.

2. Blood was collected again on the 7^{th} day 14^{th} day, 21^{st} day, 28^{th} day, 35^{th} day, 42^{nd} day and 45^{th} day, of dosing, through the retro orbital sinus of the rats.

3. The serum from the blood was separated and labeled with the animal number. The estimation of glucose level was measured enzymatically on an autoanalyser.

3.8. ASSAY OF AST AND ALT (Bergermeyer et al., 1978)

Principle:

This assay is based on the principle that AST and ALT catalyse the transfer of amino group from L-aspartate/L-alanine to a-ketoglutarate to yield oxaloacetate/pyruvate respectively. Oxaloacetate/pyruvate can oxidise NADH to NAD+ in the presence of malate dehydrogenase/lactate dehydrogenase. The decrease in absorbance at 340nm in a spectrophotometer (Genesys 10-S, USA) due to the oxidation of NADH is monitored kinetically and is proportional to AST/ALT activity.

Reagents:

Reagent 1 – Buffer

Tris (80 mmol/l pH 7.8)

L-aspartate or L-alanine (240 mmol/L)

MDH ³ 600 U/L

LDH ³ 600 U/L

Reagent 2 – Substrate

2-oxoglutarate (12 mmol/L)

NADH (0.18 mmol/L)

Working reagent

Four parts of reagent 1 were mixed with one part of reagent 2.

Procedure:

To 100µl of serum, 1000µl of working reagent was added. The tubes were mixed well and the absorbance was read after 60 seconds and the change in absorbance was measured for 2 minutes at 340nm in a spectrophotometer (Genesys 10-S, USA). AST/ALT activity is expressed as IU/L.

3.9. ASSAY OF ALP (Schlebusch *et al*., 1974)

Principle:

At alkaline pH, ALP catalyzes the hydrolysis of p-nitrophenyl phosphate to yellow coloured p-nitro phenolate and phosphate; the change in absorbance measured at 415nm is directly proportional to the enzyme activity.

REAGENTS:

1. p-nitrophenyl phosphate (PNPP)

2. Buffer

The working reagent was prepared by mixing one vial of PNPP substrate with 5.0ml buffer.

Procedure

To 20µl of serum, 1.0ml of working reagent was mixed and after one minute, the increase in absorbance was measured at 415nm in a spectrophotometer (Genesys 10-S, USA). The ALP activity is expressed as IU/L.

3.10. Lipid profile studies:

Extraction of Lipids:

The lipids were extracted from liver by the method of Folch et al. (1957). The liver tissues were dried, weighed and a known weight was homogenized with 10 ml of chloroform methanol mixture. The homogenate was filtered through Whatmann filter paper No.42 into a separating funnel. The filtrate was mixed with 0.2 ml of physiological saline and the mixture was kept overnight undisturbed.

The lower phase containing the lipid was drained off into pre-weighed beakers. The upper phase was re-extracted with excess of chloroform-methanol mixture and the extracts were pooled and evaporated under vacuum at room temperature. The lipid extract was re-dissolved in 3.0 ml of chloroform-methanol (2:1) mixture and aliquots were taken for the estimation of cholesterol and phospholipids. The total lipid contents were calculated and expressed as mg/g of fresh tissue.

a. Estimation of Total Cholesterol:

Cholesterol content was estimated by the method of Parekh and Jung (1970). About 0.1 ml of test sample was made up to 10 ml with ferric acetate-uranyl acetate reagent. 0.1 ml of the aliquot of the total lipid extract was taken and it was evaporated to dryness. The dried extract and standards were made up to 3.0 ml with ferric chloride-uranyl acetate reagent.

Then 2.0 ml of sulphuric acid-ferrous sulphate reagent was added to all the tubes and the contents were mixed well. After 20 minutes, the color developed was read at 540 nm using a Shimadzu UV spectrophotometer. Total cholesterol level was expressed as mg/dl for plasma and tissue cholesterol as mg/g of fresh tissue.

b. Estimation of Triacylglycerides (TG)

Triacylglycerol was estimated by the method of Rice (1970). Lipids were extracted with cholorform: methanol mixture (2:1 v/v). Phospholipids present in the lipid extract were adsorbed onto silicic acid and the triacylglycerol remaining in solution was saponified with alcoholic potassium hydroxide (400 mg of potassium hydroxide was dissolved in 100 ml of 95% ethanol). The liberated glycerols were oxidized by periodate to formaldehyde and the excess oxidizing power was destroyed by reaction with 0.5M sodium arsenite.

The formaldehyde formed was determined by the chromotropic colour reaction. 4.0 ml of the lipid extract was added to tubes containing 8.0 ml of saturated sodium chloride and shaken vigorously. The contents were allowed to settle for one hour and then centrifuged. The supernatant (saline-methanol phase) was discarded. The washed chloroform phase was filtered into a dry tube. 200 mg of activated silicic acid was added to chloroform phase, shaken vigorously and allowed to stand for 30 minutes. After centrifugation, 0.5 ml of the supernatant as well as tripalmitin standards was evaporated to dryness.

Then to the test, standard and blank tubes, 0.5 ml of alcoholic potassium hydroxide solution were added and the mixture was saponified in a 60°C to 70°C water bath for 20 minutes. 0.5 ml of 0.2 N sulphuric acid was added and heated in a boiling water bath for 10 minutes. After cooling the tubes, 0.1 ml of sodium metaperiodate was added and allowed to stand for 10 minutes.

The excess periodate was reduced by the addition of 0.1 ml of sodium arsenite. Then 5.0 ml of chromotropic acid reagent was added, mixed thoroughly and kept in a boiling water bath for 30 minutes. After cooling, 0.5 ml of thiourea solution was added. The colour developed was read at 540 nm against a blank in a Shimadzu spectrophotometer. Triacylglycerol content was expressed as mg/dl in plasma and mg/g in fresh tissue.

3.11. Histopathological studies

At the end of the study i.e. on 45th day the rats were sacrificed and the liver tissues were collected. The whole histopathological process was carried out in accordance with the SOPs (Standard Operating Procedures). The rats were anesthetized by using diethyl ether. Using large scissors a cut was made laterally from just below the rib cage and extended fully to sides. Again using scissors another cut was made to cut the diaphragm fully on the border of the rib (from centre to sides).

Using hemostats fasten to hold, the chest cavity was opened. Then liver was removed in a systematic fashion and a requisite tissue sample was taken for the histology. The tissue sample was kept in 10% formalin for overnight. Then the sample was dehydrated in graded alcohol. Isopropyl alcohol was used. Dehydration was done by passing the tissue through increasing concentration of alcohol. 70% alcohol for 1 hour, 90% alcohol for 1 hour, and 100% alcohol for 1 hour in an automatic tissue processor. After that it was cleaned in xylene.

Dehydrated tissues were kept in a jar of alcohol-xylene mixture for 30 min and then subjected to 2 changes of xylene of 30 min each. The liver samples were then impregnated in paraffin wax (58-60 ^{0}C). After clearing, the tissue pieces were subjected to 2 changes of melted paraffin wax of 2 hours each. Then paraffin blocks were made in which samples were embedded and allowed to cool.

Hardened blocks were taken for sectioning and staining as per the standard procedure of DSRU-RRIUM, Sgr. [RRISGR/SOP-TX/Histo/007 (Section cutting and Staining)]. All the tissue blocks were kept in freezer for 20-30 min before cutting. This process hardened the tissue blocks. Using automated microtome liver samples were cut into sections of 5µ thickness and with the help of forceps placed in the tissue flotation bath which was kept at 40^{0}C. Then the sections were lifted on slide and kept in a slide tray.

This slide tray was kept in oven at 60^{0}C for 5 min. The slide tray was then removed outside and let to cool. This process fixed the tissue section to the slide. Then again the slide tray was

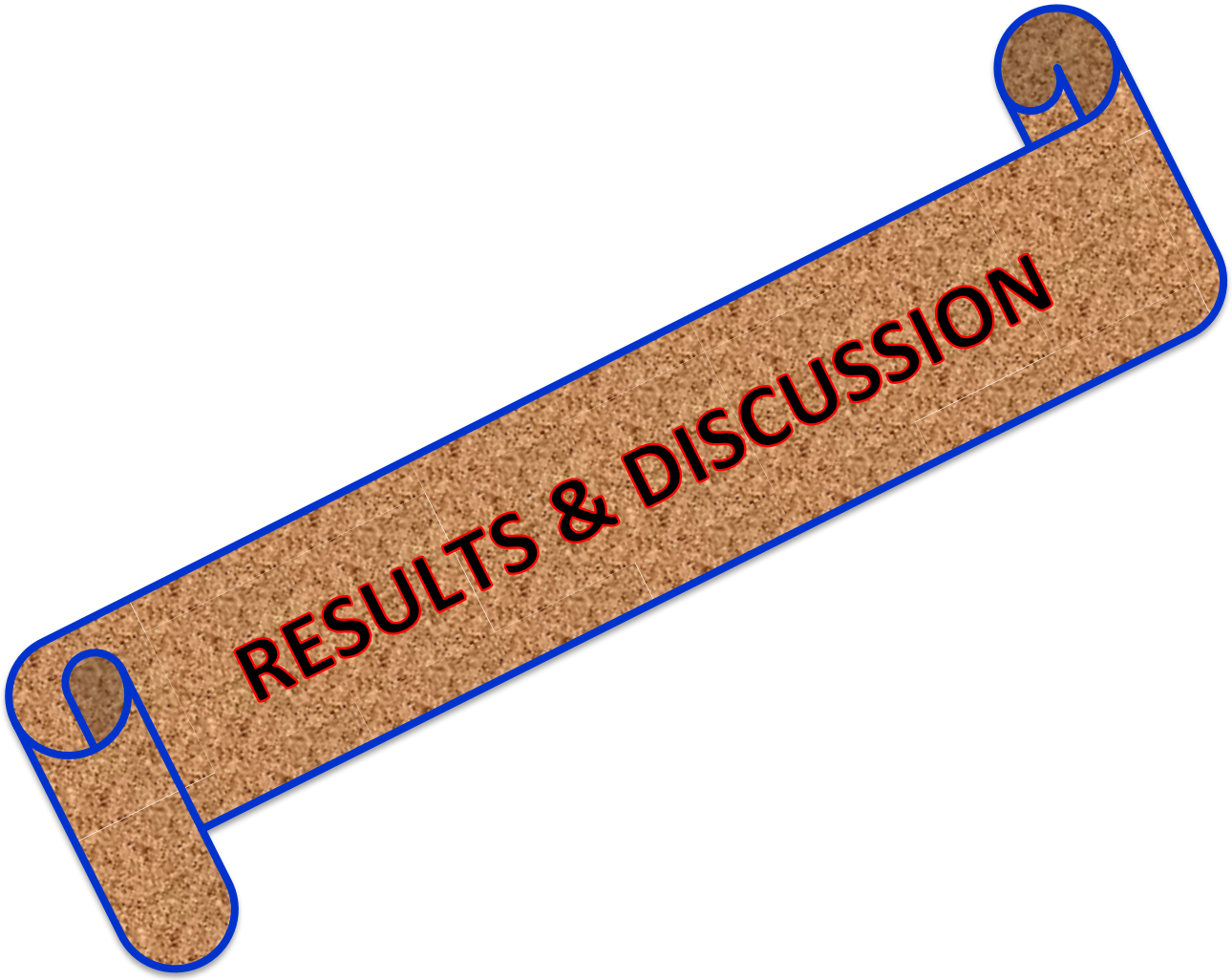
kept in oven for next 5 min. The hot slide was removed from the oven and placed in slide rack. The slide rack was dipped in a beaker containing xylene; the same was repeated in another beaker containing xylene. The slides were then subjected to staining using hematoxylin and eosin. The slide rack was passed through the decreasing concentration of alcohol. 100% alcohol 1 dip, 90% alcohol 1 dip, 80% alcohol 1 dip, 70% alcohol 1 dip and then dipped in a beaker containing tap water.

After that the slide rack was immersed in hematoxylin jar for 15-20 min and then again dipped the slide rack in a beaker containing tap water. The slides were decolorized by a single dip in 1% acid alcohol and then dipped the in a beaker containing tap water. The slide rack was dipped in Scotts tap water (3-5 dips) for bluing and the slide rack was then dipped in a beaker containing tap water. The slides were counter stained by a dip in Eosin for 1 minute and again the slides were dipped in a beaker containing tap water.

Then the slides were subjected to a single dip in 100% alcohol, 90% alcohol, 80% alcohol, 70% alcohol and in xylene. The extra stain was cleaned with gauge dipped in xylene. The stained sections were mounted using DPX and covered with the cover slip. The slides were labeled with self sticking labels. The slides were then observed under microscope and photographed in order to check the effect of the extracts on the liver and the slides were then kept safely.

3.12. Statistical analysis

All the values of body weight, fasting blood sugar, and biochemical estimations were expressed as mean ± standard deviation (S.D.) and analyzed for ANOVA and post hoc Dunnett's *t*-test. Differences between groups were considered significant at $P<0.001$ and $P < 0.05$ levels.


RESULTS & DISCUSSION

CHAPTER-IV RESULTS& DISCUSSION

4.1. Acute toxicity testing

Acute toxicity studies revealed that Oral administration of graded doses (100, 250, 500, 1000, 1500 and 2000 mg/kg bw) of the alcoholic extract of *F. arabica* to rats. The ethanolic extract of *F. arabica* was safe up to 2000mg/ kgbw and did not produce any mortality and changes in behavior, breathing, cutaneous effects, sensory nervous system responses or gastrointestinal effects during the 10 days observation period. No lethality or any toxic reactions or moribund state was observed up to the end of the study period. The obtained results signify that the use of the plant for treatment is safe. So the dose of 250 mg/ kg bw was selected to study the anti – diabetic property of the plant extract (FAEXT).

4.2. Effect of ethanolic extract of *F.arabica* on body weight of Alloxan monohydrate induced diabetic rats.

The present study was an attempt to elucidate the anti-diabetic effect of ***F.arabica*** ethanolic extract (FAEXT) which showed to be anti-hyperglycemic. Before the supplementation of FAEXT and Glibenclamide, there were no significant differences of baseline body weight of the rats. The FAEXT (250 mg/kg) and Glibenclamide (10 mg/kg) treated rats, showed significant increase in body weight as compared with diabetic groups after 6 weeks of study. Before treatment, the fasting glucose level was significantly higher ($P \leq 0.05$) in all groups when compared with normal group. After 4 weeks, groups treated with FAEXT showed dose dependent reduction of fasting glucose versus diabetic group ($P \leq 0.05$).

After 45 days of FAEXT supplementation to the diabetic rats, there was a significant elevation in insulin level in respect to diabetic control group in dose dependent manner ($P \leq 0.05$, Table 1). From the dose fixation studies, 250 mg/kg bw was found to be the most effective in reducing fasting glucose levels.

Groups	1st day	7th day	14th day	21st day	28th day	35th day	42th day	45thday
Control	**240±4.99**	**244±4.35**	**250±3.77**	**260±4**	**267±7.5**	**280±3.9**	**285±3.9**	**290±5.6**
AM-induced diabetic rats (150mg/kgbw)	**230±5.75***	**228±4.32***	**220±4.48***	**215±5.25**	**200±5**	**205±8**	**200±5.6**	**195±2.8**
FAEXT (250mg/kgbw)	**246±5.49**	**258±6.30***	**264±5.44***	**270±2.9**	**280±5.6**	**288±5**	**290±5.5**	**290±4.8**
AM-induced Diabetes + FAEXT	**226±6**	**230±6.41**	**210±5.707***	**190±4.5**	**200±8**	**215±3**	**220±6.1**	**225±4.9**
Glibenclamide (10mg/kgbw)	**232±5.98***	**244±5.48****	**254±4.62***	**265±8**	**275±5**	**268±5**	**270±2.3**	**280±2.6**

Tab.1. Effect of ethanolic extract of *F.arabica* on **body weight** of Alloxan monohydrate induced diabetic rats.

Data represented as mean ± S.D values of 6 animals each. *$p<0.001$, **$p<0.05$ (Dunnett t-test); diabetic control was compared with the normal, extract and standard treated groups were compared with the diabetic control.

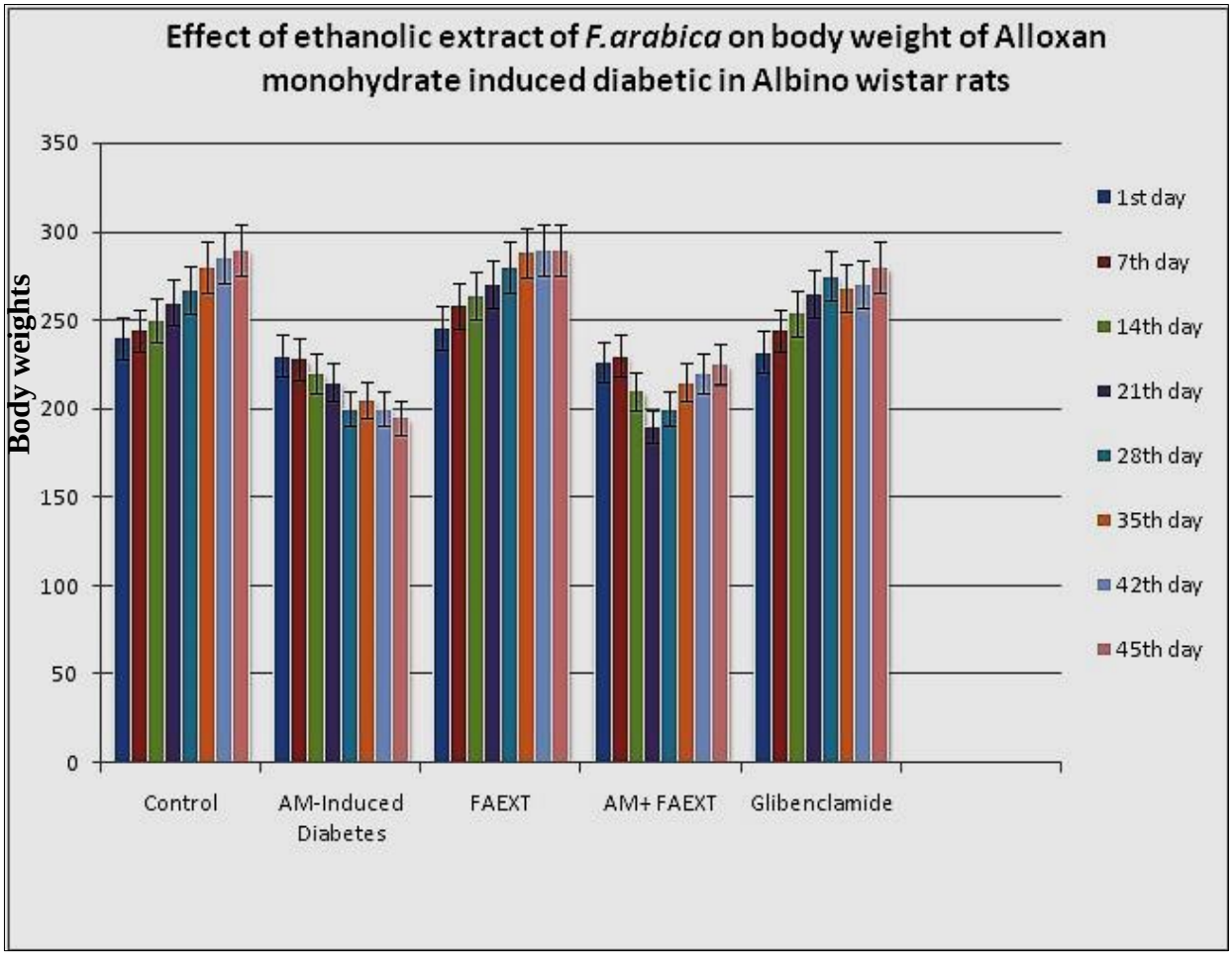


Fig.13. Effect of ethanolic extract of *F.arabica* on **body weight** of Alloxan monohydrate induced diabetic rats.

4.3. Effect of ethanolic extract of *F.arabica* (FAEXT) on fasting blood glucose levels:

As shown in Table, the induction of diabetes has caused significant initial increase in the fasting blood glucose levels of all the groups. The diabetic control group shows significant increase throughout the study period when compared with the normal control group ($p<0.001$). However, the extract treated groups and the standard treated group shows significant decrease in the fasting blood glucose levels when compared with diabetic control ($p<0.001$) which was determined on the 45th day of experiment. The effect is more pronounced in standard (10mg/kg) group, followed by ethyl acetate (500mg/kg) group, aqueous (500mg/kg) group, ethyl acetate (250mg/kg) group, aqueous (250mg/kg) group, methanol (500mg/kg) group and methanol

(250mg/kg) group as shown in Table 2. On the basis of these observations only ethyl acetate and aqueous extracts were selected for further analysis of antidiabetic activity.

Groups	**1st day**	**7th day**	**14th day**	**21th day**	**28th day**	**35th day**	**42th day**	**45th day**
Control	**84.01±4.64**	**82.21±5.58**	**80.29±6.53**	**90±5.4**	**82.6±6.2**	**85.29±5**	**90±2.9**	**92±4.9**
AM diabetes (150mg/kgbw)	**334.77±13.51***	**356±16.53***	**374±12.48***	**360.64±7**	**358.46±6.3**	**380±3.2**	**320±5.2**	**340±5.2**
FAEXT (250mg/kgbw)	**90.1±11.14**	**99±15.15***	**135.2±11.00***	**82±0.9**	**82±4.5**	**89±4.3**	**95±2.3**	**80±5.2**
AM+FAEXT	**330.1±10.2**	**250±9.67***	**240±8.99***	**225±7.5**	**256±5.4**	**229±5.7**	**195±2.9**	**158±4**
Glibenclamide (10mg/kgbw)	**90.9±14.22**	**95±16.06***	**104.4±16.88***	**96±5**	**82.8±5.02**	**84±5.2**	**85±6.8**	**80±3.2**

Table: 2 Effect of FAEXT on fasting **blood glucose levels** of alloxan monohydrate induced diabetic rats.

Data represented as mean ± S.D values of 6 animals each. *p<0.001, **p<0.05 (Dunnett t-test); diabetic control was compared with the normal, extract and standard treated groups were compared with the diabetic control.

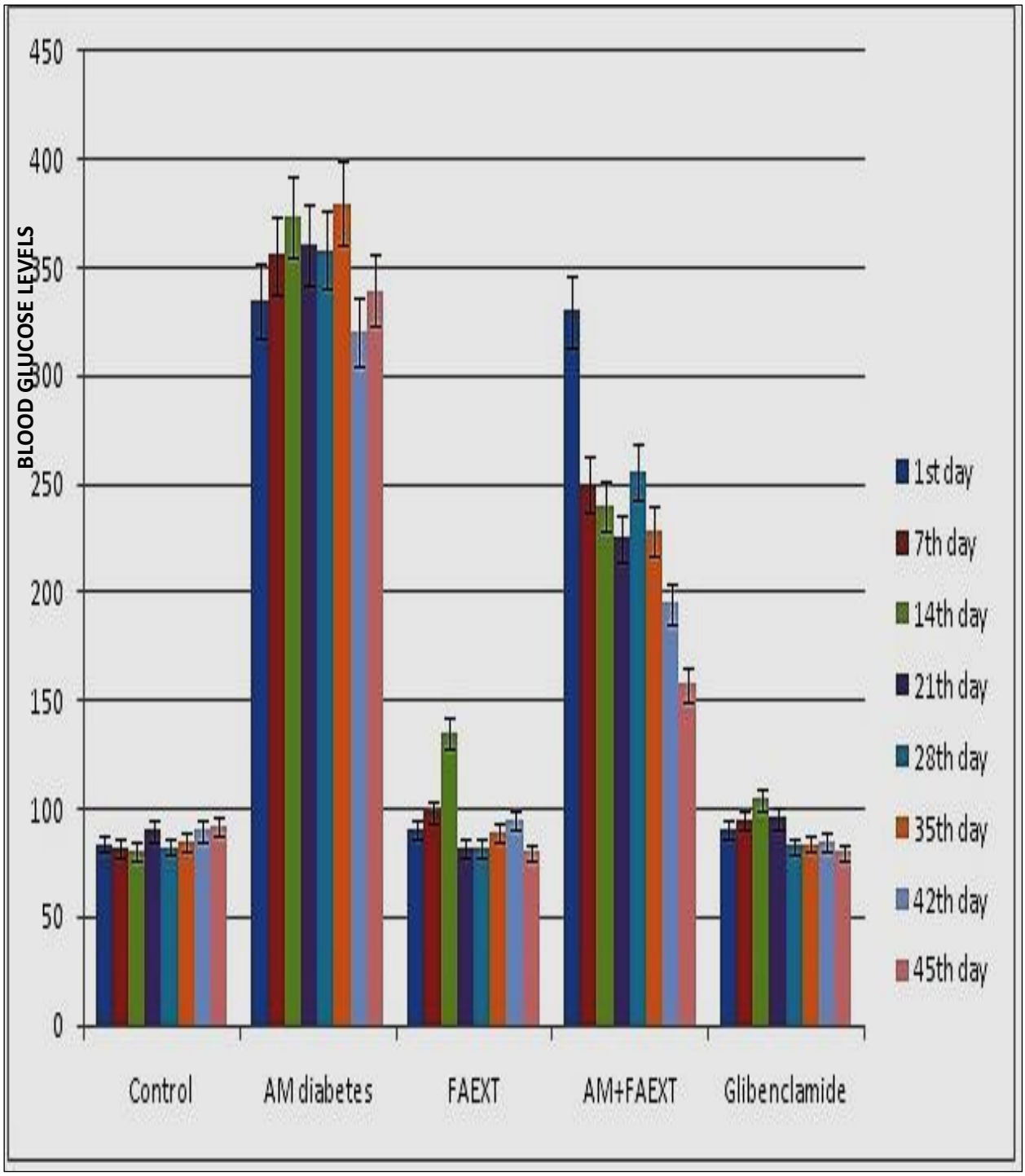


Fig.14. Fasting **blood glucose levels** of Alloxan monohydrate induced diabetic rats

4.4. Effect of ethanolic extract of *F.arabica* on AST, ALT and ALP levels:

AST, ALT and ALP, as shown in Table 3 both the extracts show significantly lower levels of AST, ALT and ALP in comparison to the diabetic control group ($p<0.001$). Here the maximum reduction was observed for standard followed by FAEXT.

Parameters	Control	AM-induced diabetic rats (150mg/kgbw)	FAEXT (250mg/ kgbw)	AM-induced Diabetes + FAEXT	Glibenclamide (10mg/kgbw)
AST (IU/100ml)	35.1±6.30*	74.07±9.41**	15.96±7.41*	42±10.27*	25±5.90
ALT (IU/100ml)	28.78±5.40*	52.2±10.11	24.01±3.80	38±2.07*	20.46±6.30**
ALP (IU/100ml)	12.9±1.4	23.6±2.1a**	20.47±1.1	14.0±0.8b**	12.5±1.5a**

Table 3. Effect of FAEXT on **AST, ALT and ALP** levels of alloxan monohydrate induced diabetic rats.

Data represented as mean ± S.D values of 6 animals each. *$p<0.001$, **$p<0.05$ (Dunnett t-test); diabetic control was compared with the normal, extract and standard treated groups were compared with the diabetic control.

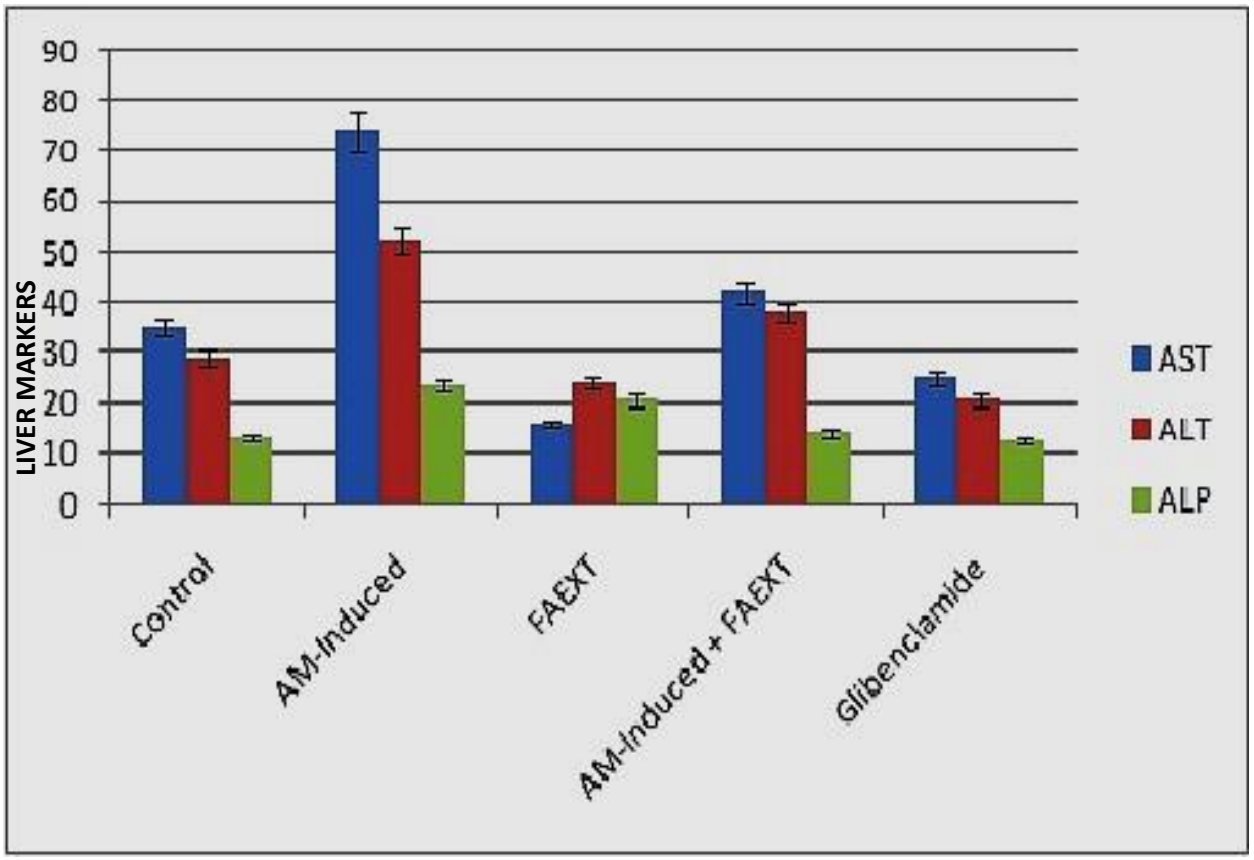


Fig.15. **AST, ALT and ALP** levels of Alloxan monohydrate induced diabetic rats.

An increase in the AST, ALT and ALP activities was recorded in diabetic rats in comparison with non-diabetic rats, indicating an altered liver function in diabetic condition. *FAEXT* significantly controlled AST, ALT and ALP values in the alloxan induced diabetic rats. In diabetic animals a change in the serum enzymes is directly related to changes in the metabolism in which these enzymes are involved. The increased levels of transaminases which are active in the absence of insulin because of increased availability of amino acids in diabetes (Bondy et al., 1949; Felig et al., 1970) are responsible for the increased gluconeogenesis and ketogenesis observed in diabetes.

In the present study, the *F.arabica* extracts significantly decreased AST and ALT enzyme activities. Hence, the improvements noticed in the levels of these enzymes are as a consequence of an improvement in the carbohydrate, fat and protein metabolism. The restoration of AST and ALT levels after treatment also indicates a revival of insulin secretion. Elevation of ALP has

been reported in diabetic rats (Mishima, 1967) and rabbits (Begum et al., 1978). This increase in ALP was significantly reversed by the extract of *FAEXT*.

4.5. Effect of ethanolic extract of *F.arabica* on Lipid profile of Alloxan monohydrate induced Diabetes in Albino wistar rats:

Groups	Lipid Profile Studies			
	Total Cholesterol (mg/dl)	**Triglycerides (mg/dl)**	**HDL (mg/dl)**	**LDL (mg/dl)**
Control	**138.4±3.51**	**78±5.01***	**128±12****	**68.56±5.6***
AM-induced diabetic rats (150mg/kgbw)	**204.77±7.51***	**256.20±8.53***	**51±7.48***	**139.1±2.9***
FAEXT (250mg/kgbw)	**99.80±6.14**	**90.30±7.15***	**162.04±5***	**90±9.1**
AM-induced Diabetes + FAEXT	**166.10±5.75**	**220.80±7.67***	**70±2.9**	**103±5***
Glibenclamide (**Standard**) (10mg/kgbw)	**99.72±8.9**	**65±5.55***	**135±5.78**	**79±5.67***

Table 4. Effect of ethanolic extract of *F.arabica* on **Lipid profile**

Data represented as mean ± S.D values of 6 animals each. *p<0.001, **p<0.05 (Dunnett t-test); diabetic control was compared with the normal, extract and standard treated groups were compared with the diabetic control.

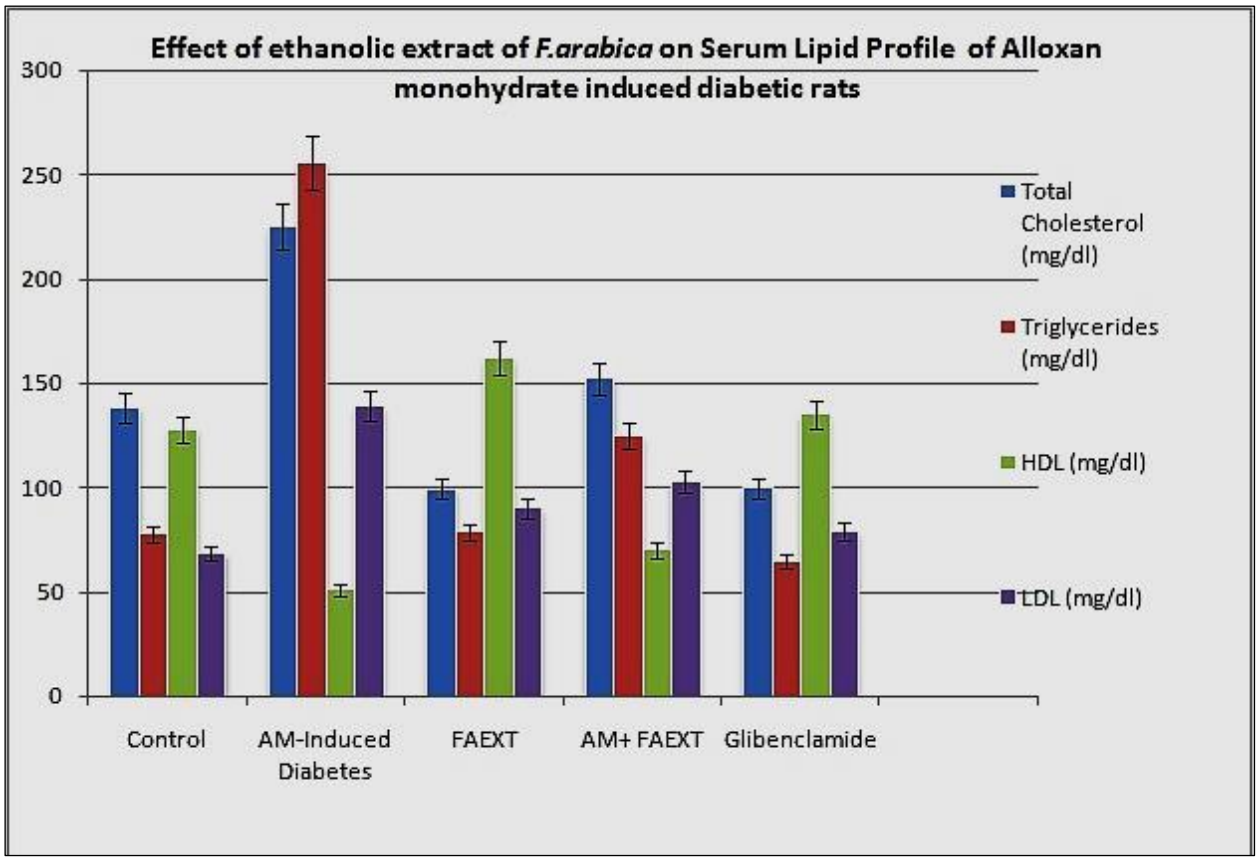


Fig.16. Effect of ethanolic extract of *F.arabica* on **Lipid profile** of Alloxan monohydrate induced Diabetes in Albino wistar rats

Moreover, hyperglycemia in diabetic rats was associated with a high serum concentration of total cholesterol and triglycerides as present in the normal diabetic conditions (Georg and Ludvik, 2000). However, the active compound from FAEXT at a dose level of 10 mg/kg reversed the diabetes-induced hyperlipidemia compared to the diabetic control group.

In FAEXT treated rats, there was a reduction in the levels of cholesterol and triglycerides, showing the hypolipidemic effect of this plant. The hypolipidemic effect may be due to inhibition of fatty acid synthesis (Sharma et al., 2003). In normal metabolism insulin activates the enzyme lipoprotein lipase and hydrolyses triglycerides and the deficiency in insulin results in inactivation of these enzymes thereby causing hypertriglyceridemia. The significant reduction of serum lipid levels in diabetic rats after treatment with extracts of FAEXT may be directly attributed to improvements in insulin levels.

4.6. Histopathological studies

Fat accumulation in liver and inflammation were reduced with the addition of active compound of FAEXT revealed. The presence of secondary metabolites that have been shown to possess antidiabetic effect in other plants (Marles and Fransworth, 1995; Saxena et al., 2004). Saponins (Abdel- Zaher et al, 2005), alkaloids (Li et al, 2004) and flavonoids (Coskul et al, 2005; Tanko et al, 2007) which were responsible for the anti-diabetic effect in other plants were also detected in the extracts of this plant.

The standard treated group also shows recovery and tends to approach the histopathology of the normal rat liver.

'A' = Normal rats,

'B'= Alloxan monohydrate (AM) (150 mg/kgbw) rats,

'C' = FAEXT 250 mg/kgbw,

'D' = AM group (150 mg/ kgbw) + FAEXT 250 mg/kgbw,

'E' = Reference control i.e. Standard drug (Glibenclamide, 10 mg/kgbw).

- CV: Central Vein; H: Hepatocytes; NC: Necrotic Changes; C: Congestion.
- Figure. Effect of FAEXT administration on liver Histopathological changes.

Development of phyto-medicines is relatively inexpensive and less time consuming; it is more suited to our economic conditions than allopathic drug development which is more expensive and spread over several years. Tissue sections from liver of normal controls showed normal architecture. The diabetic control showed dilated blood vessels, nuclear vacuolation and focal fatty infiltration.

Treatment with the active compound FAEXT at lower dose improved the lesions. Mild necrosis was observed in diabetic rats which received Alloxan monohydrate. Necrosis was seen at higher doses of both the extracts on non-diabetic rats as well. Toxic changes in the histology of liver were observed cytoplasmic vacuolation, hydropic changes, and inflammation of portal veins. Eosin (a red fluorescent dye that is a bromine derivative of fluorescein). haematoxylin (Tissues are stained in aqueous hematoxylin after mordanting in iron ammonium sulfate (iron alum)).

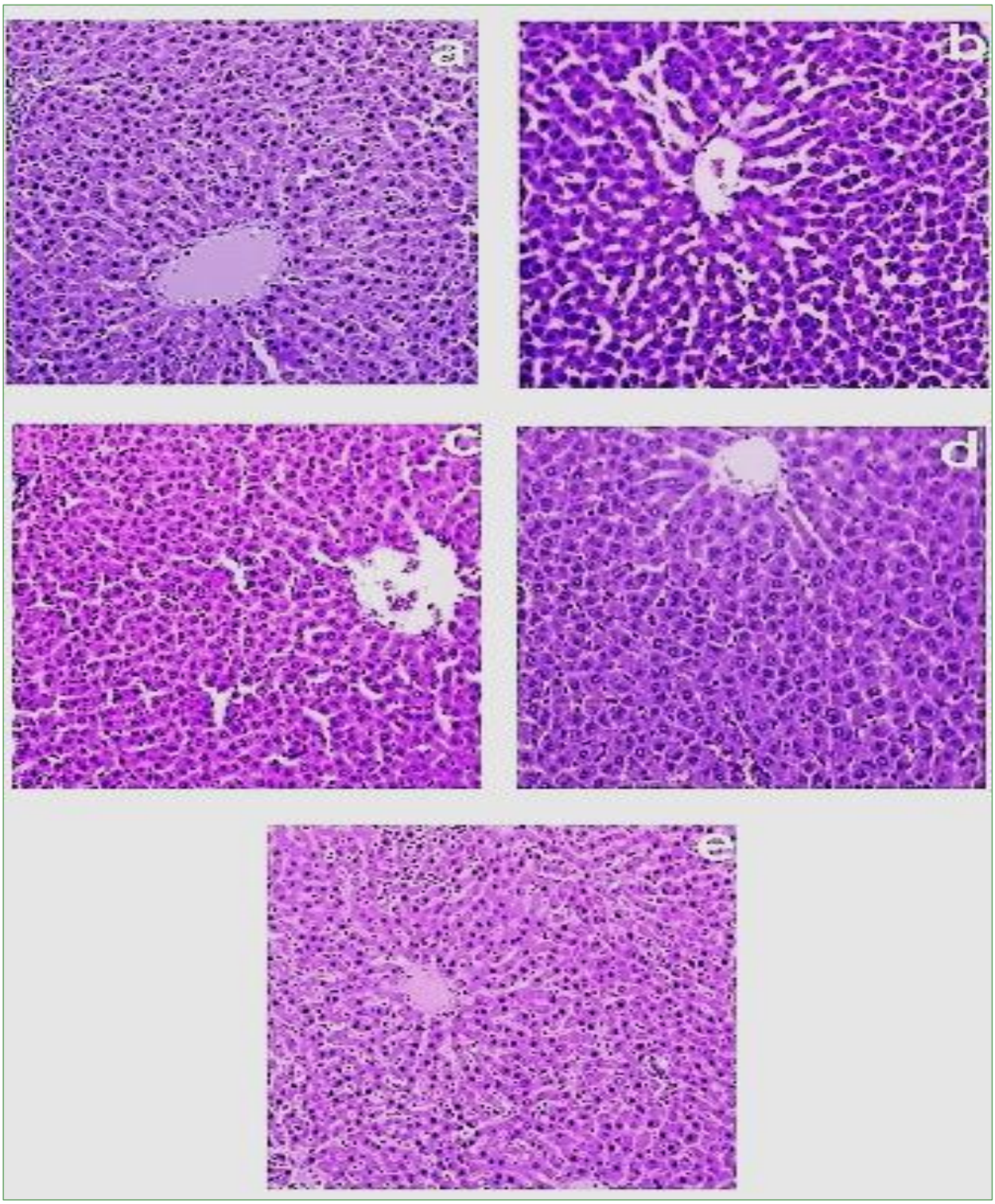
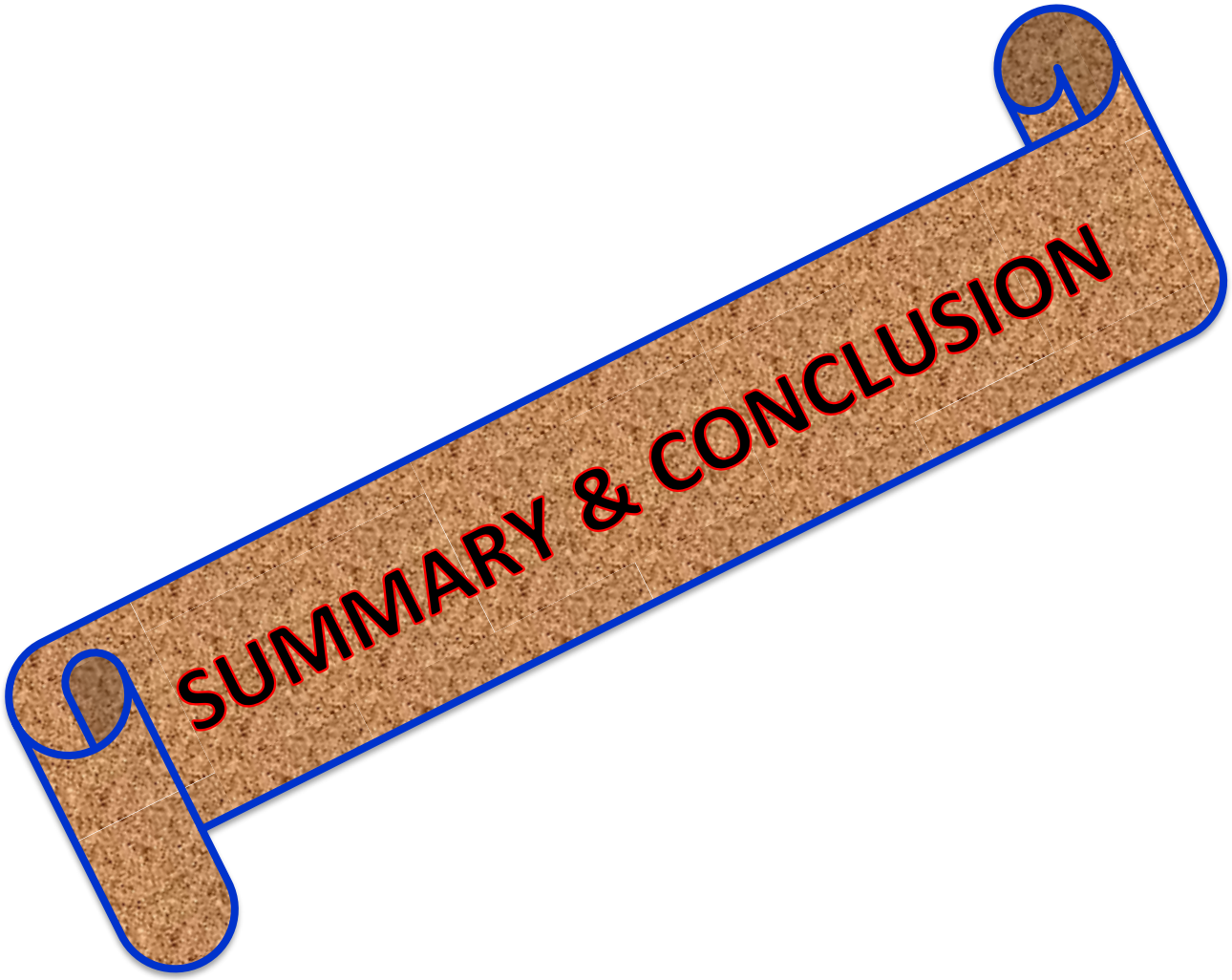


Fig. 17. (A, B, C, D, E): Micrographs of rat liver stained by haematoxylin and eosin of A, B, C, D & E group rats.


SUMMARY & CONCLUSION

CHAPTER-V SUMMARY AND CONCLUSION

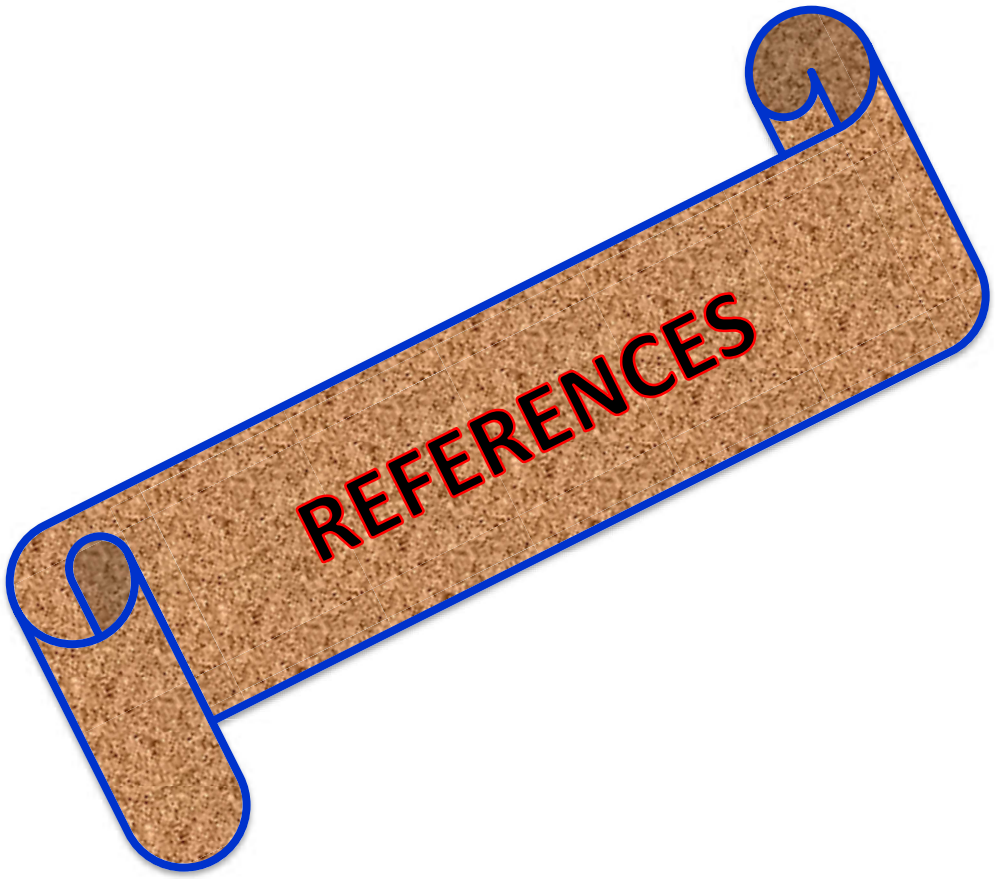
- The alcoholic extract of *F.arabica* showed anti-diabetic activity against Alloxan monohydrate induced Diabetes in Albino wistar rats.
- In conclusion, the plant is safe for use as no mortality was recorded in the acute toxicity test. The study was performed to find out beneficial Anti-diabetic effects of the *F.arabica* through animal studies, revealed that the plant extract is having protective effect against Diabetes.
- Alloxan monohydrate was used induce the diabetes and Glibenclamide was used as standard drug.
- There are increase in the levels of Ch, TG, LDL and decrease of HDL level in group B compared to control group A by using AM induced diabetes.
- Decrease of Ch, TG, LDL levels and increase of HDL level in group C compared to the diabetic control group B by using FAEXT showing the hypolipidemic effect of this plant .
- In Glibenclamide treated rats, there are reduction in the levels of Ch, TG, LDL and increase level in HDL in group E compared to the diabetic control group B . The hypolipidemic effect may be due to inhibition of fatty acid synthesis.
- FAEXT (250mg/kgbw) was showed beneficial effects on blood glucose levels of normal in rat models and we found best results and the results also reveal that the significantly protect from other metabolic aberrations found in diabetes, physiological as well as biochemical aberrations.
- Effective blood glucose and hypertension control is the key for preventing or reversing diabetic hypertensive complications. The results indicated that the FAEXT is most potent in lowering the fasting blood glucose level of the diabetic rats; the effect is dose dependent. Moreover, the extract showed improvement in parameters like body weights and fasting blood Glucose levels.
- The FAEXT also lower serum ALT, AST, and ALP levels which show the effect of the active compound in reversing the organ damage due to diabetes which is clearly observed by high levels of AST and ALT in diabetic control.

- Lipids are very susceptible to attack by free radicals, and oxidized LDL species appear to contribute to the atherosclerosis pathobiology within the artery wall. In recent years, several compounds from vegetables and fruits i.e., food especially rich in natural antioxidants like polyphenols, have been used to prevent oxidized LDL (oxLDL) formation. The FAEXT also lower the levels of serum lipids like triglycerides, LDL and cholesterol (Lipid profiles) to protective of atherosclerosis.
- Histopathological examination of liver showed the recovery of damaged tissue when sections of treated groups are compared with diabetic control.

Besides drugs in modern medicine, several species of plants have been described in the scientific and popular literature as having hypoglycemic activity. Because of their perceived effectiveness, minimal side effects in clinical experience and relatively low costs, herbal drugs are prescribed widely even when their biologically active compounds are unknown.

RECOMMENDATION

In order to maintain health where phytochemicals are involved, a daily recommended allowance similar to other nutrients is required. From the present study we observed that the ethanolic extract of the *F.arabica* (250mg/kgbw) is safe and showed protection against Alloxan monohydrate induced Diabetes. Therefore, there is a need for dosage allowance for each bioactive compound from *Fagonia arabica* (L.).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