EVALUATION OF ANTIHYPERGLYCEMIC EFFECT OF ALOE VERA GEL EXTRACT IN NORMAL RATS AND STREPTOZOTOCIN INDUCED DIABETIC RATS

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TAMILNADU.

Madurai

10.2017

CERTIFICATE

This is to certify that the dissertation entitled **"EVALUATION OF ANTIHYPERGLYCEMIC EFFECT OF ALOE VERA GEL EXTRACT IN NORMAL AND STREPTOZOTOCIN INDUCED DIABETIC RATS"** is a bonafide record of work done by **Dr. S. MEENAMBAL**, under the guidance and supervision of **Dr. K.RAADHIKA, M.D.**, Associate Professor, in the Institute of Pharmacology, Madurai Medical College, Madurai during the period of her postgraduate study of M.D Pharmacology from 2015-2018.

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This is to certify that the dissertation entitled **"EVALUATION OF ANTIHYPERGLYCEMIC EFFECT OF ALOE VERA GEL EXTRACT IN COMPARISON WITH GLIBENCLAMIDE IN NORMAL AND STREPTOZOTOCIN INDUCED DIABETIC RATS"** is a bonafide record of work done by **Dr. S MEENAMBAL**, under my guidance and supervision in the Institute of Pharmacology, Madurai Medical College, Madurai during the period of her postgraduate study of M.D Pharmacology from 2015-2018.

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DECLARATION

I, **Dr.S.MEENAMBAL** solemnly declare that the dissertation titled **"EVALUATION OF ANTIHYPERGLYCEMIC EFFECT OF ALOE VERA GEL EXTRACT IN NORMAL AND STREPTOZOTOCIN INDUCED DIABETIC RATS"** has been prepared by me under the able guidance and supervision of **Dr. R. PARAMESWARI, M.D.,** Director and Professor, Institute of Pharmacology, Madurai Medical College, Madurai, in partial fulfilment of the regulation for the award of M.D Pharmacology degree examination of the Tamilnadu Dr.MGR Medical University, Chennai to be held in April 2018.

This work has not formed the basis for the award of any degree or diploma to me, previously from any other university to anyone.

Place: Madurai **Dr. S. MEENAMBAL**

Date:

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INTRODUCTION

INTRODUCTION

Diabetes mellitus is a heterogeneous disease which is mainly characterized by altered cellular metabolism arising from defects in insulin secretion or its action or both. According to American Diabetes Association, Diabetes and chronic hyperglycemia results in long- term damage to various organs and also cause organ dysfunction especially in the eyes, kidney, nerves, hearts and blood vessels and other complications such as atherosclerosis, coronary heart disease, diabetic retinopathy, nephropathy and neuropathy[1].

Diabetes mellitus has now become an epidemic. At present the worldwide incidence is 5% in the general population. Globally 100 million among the world's population are suffering from diabetes. The number is rampantly increasing and it is expected to hike upto 366 million[2]. Altered life style has been attributed for this increasing prevalence which includes decrease in physical activity, weight gain, stress and changes in food habbits[3]. In conventional therapy, type I diabetes is treated with exogenous insulin and type 2 with oral hypoglycemic agents. Though different types of oral hypoglycemic agents are available along with insulin for the treatment of diabetes, there is an increase demand by patients to use natural products with antidiabetic activity. Since time immemorial, patients with noninsulin-dependent diabetes have been treated orally in native medicine with a variety of plant extracts. In India, a number of plants are mentioned in ancient literature *(Ayurveda*) for the cure of diabetic conditions[4].

Several theories have been postulated to explain the pathogenesis of diabetes. These encompass auto oxidation of glucose and progressive non-enzymatic glycation of proteins. Enhanced glucose influx via polyol pathway which ultimately results in increased formation of glycosylation end products and leading cause of diabetic complications in both types 1 and 2 diabetes[5]. ROS produced by protein glycation and glucose oxidation mediates the pathogenic effects of high glucose.

Reactive Oxygen Species (ROS) leads to molecular damage and cellular damage by directly facilitating numerous cellular stress-sensitive pathways such as JNK/SAPK, NF-κB, p38 MAPK, and hexosamine pathway resulting in late complication of diabetes[6]. Also ROS is responsible for β-cell dysfunction and insulin resistance via same pathways. Eventhough innumerable modern medicines are available for management of diabetes, none of them is free from side effects. Hence treatment of diabetes is still remains an unmet challenge.

Antidiabetic drug, glibenclamide (GL) classified under sulfonyl ureas promotes insulin secretion by inhibiting ATP-sensitive K+ (K_{ATP}) channels in the beta cells of pancreas. GL (also known as glyburide) is a derivative of sulfonyl cyclo hexylurea. Apart from the antidiabetic property, it also possesses analgesic, anti-neoplastic and antiplatelet activities. Also it has vasodilatory effect by releasing nitric oxide which is due to its stimulatory effect on endothelial Ca^{2+} levels causing endothelium dependent relaxation activity[7]. Interestingly, research from the antioxidant studies shows that GL is efficient to counteract the reactive oxygen species mediated oxidative stress. The side effects

of oral hypoglycemic agents includes hypoglycaemia, weight gain, nausea, diarrhoea, hepatotoxicity, lactic acidosis, vitamin B12 deficiency, photosensitivity, skin rash, blood dyscrasias, cholestatic jaundice, risk of urinary bacterial and fungal infections.

This leads to the search for natural products with antidiabetic activity with fewer side effects. Numerous herbs and plant products have been shown to possess anti diabetic property. Therapeutic use of plants is deployed on the premise that plants contain natural substances, which promote health and alleviate illness. Since ancient times, number of natural products has been used to check hyperglycemia. There is continuous search for alternative drugs. Therefore, it is prudent to look for options in herbal medicines for diabetes as well. Herbal medicines have long been used effectively in treating diseases in Asian communities and throughout the world[8].

The World Health Organization (WHO) has also endorsed the screening of herbal remedies for the management of diabetes mellitus. The ethnobotanical information states that nearly 800 plants has antidiabetic property. Nowadays numerous plant extracts have been evaluated in the field of diabetic research. It has ultimately resulted in many herbal products with antidiabetic activity which can counter the high cost and poor availability of the medicines.

Various natural products like *Anacardium occidentale, Canavalia ensiformis, Catharanthus roseus, Coscinium fenestratum, Murraya koenigii* and many other natural products has been known to possess hypoglycemic activity.

Aloe vera (Barbados Aloe) is one of herbal plant shown to possess anti diabetic property. Aloe vera, often known as aloe or Gwar patta (Hindi), is classified under the family Asphodelaceae or aloe family. It has got a wide spectrum of acivitiy which includes wound healing, antifungal, hypoglycemic or antidiabetic, anti inflammatory, anticancer, immunomodulatory and gastro protective property. Aloe vera consists of many phytochemicals, vitamins, nutrients and anti-oxidants. Fresh aloe gel from the inner leaf parenchyma constitutes 96% water, polysaccharides (mucilage). This mucilage contains D-glucose and D-mannose, tannins, steroid, enzymes, plant hormones, amino acids, vitamins and minerals[9]. The aloe vera gel extract also contains appreciable amount of trace elements (Cr, Mn and Zn). Gel from the leaves of Aloe vera is responsible for many of its health benefits.

Diabetes mellitus is induced in experimental animals by using chemicals like Streptozotocin (STZ), a β- cytotoxin. It produces diabetes in a wide variety of animal species including rat by selectively damaging the insulin-secreting β-cells of the pancreas. Intraperitoneal injection of STZ causes fragmentation of DNA of β-cells of pancreas which increase ADP-ribose and decreases NAD which ultimately leads to the destruction of β- cells resulting in the clinical symptoms of hyperglycemia and hypoinsulinaemia[10]. Oxidative stress has been attributed to play a significant role in the etiopathogenesis of diabetes and its complications[11].

To bridge the existing gap and to gain knowledge about the role of complimentary alternative medicine, we have performed a study on

antihyperglycemic effect of aloe vera gel extract in normal rats and streptozotocin induced diabetic rats.

Hence, the aim of this present study is to investigate the effects of aloe vera gel extract on antihyperglycemic activity.

AIM AND OBJECTIVES

AIM

To evaluate the antihyperglycemic effect of aloe vera gel extract in normal rats and Streptozotocin induced diabetic rats by tail venepuncture method.

REVIEW OF LITERATURE

REVIEW OF LITERATURE

"History will undoubtedly record December 20th 2006 as turning point in the fight against diabetes. On this day the United Nations General Assembly passed a landmark resolution recognising diabetes as a chronic debilitating and costly disease associated with major complications that pose severe risks for families countries and the entire world."

- Professor Martin Silink, President of International Diabetes Federation

Diseases like diabetes and coronary vascular disease (CVD) have increased several fold due to immense change in pace and life style. India has the world's largest population suffering from Diabetes Mellitus. Worldwide estimates exhibit that people suffering from diabetes will rise to 366 million in 2030 from 171 Million in 2000[12]. In India, according to the 'Diabetes Atlas' 40 million people were registered with diabetes in 2007, which is anticipated to rise almost 70 million by the year of 2025, making every 5th diabetic subject in the world to be Indian. India has more vulnerability with a huge burden of diabetes due to chronic complications such as diabetic nephropathy which makes people more cripple[13]. The two most important "Life Style Diseases" commonly encountered in the community are diabetes mellitus and hypertension. The most common cause of death in the diabetic patient is heart disease[14].

Type 2 diabetes is by no means a novel disease. There is no definite description of diabetes found in the Corpus Hippocraticum, except inconclusive

descriptions by Galenus and Aretaios. It took centuries before, Thomas Willis (in 1674) described the sweet nature of urine in diabetes and Matthew Dobson (in 1776) identified sugar in the urine. In contrast, a large body of evidence points to the common presence and diagnosis of diabetes in ancient India and China, seemingly the result of genetics, lifestyle and acuity of the respective physicians.

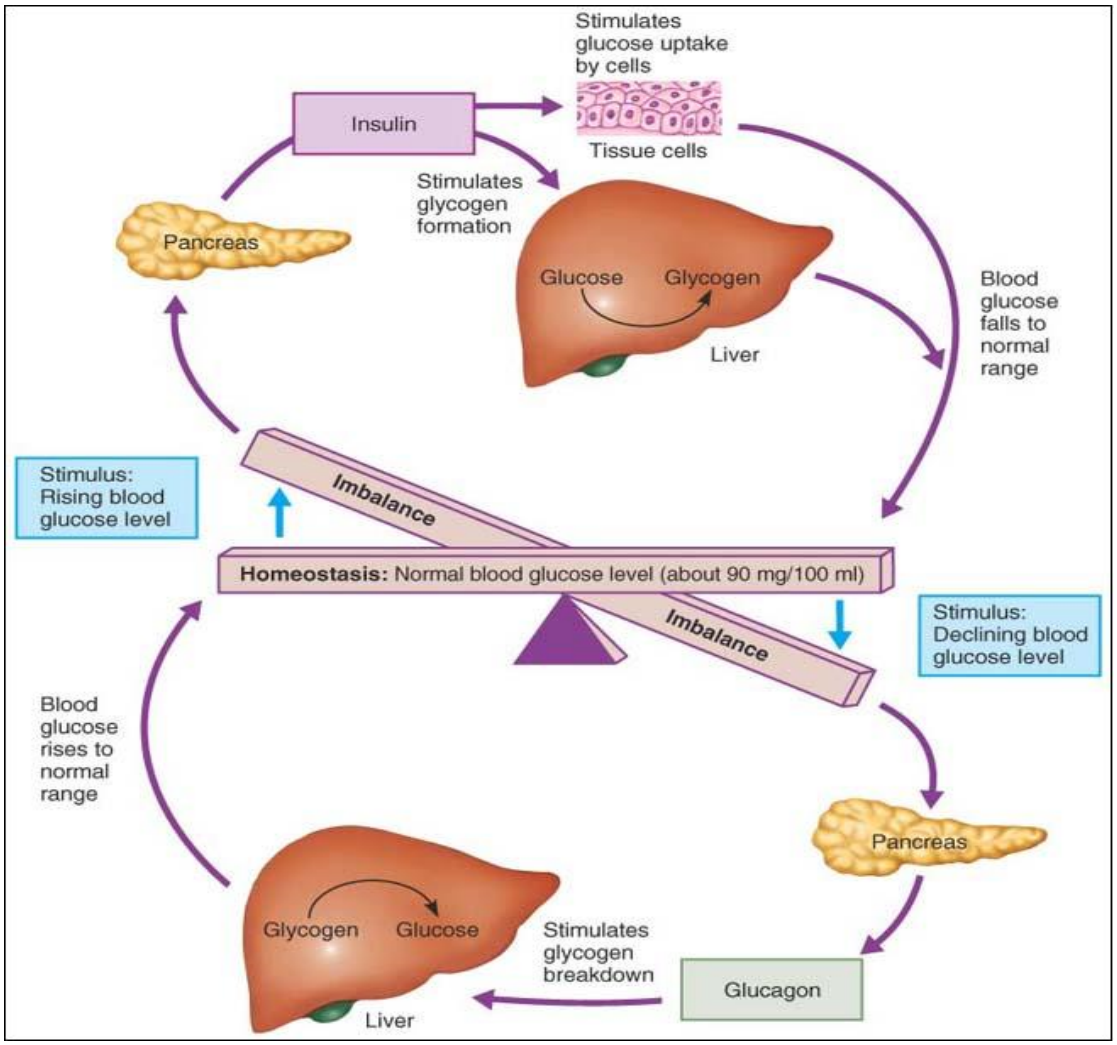
The Indian Sanskrit medicine literature was mentioned the characteristic sweet urine in diabetes likely written between 300 BC and 600 CE[15]. The ancient physicians described "sugar cane urine" (Iksumeha) or "Honey Urine" (Madhumeha and Hastimeha) as well as "urine flow like elephant in heat"; furthermore, they mentioned the observation that ants and insects rush to this type of urine[16] - suggesting that the observations concerned true glucosuria and diabetes. The three cardinal symptoms are polyphagia, polyuria and polydipsia, even secondary sequelae of diabetes such as abscess formation, carbuncles, lassitude and floppiness. Suggested interventions ranged from administration of honey and sugar in patients with Iksumeha and Mathumeha (illogical) to the rational advice of active physical exercise with long marches and riding on elephants.

EPIDEMIOLOGY

Against this long historical background, the recent abrupt rise in the prevalence of metabolic syndrome and of type 2 diabetes mellitus worldwide is new, and which is extremely patent in Asian countries and is particularly dramatic in India[17,18.]. This gave India the suspicious distinction of the "Diabetes capital of the world." Diabetic patients in India tend to have higher waist circumference despite lower body mass index, pronounced insulin resistance,

lower adiponectin levels and higher inflammatory markers. A high prevalence of the glucose intolerance and of metabolic syndrome, both precursors of diabetes has been well documented in the Indian population. The prevalence of overt diabetes is particularly high in Indian elderly - but of great concern is the frequency of obesity, prediabetes and overt diabetes in the young. Diabetes is also particularly frequent in the rural populations of India[19,20]. Increasing age, obesity, alcohol use and a family history of T2DM independently predicted the development of diabetes[21].

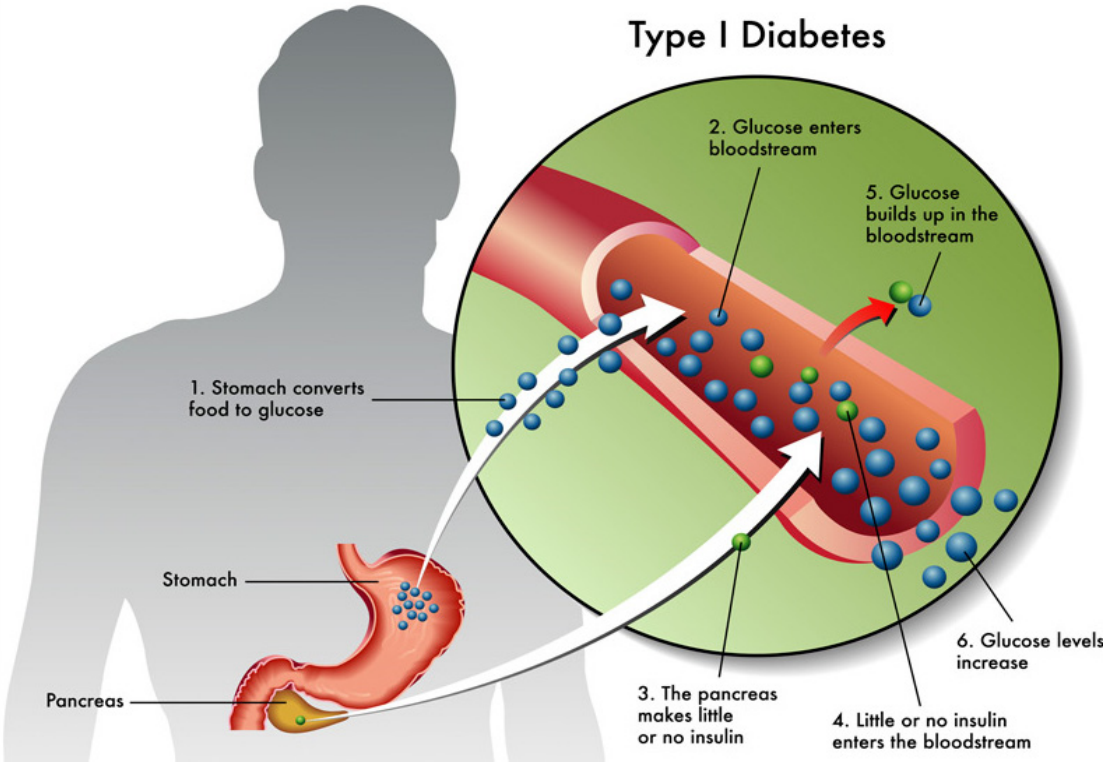
Figure 1: Glucose homeostasis



Diabetes mellitus is an endocrine as well as metabolic disorder. It may also be recognised as a disorder that ultimately affects the cardiovascular system in the form of acceleration of atherosclerosis due to changes in the basement membrane of the vascular endothelium as a result of hyperglycemia, thus leading to the cardiovascular complications such as angina pectoris and hypertension. Thus it is better to consider the diabetes mellitus as endocrine, metabolic and cardiovascular disorder as it affects all the vital systems of the body such as eyes, kidney, heart, nerves and blood vessels[22].

Diabetes mellitus can be classified into many types mainly type 1 and type 2 diabetes. Type 1 diabetes is managed with insulin and type 2 diabetes by oral hypoglycemic agents and also with insulin.

Figure 2 : Type I Diabetes



CLASSIFICATION[23]

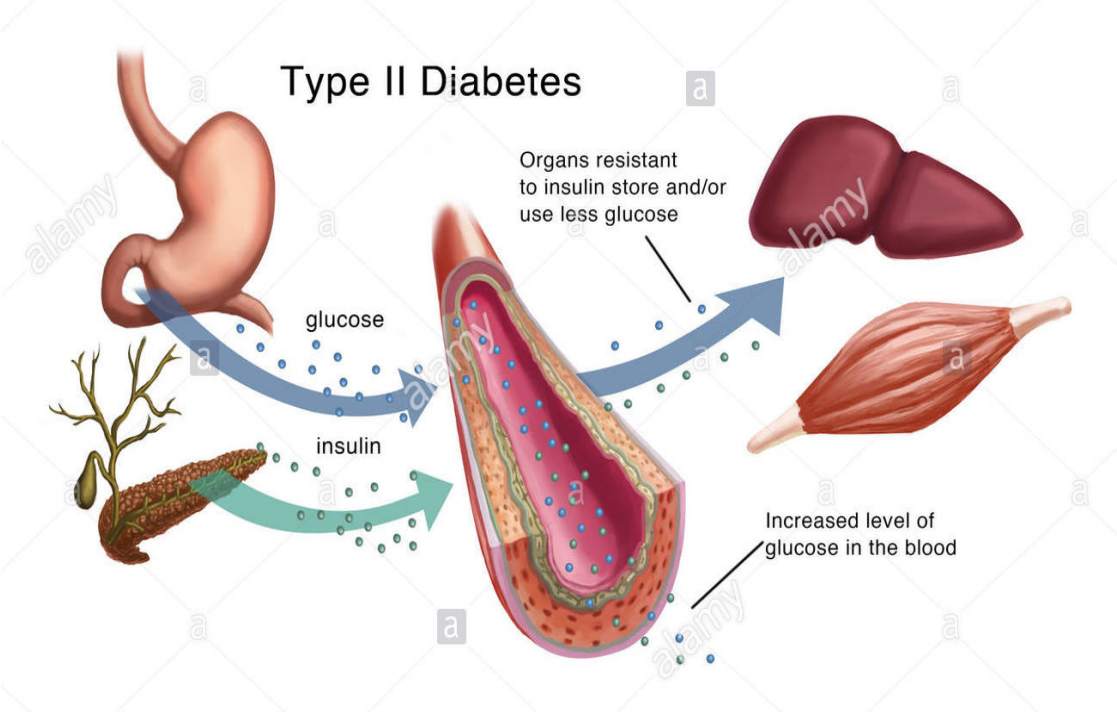
I. Type 1 Diabetes (beta cell destruction, usually leading to absolute insulin deficiency)

   A. Immune mediated

   B. Idiopathic

II. Type 2 Diabetes (may range from predominantly insulin resistance with relative insulin deficiency to a predominantly insulin secretory defect with insulin resistance)

Figure 3: Type II Diabetes



III. Other specific types of diabetes

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

      1. Hepatocyte nuclear transcription factor(HNF) 4alpha(MODY)

      2. Glucokinase(MODY 2)

3. HNF -1 alpha (MODY 3)
4. Insulin promoter factor (IPF) 1 (MODY 4)
5. HNF -1 Beta (MODY 5)
6. Neuro D1 (MODY 6)
7. Mitochondrial DNA
8. Proinsulin or insulin conversion

B. Genetic defects in insulin action:
   1. Type A insulin reistance
   2. Leprechaunism
   3. Rabson-Mendenhall syndrome
   4. Lipodystrophy syndromes

C. Diseases of the exocrine pancreas:
   1. Pancreatitis
   2. Pancreatectomy
   3. Neoplasia
   4. Cystic fibrosis
   5. Hemochromatosis
   6. Fibrocalculus pancreatopathy

D. Endocrinopathies:
   1. Acromegaly
   2. Cushing syndrome
   3. Glucagonoma
   4. Pheochromocytoma

5. Hyperthyroidism
6. Somatostatinoma
7. Aldosteronoma

E. Drug or chemical induced:
1. Vacor
2. Pentamidine
3. Nicotinic acid
4. Glucocorticoids
5. Thyroid hormone
6. Diazoxide
7. Beta adrenergic agonists
8. Thiazides
9. Phenytoin
10. Alpha interferon
11. Protease inhibitors
12. Clozapine
13. Beta blockers

F. Infections:
1. Congenital rubella
2. Cytomegalovirus
3. Coxsackie

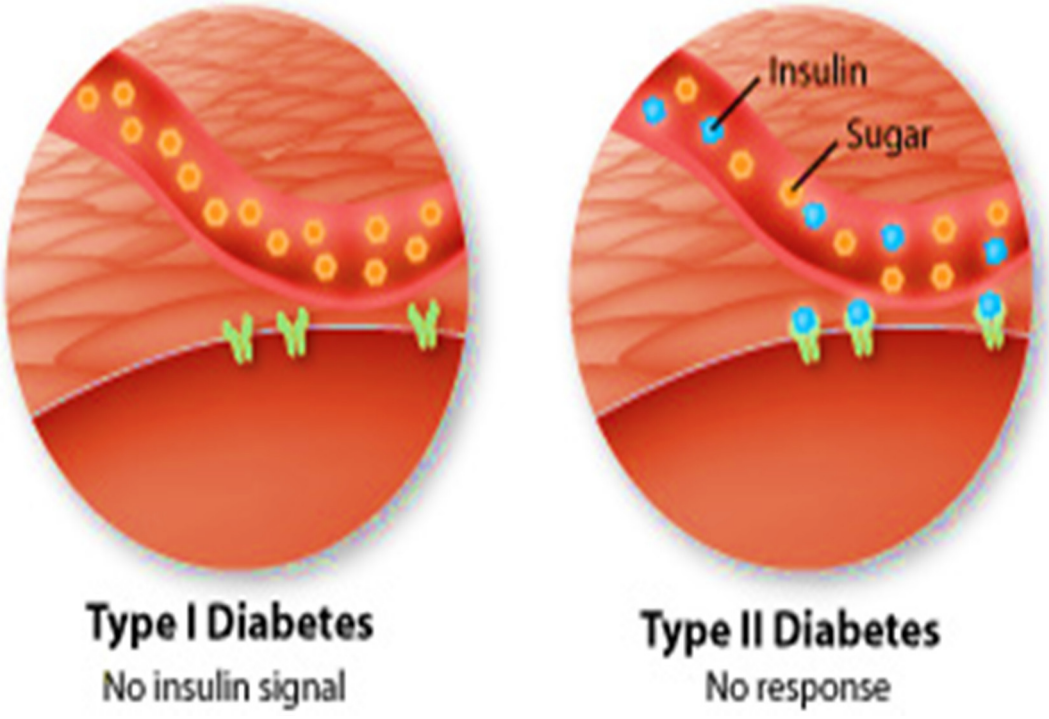
G. Uncommon forms of immune mediated diabetes:
1. Stiff-man syndrome
2. Anti insulin receptor antibodies

H. Other genetic syndromes sometimes associated with diabetes:

1. Down's syndrome
2. Klinefelter's syndrome
3. Turner syndrome
4. Wolfram's syndrome
5. Friedreich's ataxia
6. Huntington's chorea
7. Laurence – Moon Biedl syndrome
8. Myotonic dystrophy
9. Porphyria
10. Prader – willi syndrome

IV. Gestational diabetes mellitus (GDM)

Figure 4: Differentiating Type 1 and Type 2 Diabetes Mellitus



PATHOGENESIS

NORMAL GLUCOSE HOMEOSTASIS

The human body is dependent on a strict control of its blood glucose levels in order to secure normal body function[24]. After ingestion of mixed meal, 50% of glucose is utilized in brain, 25% by splanchnic and gastrointestinal systems. Both are non-insulin dependent tissues. Only remaining 25% is taken by skeletal muscle and adipose tissue which are insulin dependent.

So in a fed state there is hyperglycemia and hyperinsulinemia. This stimulates increased glucose uptake by muscles and decreased hepatic glucose production.

Adipocytes regulate release of free fatty acids from stored triglycerides. They decrease the release of free fatty acids and increase glucose uptake in a fed state. All these effects are facilitated by insulin. It also indirectly decreases the glucagon secretion there by decreasing hepatic glucose production.

Factors responsible for maintenance of normal glucose tolerance in healthy subjects:

A. Insulin secretion
B. Tissue glucose uptake
    a. Peripheral (muscle)
    b. Liver and gut (splanchnic)
C. Suppression of hepatic glucose production
    a. Decreased FFA
    b. Decreased glucagon

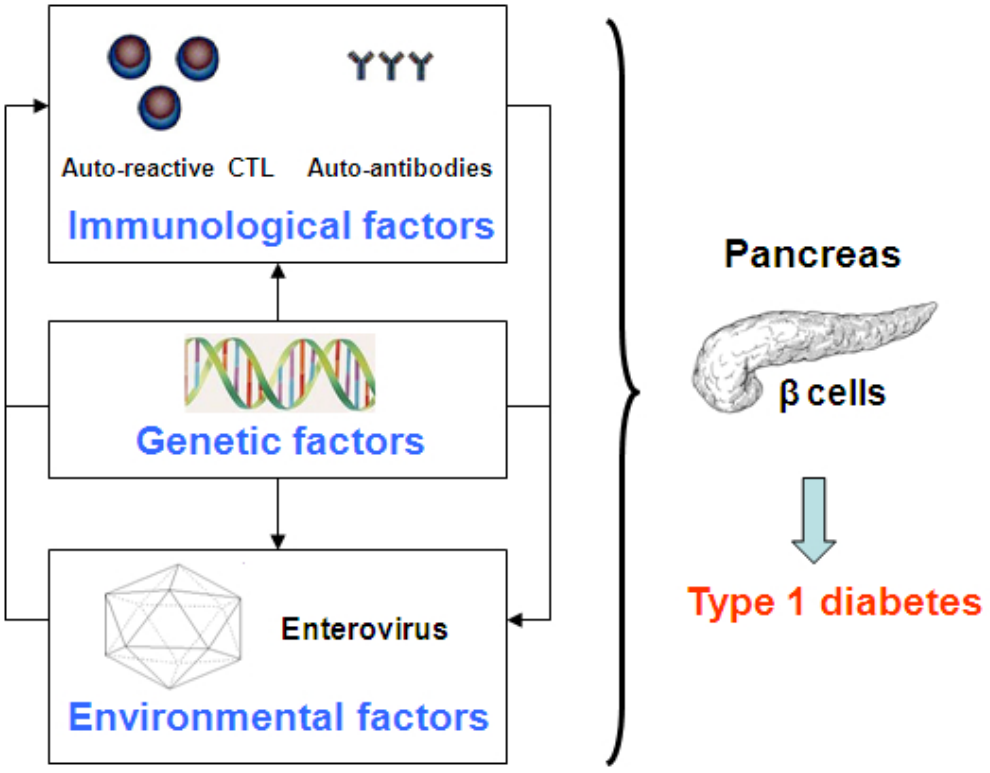
While both type 1 and type 2 diabetes result in hyperglycemia, the pathophysiology and etiology of the diseases are distinct and require us to consider each type of diabetes independently[25].

TYPE-1 DIABETES MELLITUS

This type of diabetes results from a severe absolute lack of insulin caused by reduction in beta cell mass, with preponderance for insulin dependence which can lead to development of serious complications such as ketoacidosis and coma[26].

There are three interlocking mechanisms responsible for islets cell destruction namely genetic susceptibility, auto immunity and environmental insult.

Figure 5: Mechanism of type I diabetes

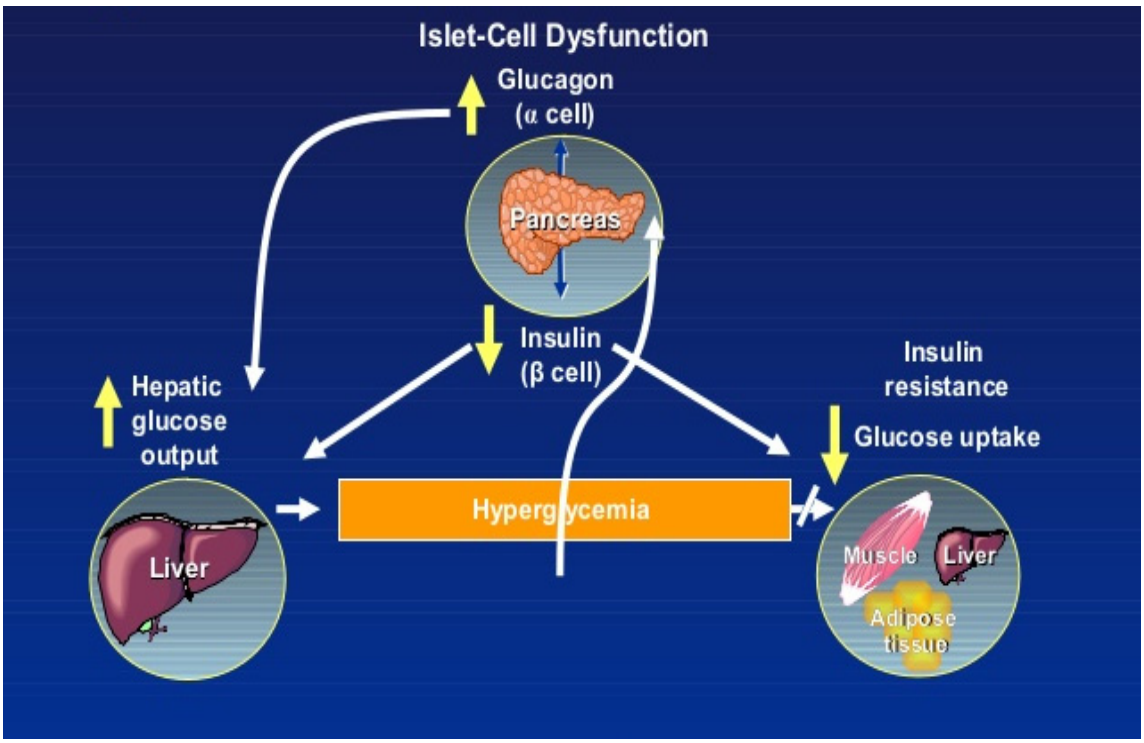


The major genetic susceptibility determinants are the highly polymorphic HLA loci on chromosome 6p21—more specifically the class II loci, HLA-*DRB1*, HLA *DQB1/DQA1* and, to a lesser extent, HLA-*DPB1/DPA1*[27]. The auto immune reaction either develops spontaneously or triggered by environmental insult.

TYPE-2 DIABETES MELLITUS

No evidence of auto immune mechanism is involved in the pathogenesis of type-2 Diabetes Mellitus. Life style clearly plays a role. Genetic factors are even more important than in type-1 Diabetes Mellitus. It appears to result from multiple genetic defects or polymorphism, each contributing its own predisposing risk and modified by environmental factors. HLA-DQA1*0301 and HLA-DQA1*0501 alleles are markers of susceptibility for T2DM[28].

Figure 6: Mechanism of type II diabetes



Beta cell mass is decreased in obese and lean type-2 diabetes mellitus.

The two metabolic effects that characterize type-2 diabetes mellitus are,

1. A derangement in beta cell secretion of insulin
2. A decreased response of peripheral tissues to respond to insulin (insulin resistance)[29]

While much has been learned in recent years, the pathogenesis of type-2 diabetes mellitus remains enigmatic. There is no evidence that auto immune mechanisms are involved.

Among the identical twins, the concordance rate is 60% to 80%. Thus, identical twin pairs show a higher concordance rate for diabetes than do fraternal twins[30].

To summarize, type-2 diabetes mellitus is a complex, multifactorial disorder involving both impaired insulin release leading to relative insulin deficiency and end organ insensitivity. Insulin resistance frequently associated with obesity, produce excess stress on beta cells, which may fail in the face of sustained need for a state of hyperinsulinemia. Genetic reactors are definitely involved but how they fit into this puzzle remains mysterious. TCF7L2 is the strongest susceptibility locus associated with beta cell dysfunction[31].

CLINICAL FEATURES

The symptoms of diabetes mellitus includes[32]

1. Extreme tiredness
2. Sudden weight loss

3. Drowsiness
4. Dry mouth
5. Thirst
6. Increased hunger
7. Increased frequency of urination
8. Blurring of vision

Frequent urination and increased thirst: When excess glucose builds up in the bloodstream, fluid is pulled from the body's tissues. Excessive thirst occurs, causing people with type 2 diabetes to drink and urinate more.

Increased hunger: In type 2 diabetes the body does not have enough insulin to send glucose to cells. This means the muscles and organs are depleted of energy, resulting in increased hunger.

Weight loss: Insufficient insulin forces the body to start burning fat and muscle for energy. This causes weight loss.

Fatigue: When cells are left without enough glucose, the body becomes tired.

Uncontrolled diabetes is associated with an increased susceptibility to infections and patients may present with skin sepsis (boils) and genital candidiasis and complain of pruritis vulvae and balanitis.

The difference between signs and symptoms of type I and type II diabetes mellitus includes :

SIGNS & SYMPTOMS	Type-1 DM	Type-2 DM
Polyuria and thirst	++	+
Weakness and fatigue	++	+
Polphagia with decrease in weight	++	-
Recurrent blurred vision	+	++
Vulvovaginitis or Pruritis	+	++
Peripheral neuropathy	+	++
Nocturnal enuresis	++	-
Asymptomatic	-	++

The physical signs in patients with type-2 diabetes mellitus at diagnosis depend on the mode of presentation. More than 70% are over weight and obesity may be central (truncal or abdominal). Hypertension is present in 50% of patients with type-2 diabetes mellitus. Hyperlipidemia is also common. Sometimes patients present with one or more of the long term complications of diabetes mellitus. They may complain of parasthesia, pain and muscle weakness in the legs with signs of peripheral neuropathy or foot ulceration or deterioration of vision from cataract or retinopathy. Signs of macrovascular complications are common and may include diminished or impalpable pulses in the feet, bruits over the carotid or femoral arteries and ischaemic toes. Cutaneous features of diabetes include a dermopathy with trophic brownish scar on the skin and necrobiosis lipoidica diabeticorum.

Reducing hyperglcemia in patients with type-2 diabetes mellitus is done with antidiabetic drugs, diet and exercises that ameliorate the underlying defect responsible for the disease.

From a therapeutic view the following points are pertinent:

1. All patients with type-2 diabetes mellitus who are hyperglycemic have some amount of deficiency of insulin secretion.
2. The higher the fasting plasma glucose level, the greater the degree of insulin deficiency.
3. Insulin resistance is present in some patients but not in all patients with type-2 diabetes mellitus.
4. The magnitude of insulin resistance varies considerably among the patients with type-2 diabetes mellitus and is significantly influenced by obesity.

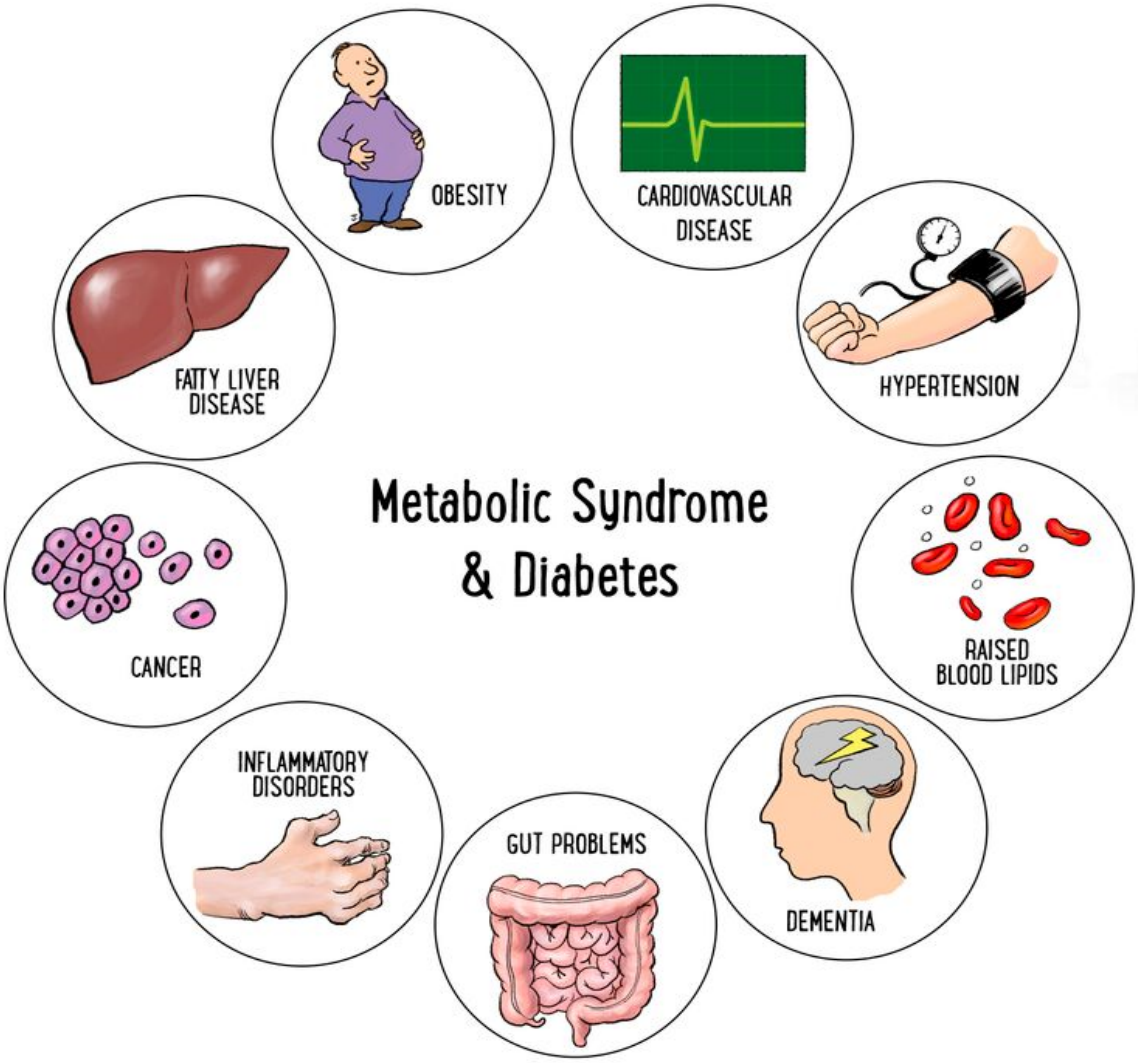
An estimate of the relative roles played by insulin resistance and insulin deficiency in the pathogenesis of hyperglycemia in a particular patient may be useful in deciding which therapeutic modalities are likely to be more effective.

METABOLIC SYNDROME

It describes risk factors that determine who will develop type-2 diabetes mellitus. They are hypertriglyceridemia, low HDL cholesterol, hypertension, abnormal fasting glucose level and abnormal visceral obesity. Hypertension and prehypertension can increase the risk of developing cardiovascular disease (CVD) and diabetes[33]. It is diagnosed by glucose tolerance test (GTT) and

treated with life style modifications and treatment of obesity. It prevents progression of impaired glucose tolerance to overt diabetes.

Figure 7: Metabolic syndrome

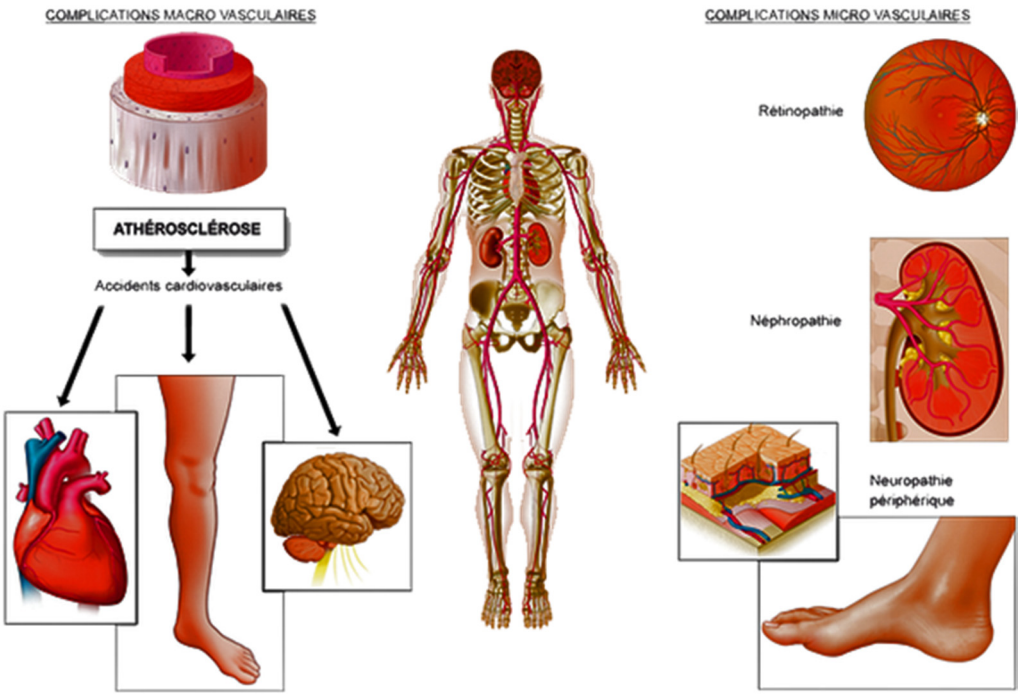


COMPLICATIONS OF DIABETES MELLITUS[34]:

ACUTE COMPLICATIONS:

- Diabetic ketoacidosis
- Hyperosmolar non ketotic diabetic coma

Figure 8: Complications of Diabetes mellitus



CHRONIC COMPLICATIONS

- Hypertension
- Renal failure
- Cataract
- Autonomic neuropathy
- Peripheral neuropathy
- Myocardial infarction
- Cerebro vascular accidents
- Gangrene of lower extremities

TREATMENT OF DIABETES MELLITUS

MODALITIES OF TREATMENT

Diet

Physical exercise

Insulin

Oral antidiabetic agents

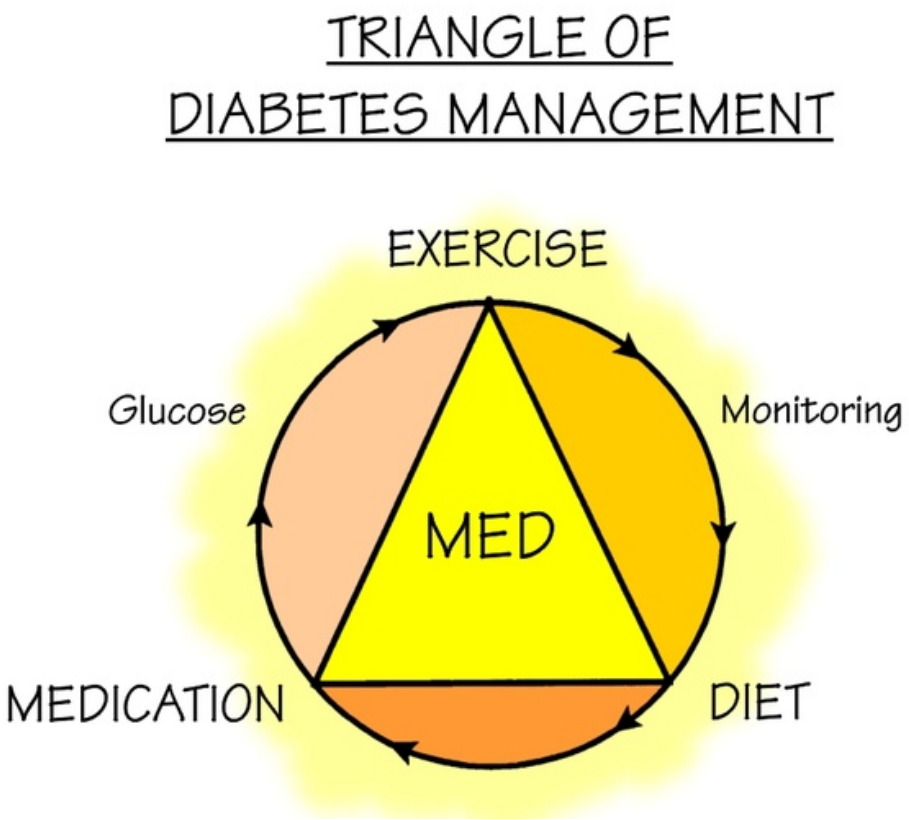
DIET

Nutrition therapy is recommended for all people with type 1 and type 2 diabetes as an effective component of the overall treatment plan. In type 2 diabetes mellitus, strict adherence to diet is necessary since the endogenous insulin reserve is limited.

Caloric content of the diet should be first determined according to the needs of the patients. Protein content should be probably 10-15% of daily calories. Saturated fat provide less than 10% of daily calories. Polyunsaturated fat provide less than or equal to 10% of daily calories. Remaining calories are to be divided between carbohydrates and monounsaturated fat based on medical needs and personnel tolerance. Dietary fibre may play a role within insulin sensitivity and glucose tolerance[35].

After determining all the above mentioned factors we must decide the dietary substances. Initially dietary prescription for diabetic patients were rigid and difficult to follow. Now the concept is to provide flexibility in use of ordinary foods. This gives good compliance of both patients & family members.

Figure 9: Triangle of Diabetes management



Increasing the fiber content of the diet will be very useful. It is the long term overall diet pattern that count. Deviation of one or two meals does not matter much.

When the dietary modality is not adequate, we must go for oral anti-diabetic drugs for type 2 diabetes mellitus.

The components of optimal medical nutrition therapy (MNT) includes fruits, vegetables, fibre containing foods, and low fat. MNT is a term used by the American Diabetes Association, it explains the coordination of caloric intake with other aspects of therapy. MNT is an effective and increasingly affordable method to prevent type 2 diabetes and to treat both type 1 and type 2 diabetes[36].

The consumption of foods with a low glycemic index tends to reduce postprandial blood glucose level and improve glycemic control. Reducing calorie and non-nutritive sweeteners are useful.

EXERCISE

Regular exercise improves by[37]

- ✓ Increase in the number of insulin receptors
- ✓ Increase in the sensitivity of insulin receptors
- ✓ Elevation of 2 to 3 DPG levels in the RBC and reduce HbA1C
- ✓ Promote oxygen delivery to the peripheral tissues

The benefits of exercise as identified by ADA are:

- Improved glycemic control,
- Enhancement of weight loss and weight maintenance
- Prevention of cardiovascular disease

YOGA

The science of yoga is an ancient one. It is a rich heritage of our culture. The beneficial effects of yoga are:

- Improves glycemic control
- Elimination of stress
- Reduction of insulin resistance

INSULIN

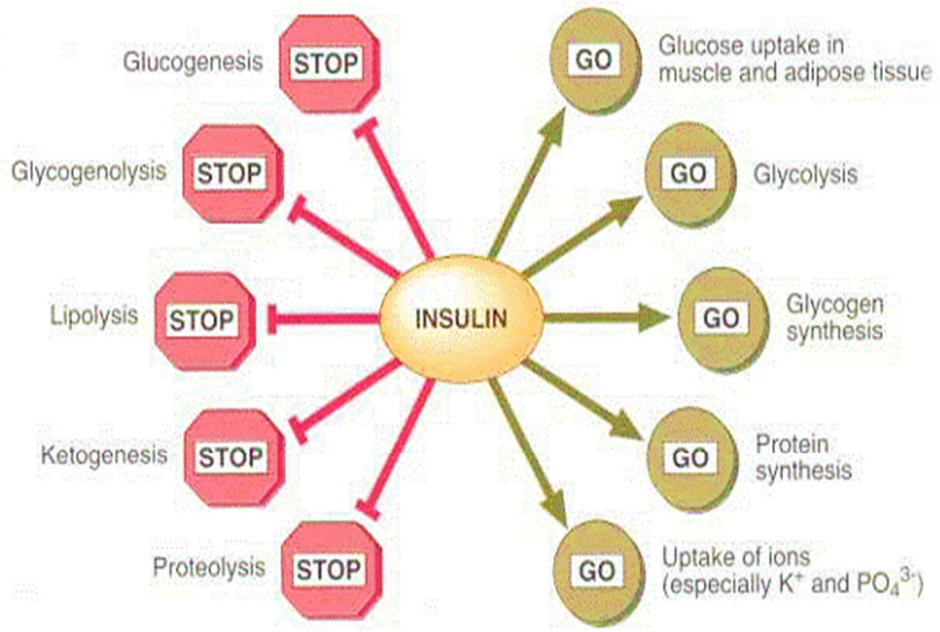
Insulin was discovered in 1921 by Banting and Best[38]. It is a polypeptide having 51 amino acids. It contains two chains A (21 amino acids) and B (30 amino acids). It was initially obtained from the animals as bovine and porcine insulin. Insulin is normally given for type 1 diabetes mellitus and some patients of type 2 diabetes mellitus with insulinopenia, whose hyperglycemia does not respond to diet therapy either alone or combined with oral anti-diabetic drugs[39]. It is injected subcutaneously several times a day into anterior abdominal wall, upper arms, outer thighs and buttocks.

Effects of insulin

It elicits a remarkable array of biological responses. The important target tissues of insulin are liver, muscle and adipose tissue. The effets include

- Decrease in blood glucose
- Decrease in protein synthesis
- Inhibition of breakdown of fat

Figure 10: Effects of insulin

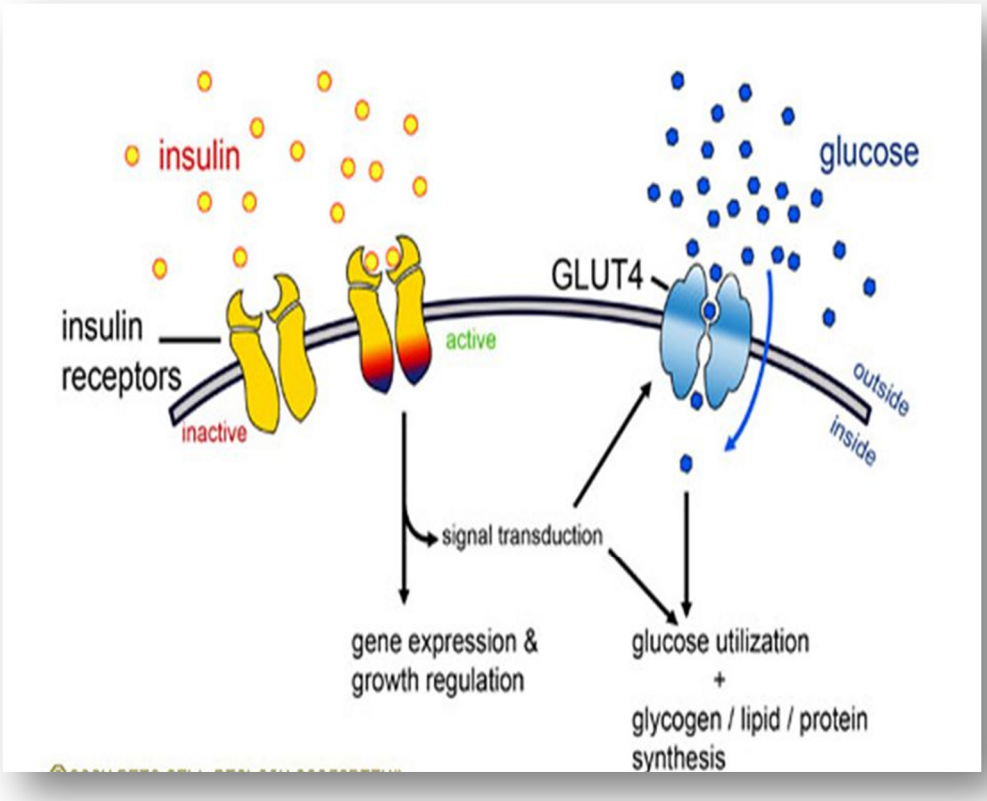


Molecular mechanisms of insulin action

Insulin acts through a cell surface receptor. Insulin receptor is a ligand activated tyrosine kinase. When insulin binds to its receptor it leads on to rapid intra molecular auto phosphorylation of several tyrosine residues. The activated receptor kinase initiate a cascade of events by phosphorylating insulin receptor substrates (IRS 1-4)[40]. The effects of insulin receptor appear to be mediated by IRS -2.

In turn it activate other kinases including protein kinases and produce the physiological effects of insulin.

Figure 11: Mechanism of insulin



Classification:

1. Ultra short acting:

   Insulin lispro

   Insulin aspart

   Insulin glulisine

2. Short acting:

   Regular insulin

   Semi lente insulin

3. Intermediate acting:

   Neutral protamine Hagedorn

   Lente insulin

4. Long acting:

    Ultra lente insulin

    Insulin glargine

    Insulin detemir

Insulin lispro:

It is identical to human insulin except at positions B28 and B29 were proline and lysine are reversed.

Insulin aspart:

It is formed by replacement of proline at the 28^{th} position with aspartic acid.

The important advantage with insulin lispro and insulin aspart is that they readily dissociate into monomers shortly after injection. So they can be given just 5 minutes before food and thereby reducing the risk of hypoglycemic attacks. Insulin lispro can be given in insulin pumps.

Insulin glargine:

Two modifications are made in human insulin. Two arginine residues are added to C terminus of B chain and asparginase molecule in position 21 on A chain is replaced with glycine.

The advantage of this preparation is that it produces less hypoglycaemia and sustained peakless absorption profile.

Conventional insulin regimen involves the administration of one or two injections of intermediate acting insulin with or without addition of small amount

of regular insulin. Multiple subcutaneous and continuous infusions are needed in intensive treatment schedules. The problem of hypoglycaemia is common in insulin dependent diabetes mellitus. Hypoglycemic attacks are dangerous and if frequent, leads to serious and fatal outcome. So that goal of insulin therapy is to keep the upper limit of postprandial blood sugar at 200 mg/dl.

Insulin therapy:

In type 2 diabetes mellitus:

Combination:

Use of daytime oral antidiabetic agents and single injection of intermediate and long acting insulin at bed time.

Advantages:

- Patient compliance better with single injection.
- Less weight gain.
- Suppress excessive hepatic glucose production at night.

Other methods:

Multiple regimen:

Pre breakfast, pre- dinner dose of mixing intermediate and fast acting insulin is useful in obese type 2 diabetes mellitus patients.

Basal Bolus Regimen:

There is a continuous basal insulin throughout the day with increased insulin at meals by bolus. Pre meal regular insulin and bed time NPH or glargine or Lente or ultra-Lente can be given as basal insulin.

Insulin lispro or aspart can be taken 5 minutes before food as they are quick acting. So the risk of hyphoglycemica is less.

This type of intensive insulin regiments reduce microvascular complications.

Self-monitoring of blood glucose should be done by insulin treated patients to find out asymptomatic hypoglycaemia. But patient compliance is poor.

Role of Newer Insulin:

Basal insulin should have a peak-less pharmacodynamic profile, at least 24 h of duration of action, very low risk of hypoglycemia, should be tolerated well in both type 1 and type 2 diabetes mellitus, and should have predictable action without any intra and inter individual variability[41].

Inhaled Insulin

This newer form has recently been approved by FDA[42]. Patients often have a fear of needles and may also object to injection therapy as being inconvenient and unacceptable. Consequently, the pulmonary route has been investigated as an alternatives, less invasive method of insulin administration. Inhaled insulin has a faster onset of action than both insulin lispro and regular insulin, and its duration of action is longer than the insulin lispro and similar to regular insulin. These characteristics make inhaled insulin suitable for administration before meals to control postprandial hyperglycemia. No adverse

effects are reported so far. Smokers would not be able to use it because of higher absorption and higher incidence of hypoglycemia[43].

In comparison to animal insulin human insulin is

- Less antigenic / less allergic reaction
- Less insulin resistance
- More potent
- More hypoglycemia
- Higher cost

Adverse effects[44]:

- Hypoglycemia
- Insulin allergy
- Lipodystrophy
- Insulin resistance
- Insulin oedema

ORAL ANTI- DIABETIC AGENTS

Introduction:

Oral anti- diabetic agents are of use in type- 2 diabetes Mellitus when it is not controlled by diet and exercise. These drugs can bring the blood glucose level back to normal in mild disease. In severe disease insulin is necessary.

Drugs[45]**:**

1. Sulfonyl ureas

2. Biguandies

3. Meglitinides

4. Thiazolidinediones

5. Alpha glucosidase inhibitors

Historical Aspects:

Sulfonyl ureas were discovered serendipitously in contrast to insulin which was discovered due to systematic investigations. In 1942 Janbon and colleagues noted that some sulphonamides caused hypoglycaemia in experimental animals. These observations were soon extended and it led to the development of the entire class of sulfonylureas.

During 1920's Biguandies were introduced but overshadowed by the discovery of Insulin. In 1997, the first member of a new class of oral insulin secretogogues called "Meglitinides" were introduced. "Thiazolidinedione's" were introduced in 1997 as the second major class of insulin sensitizers.

SULFONYL UREAS[46]:

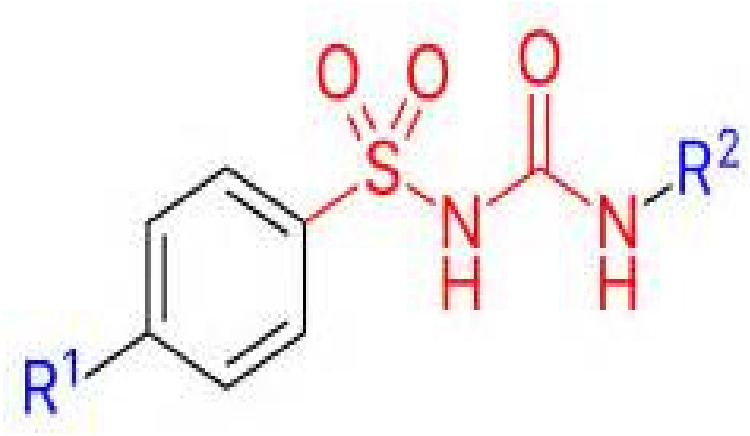
First Generation

- Tolbutamide
- Chlorpropamide
- Tolazamide
- Acetohexamide

Second Generation

- Glibenclamide (Glyburide)
- Glipizide
- Gliclazide
- Glimepride

Figure 12: Structure



Mechanism of Action of Sulfonyl Ureas

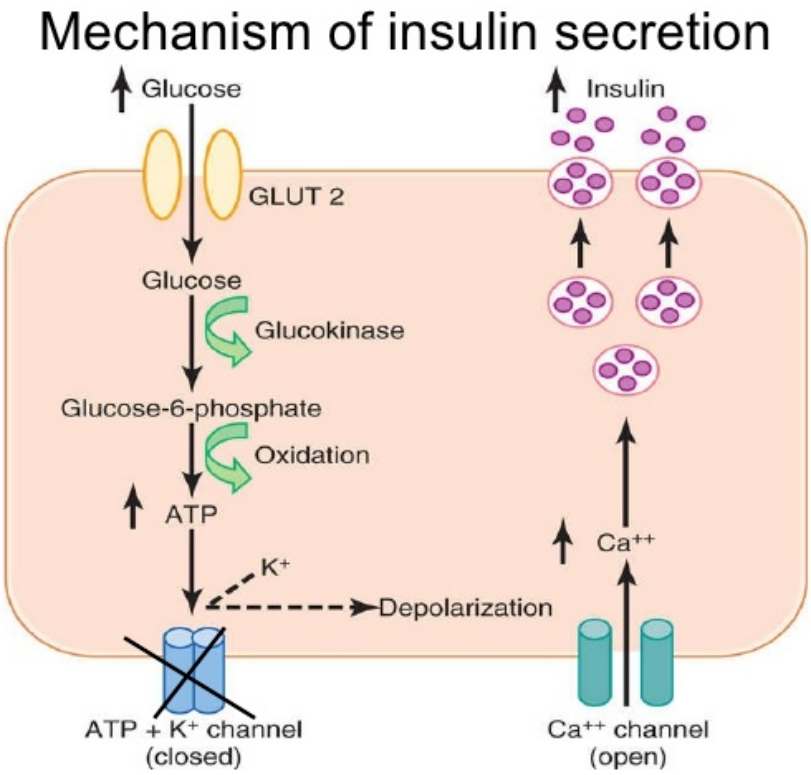
Their effects in the treatment of diabetes Mellitus are more complex.

They stimulate insulin release from the pancreatic beta cells. They reduce hepatic clearance of the hormones. They enhance insulin in cells and stimulate glucose transporters. They suppress hepatic gluconeogenesis.

The effects of the sulfonyl ureas are initiated by binding to and blocking an ATP sensitive K+ channel.

Reduced K+ Conductance causes membrane depolarization and influx of Ca^{2+} through voltage sensitive Ca^{2+} channels which causes insulin release.

Figure 13: Mechanism of sulphonylureas



Pharamacokinetics of sulfonyl ureas

All are effectively absorbed from gastro- intestinal tract. Food and hyperglycemia can inhibit the absorption. So they should be ingested 30 minutes before food. They are largely bound to plasma proteins especially albumin. All sulfonyl ureas are metabolized by the liver and metabolites are excreted by the kidneys. Sulfonyl ureas should be administered with caution to patients with either renal or hepatic insufficiency.

Adverse Reactions of Sulfonyl ureas

- ✓ Hypoglycemia
- ✓ Non – Specific skin rashes
- ✓ Nausea, Vomiting

- ✓ Hemolytic Anaemia
- ✓ Agranulocytosis
- ✓ Hepatitis

Contraindications of Sulfonyl ureas

- ✓ Pregnancy
- ✓ Lactation
- ✓ Renal Insufficiency
- ✓ Hepatic Insufficiency

BIGUANDIES[47]

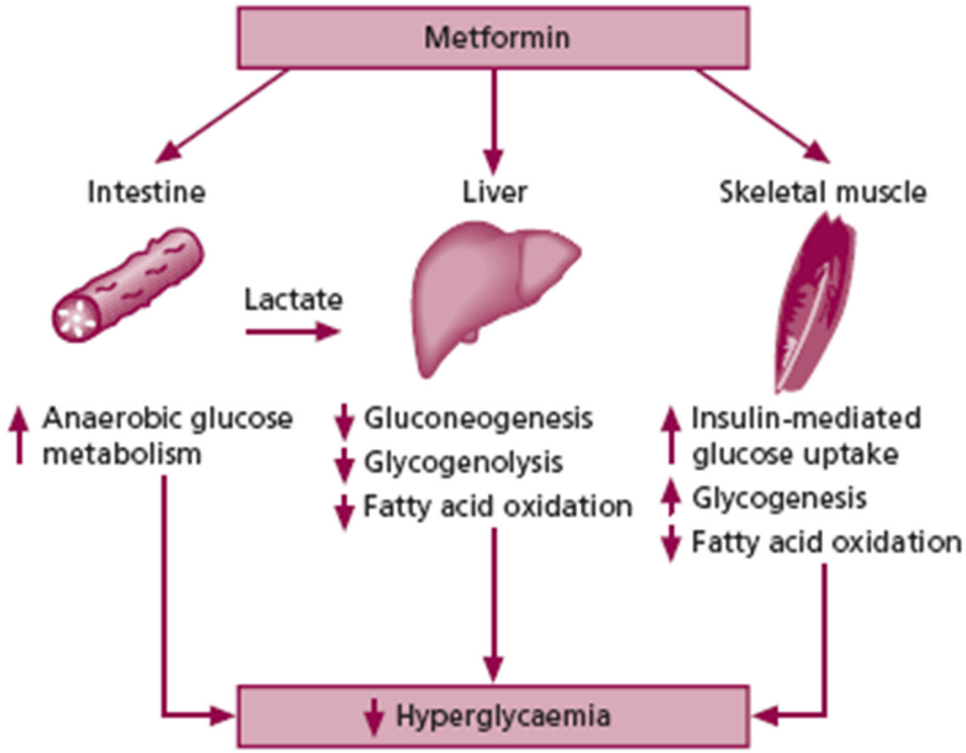
1. Metformin
2. Phenformin
3. Buformin

Mechanism of Action of Biguandies

They are euglycemic. They enhance glucose removal from blood and inhibit gluconeogenesis. They are taken with or after food. They are given alone or with sulfonylureas. Presence of insulin is required for their action.

- ✓ Reduce glucose production by inhibiting gluconeogenesis from liver
- ✓ Facilitating insulin action in muscle and fat
- ✓ Decreased glucose absorption from intestine
- ✓ Increase insulin sensitivity (Insulin sensitizer)

Figure 14: Mechanism of Biguanide



It should be used cautiously as they are prone to develop lactic acidosis. They are contraindicated in

- Hypersensitivity
- Severe pulmonary disease
- Congestive cardiac failure
- Recent myocardial infraction.

Its main advantage is no weight gain. It is particularly useful in obese diabetic patients. It is also used for hirsuitism in women with polycystic ovary syndrome by reducing androgen level and increasing insulin sensitivity. Infertility may be treated.

In type -1 Diabetes Mellitus, it is given along with insulin to decrease insulin resistance.

Phenformin and Buformin were withdrawn due to severe lactic acidosis.

MEGLITINIDES[48]

1. Repaglinide
2. Nateglinide

They are insulin secretogogues like sulfonyl ureas. They are useful in postprandial hyperglycemia exertions. They should be taken before food. Nateglinide with rapid onset and short duration of actions is useful for this purpose. Repaglinide acts by blocking ATP sensitive potassium channels in the beta cells of pancreas and increasing calcium influx, thus releasing insulin by exocytosis.

Peak effect is observed in 1 hour of administration so if meal is delayed there are more chances of hypoglycaemia with repaglinide. The chances of hypoglycaemia with nateglinide are least among all insulin secretogogues including sulphonylureas[49]. Both the agents are metabolized by CYP3A4 and precaution should be taken in patients with poor hepatic function.

THIAZOLIDINEDIONES

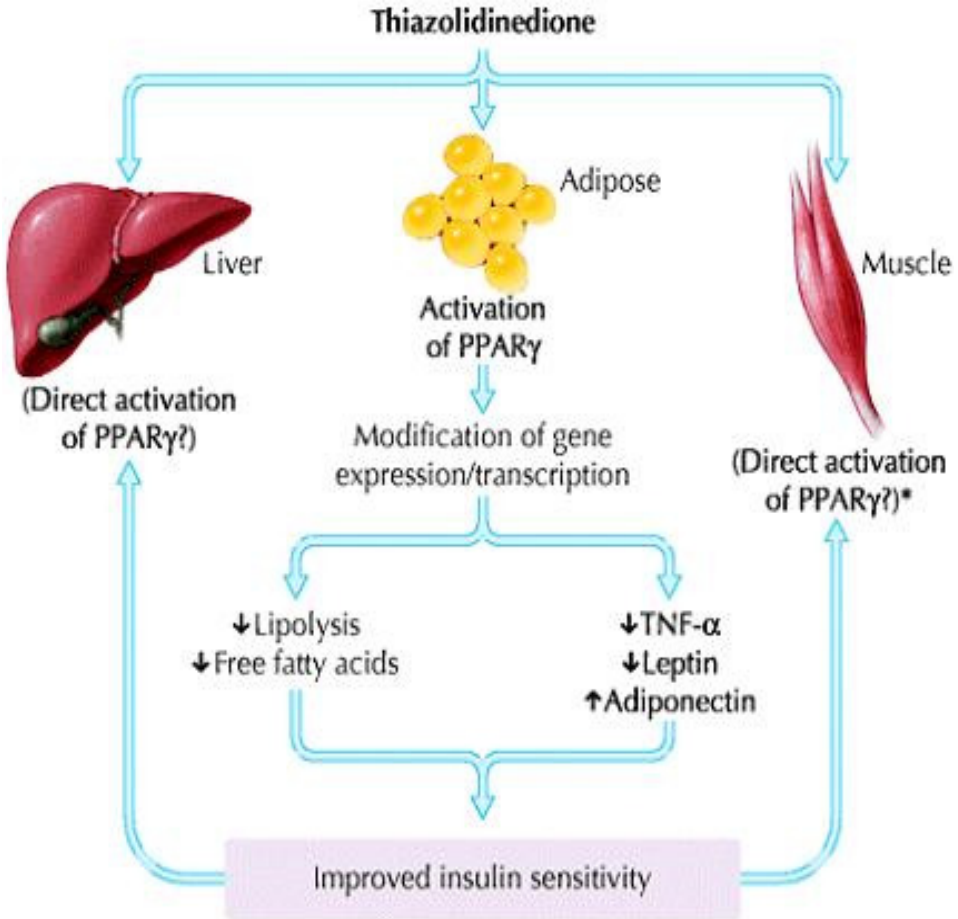
It was discovered by serendipity from ciglitazone as hypolipidaemic drug and noticed that this drug reduced blood glucose level but it was found to be hepatotoxic.

Troglitazone was the first agent of this class and withdrawn due to severe hepatotoxicity, an idiosyncratic reaction. Later on two drugs were introduced and they do not have hepatotoxicity. They are

1. Pioglitazone
2. Rosiglitazone

They are insulin sensitizers. They need insulin for their action. They act through peroxisome proliferative - activated receptors gamma (PPAR-γ) they alter adipokinase release, increase adenopectin which enhance insulin sensitivity[50].

Figure 15 : Mechanism of thiazolidinedione



a. Reduce insulin resistance in peripheral tissues
b. Reduce glucose production in the liver
c. Increased glucose transport into muscle and adipose tissues
d. Shift fat from viscera to subcutaneous tissue

Other effects

a. Increases HDL
b. Reduces TGL

They are used as adjuvant to diet and exercises. They should be started with low dose. They reduce insulin requirements. They are combined with metformin or sulfonyl ureas.

Adverse effects

- Hepatotoxicity after prolonged use
- Oedema
- Anaemia
- Heart failure
- Risk of fracture in elderly (Rosiglitazone)

Both are long acting so, once a day administration is sufficient. For fear of hepatotoxicity, liver function test are to be done every 6 months. It is also contraindicated in cardiac failure patients. Rosiglitazone has been associated with myocardial infarction and due to this reason it is banned in India (2010).

The risk of bladder cancer with pioglitazone appears to be related to cumulative dose and duration of exposure[51].

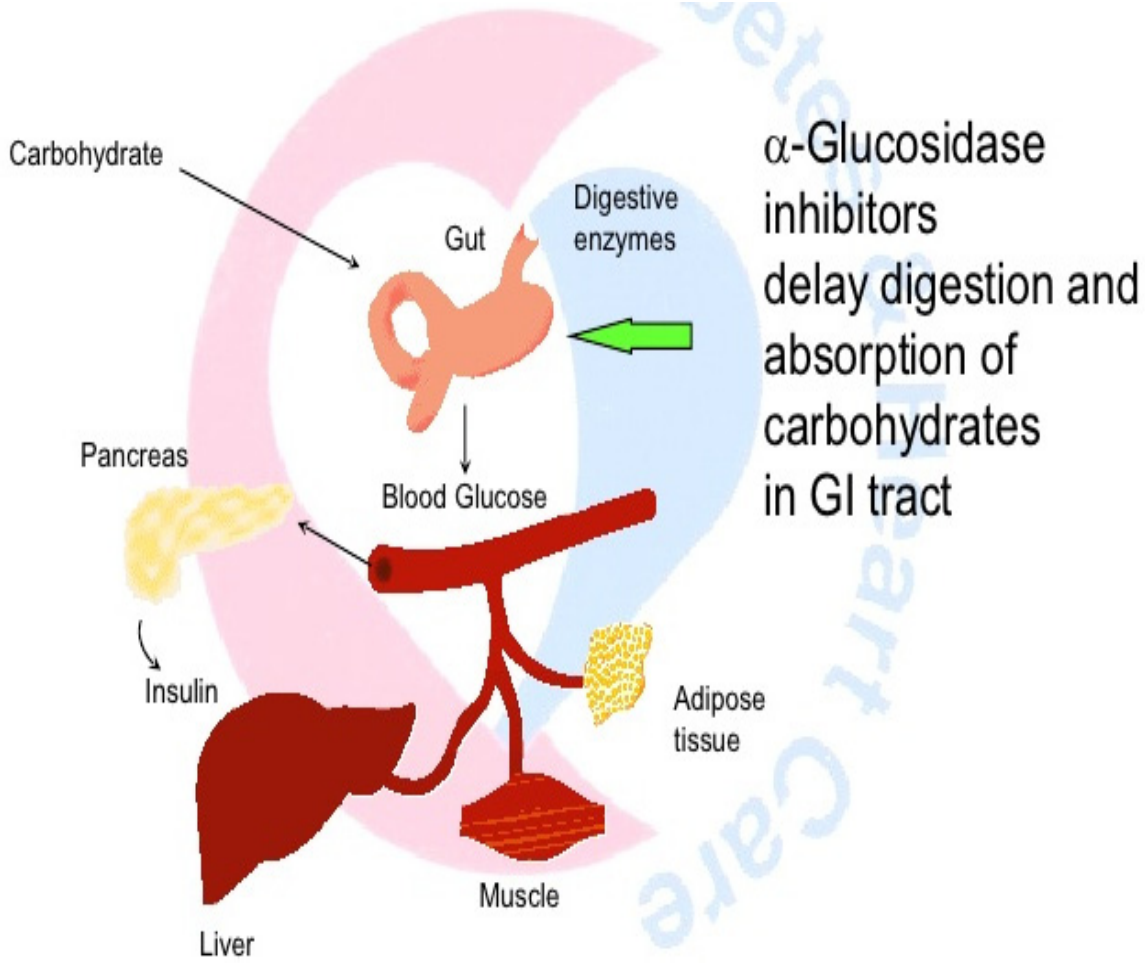
ALPHA GLUCOSIDASE INHIBITORS

1. Acarbose
2. Miglitol
3. Voglibose

Alpha glucosidase enzyme facilitates digestion of oligosaccharides and disaccharides into monosaccharides, thus it is absorbed from the small intestine. Acarbose was the first agent of this class. They act by inhibiting alpha glucosidase at brush border of intestine and thereby decreasing absorption of sugars. So they should be taken just before each meal. The enzymes glucoamylase and sucrase are also inhibited[52].

Regular use tends to lower HbA1C. It can be used as monotherapy in the elderly patients or in a patient having mainly postprandial hyperglycemia. They are combined with insulin or sulfonyl ureas.

Figure 16 : Mechanism of alpha glucosidase inhibitors



Adverse effects

- Flatulence
- Diarrhoea
- Abdominal pain

Precaution should be taken while treating hypoglycaemia who are taking other hypoglycaemic agents with acarbose. It should be corrected by giving glucose and not by sucrose, because its breakdown is already blocked.

Contraindications

- Inflammatory bowel disease
- Intestinal obstruction

NEWER DRUGS

Incretin - mimetic

1. Exenatide
2. Liraglutide
3. Albiglutide
4. Dulaglutide

Dipeptidyl peptidase 4 inhibitors

1. Sitagliptin
2. Vildagliptin
3. Saxagliptin
4. Linagliptin

Amylin analogue

1. Pramlintide

Sodium glucose cotransporter 2 (SGLT-2) inhibitors

1. Canagliflozin
2. Dapaglifozin
3. Serglifozin
4. Empagliflozin

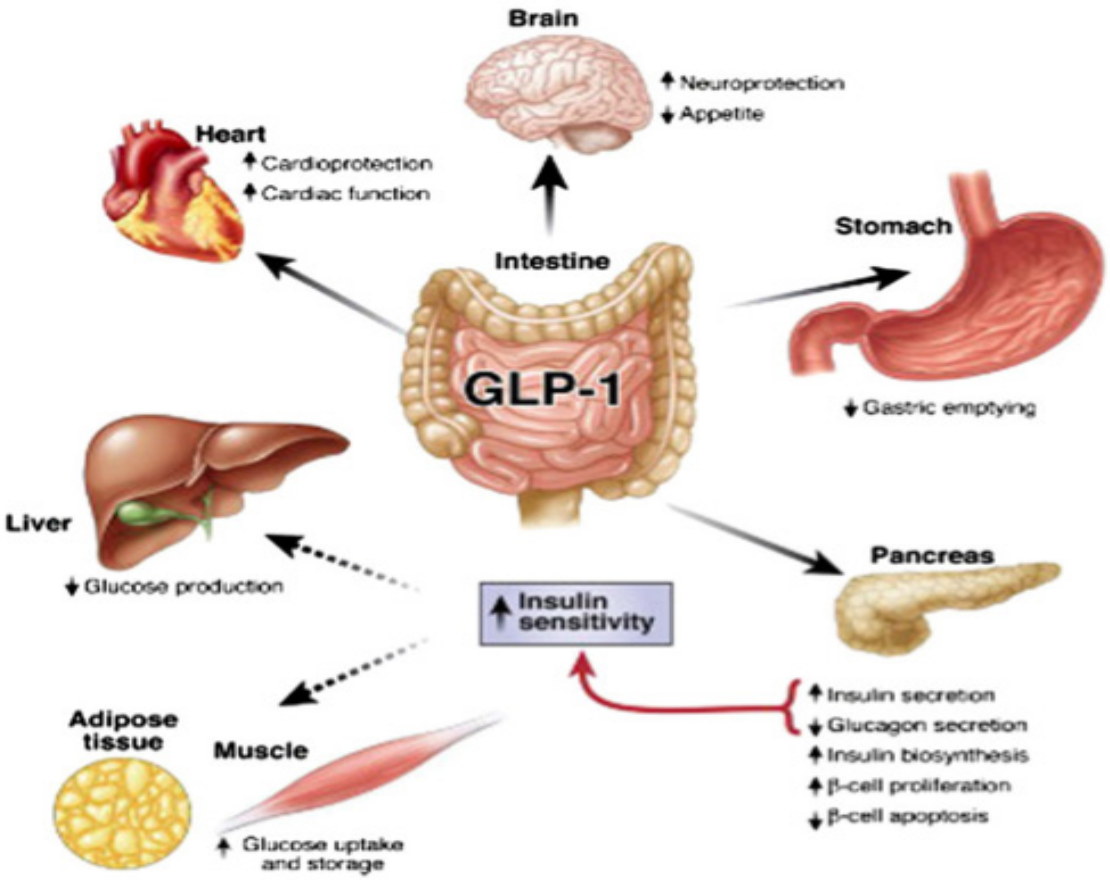
Other hypoglycaemic drugs

1. Colesevelam hydrochloride
2. Bromocriptine

Exenatide

It is a synthetic analogue of glucagon like peptide 1 (GLP-1). It is the first incretin therapy to become available for the treatment of Diabetes mellitus. It is orally inactive, hence approved as an injectable (subcutaneous) and can be used as an adjuvant therapy in individuals with type 2 Diabetes mellitus treated with metformin or sulphonyl ureas who still have suboptimal glycaemic control[53].

Figure 17 : Mechanism of GLP-1 analogue



- Stimulates insulin secretion from beta cells of pancreas
- Slows the rate of nutrient absorption by slowing gastric emptying
- Decrease appetite by acting at hypothalamus
- Decrease glucagon release
- Lowers HbA1C level
- Reduces the risk of heart disease

Exenatide and liraglutide must be administered subcutaneously. Liraglutide is highly protein bound and has a long half life, allowing for once daily dosing. Exenatide is absorbed orally from arm, abdomen and thigh,

reaching peak concentration in approximately 2 hours with a duration upto 10 hours. It is injected within 60 minutes before a meal.

Adverse effects

- Nausea
- Anorexia
- Diarrhoea
- Necrotising and hemorrhagic pancreatitis

Disadvantages

- Expensive
- Parenteral preparation (s.c)
- Risk of hypoglycemia

Sitagliptin and Vildagliptin

Both are orally active selective inhibitor of dipeptidyl –peptidase 4 (DPP-4)[54], the enzyme that degrades incretin and other GLP-1 like molecules. As a result GLP-1 levels are increased and its actions are prolonged. Efficacy is linked with endogenous GLP-1. These have longer plasma half life. It is approved for use in type 2 Diabetes mellitus and used as an adjuvant in combination with sulphonylureas or metformin.

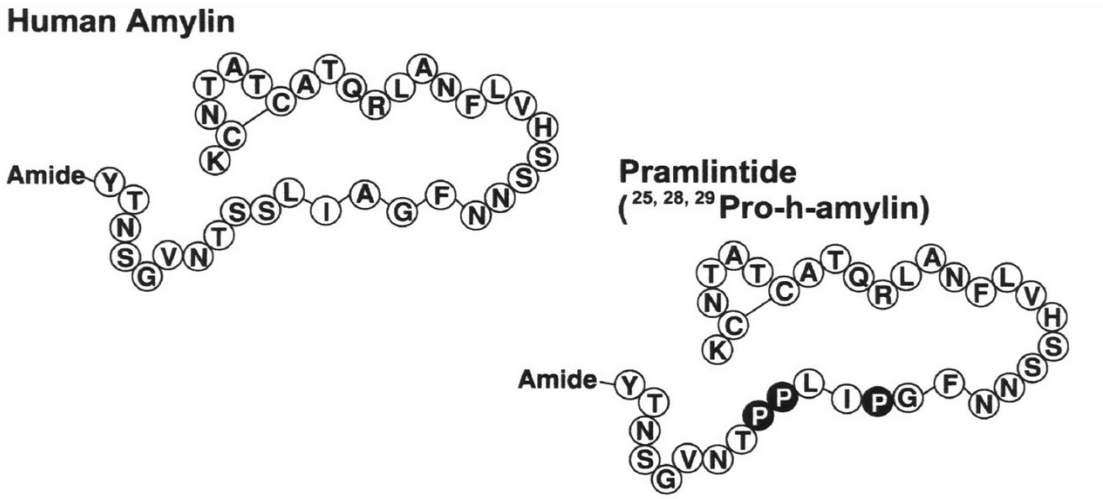
Adverse effects

- Gastrointestinal side effects
- Nasopharyngitis
- Upper respiratory tract infection
- Allergy

Pramlintide[55]

It is a soluble injectable synthetic analogue of amylin that modulates postprandial glucose levels. It is approved for pre-prandial use in individuals with type 1 and type 2 diabetes mellitus.

Figure 18 : Structure of pramlintide



It is administered in addition to insulin in those who are unable to achieve their target post-prandial blood glucose levels. It suppresses glucagon release via undetermined mechanisms, delays gastric emptying and has central nervous system mediated anorectic effects. It is rapidly absorbed after subcutaneous administration. Peak levels within 20 minutes and the duration of action is not more than 150 minutes. It is renally metabolized and excreted even with low creatinine clearance. It has not been evaluated in dialysis patients. The most reliable absorption is from the abdomen and thigh. It should be injected immediately before eating. Doses range from 15mcg to 120mcg. Therapy should be initiated with the lowest dose and titrated upwards. It should always be injected separately and it should not be mixed with insulin.

Adverse effects

- Gastrointestinal side effects
- Hypoglycaemia

Sodium glucose cotransporter -2 inhibitors (SGLT-2)

Dapaglifozin, serglifozin and remoglifozin are newer antidiabetic drugs that have successfully cleared phase 3 trials. The kidney continuously filters glucose through glomerulus and this glucose is reabsorbed back from the proximal tubule by a transporter called SGLT-2.

These drugs act by inhibiting SGLT-2, decreases the amount of glucose absorption from the proximal tubule and increases its excretion in urine[56].

Advantages

- ✓ Weight loss
- ✓ No hypoglycaemia (not inducing insulin secretion)
- ✓ Improve insulin resistance
- ✓ Beneficial in patients of DM with hypertension because of their diuretic effects.

Disadvantages

- ✓ More polydipsia
- ✓ Increased risk of urinary bacterial/fungal infection
- ✓ Risk of sodium loss

THE GOALS OF THERAPY[57]

With all these modalities, the aim of treatment is to achieve almost near normal glucose metabolism. By keeping the body weight and blood glucose concentration at ideal level, metabolic profile is improved. This helps to lower the incidence of vascular disease and specific diabetic complications.

For both type 1 and type 2 diabetes mellitus, the goals are to

- Eliminate symptoms due to hyperglycaemia
- Reduce the long term complications of DM
- To achieve as normal a lifestyle as possible

Figure 19: Modalities of treatment

Treatment goals for adults with Diabetes

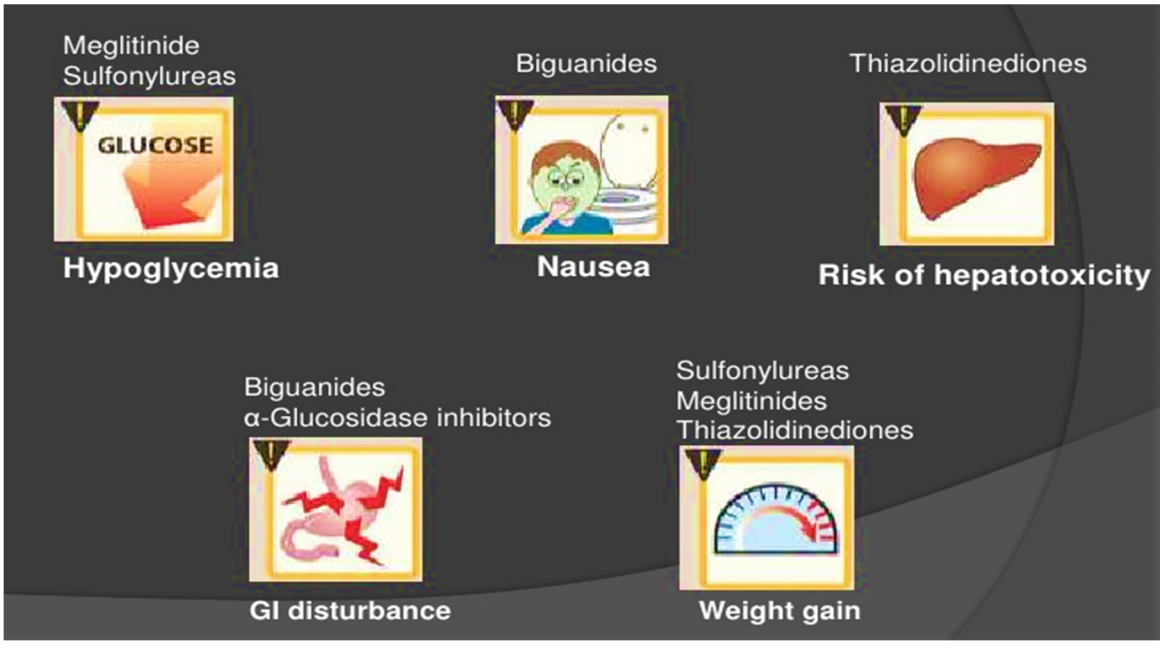
HbA1C	<7.0%
Preprandial capillary plasma glucose	80-130mg/dl
Postprandial capillary plasma glucose	<180mg/dl

Drawbacks of oral antidiabetic drugs

They are ineffective in 20% of newly diagnosed patients of type 2 diabetes mellitus and in some patients the initial response decreases after several years.

Their use is complicated due to hypoglycaemic attacks and significant drug interactions. The use of OHA produce unwanted side effects[58]. They should be used cautiously in patients with liver diseases particularly biguanides and thiazolidinediones.

Figure 20 : Drawbacks of oral antidiabetic agents



So search still continues for better hypoglycaemic agents.

- None of them (OHA) can maintain blood glucose level within normal limits around the clock.
- In large doses they pose the problem of hypoglycaemia
- Often insulin resistance may complicate the disease management
- Lactic acidosis is seen with biguanides
- Vitamin B12 and folate deficiency
- Gastrointestinal disturbances
- Altered liver function test

On account of the above limitations of modern drug therapy in the treatment of diabetes, there is a switch over to old remedies, which have the healing powers. They include the use of plants and other natural products which are capable of alleviating diabetes mellitus. It has emerged as the recent trend world over.

PLANT DERIVED DRUGS

Medicinal substances of plant origin are known to mankind for several centuries. Majority of medicines were formulated from herbs until the previous century. Current day pharmaceutical industry still depends on plant sources for the synthesis of many lead compounds as a vital part in new drug development. Plant derived drugs are called phytomedicines.

According to World Health Organization (WHO), more than 10,000 plant species are in use worldwide. 20 -50% of patients consume herbal medicines.

They are taken either as a whole or its components, extracts, tablets, capsules, syrups.

India is known for its medicinal plants. Thousands of medicinal plants have been identified. These include herbal, medicinal and aromatic plants. India has indigenous medical systems like ayurveda, siddha and unani medicine where herbal medicines are mainly used.

Traditional medicine is being integrated worldwide along with the primary health care because of its easy accessibility to all and acceptability. Medicines used in alternative therapies are not approved drugs with proven safety, efficacy and quality. These medicines are having traditional values and are used empirically.

Mostly female patients with chronic medical problems seem to choose herbal medicines. Diabetes mellitus, sleep disorders, obesity, benign prostatic hypertrophy, chronic pain, erectile dysfunction, common cold, cough, cancer, HIV, arthritis are some of the diseases where herbal medicines are commonly used.

Herbal medicines are widely used because of easy availability and the belief that ‘naturalness’ of the phytomedicines always protects rather than cause adverse effects. Due to lack of regulation regarding their production, they are prone to cause adverse effects due to contaminants and adulterants. Since no medicine is free from side effects, herbal medicines must also be subjected to proper clinical trial.

ALOE VERA

Figure 21: Aloe Vera

In traditional medicines, aloe vera has been used either alone or along with other herbal remedies for the treatment of Diabetes mellitus.

ALOE VERA

Aloe vera locally known as kawar gandal and it is a cactus-like plant with green, dagger-shaped leaves. The name was derived from the Arabic 'alloeh' meaning 'bitter'[59], because of the bitter liquid found in the leaves. It is also known as 'lily of the desert'

Aloe vera is a plant species of the genus Aloe. It grows wild in tropical climates around the world and is cultivated for agricultural and medicinal uses. It grows successfully indoors as a potted plant.

It is found in many consumer products including beverages, skin lotion, cosmetics, or ointments for minor burns and sunburns. There is trivial scientific evidence of the effectiveness or safety of Aloe vera extracts for either cosmetic or medicinal purposes.

The plant

Aloe vera is a very short-stemmed plant growing to 60–100 cm tall, spreading by offsets. The leaves are thick, fleshy and it looks green to grey-green. Some varieties showing white flecks on their upper and lower stem surfaces. The margin of the leaf is serrated and has small white teeth.

Figure 22 : Scientific classification of Aloe vera

Scientific classification	
Kingdom:	Plantae
Clade:	Angiosperms
Clade:	Monocots
Order:	Asparagales
Family:	Asphodelaceae
Subfamily:	Asphodeloideae
Genus:	*Aloe*
Species:	***A. vera***

Parts used

Aloe vera gel

Distribution

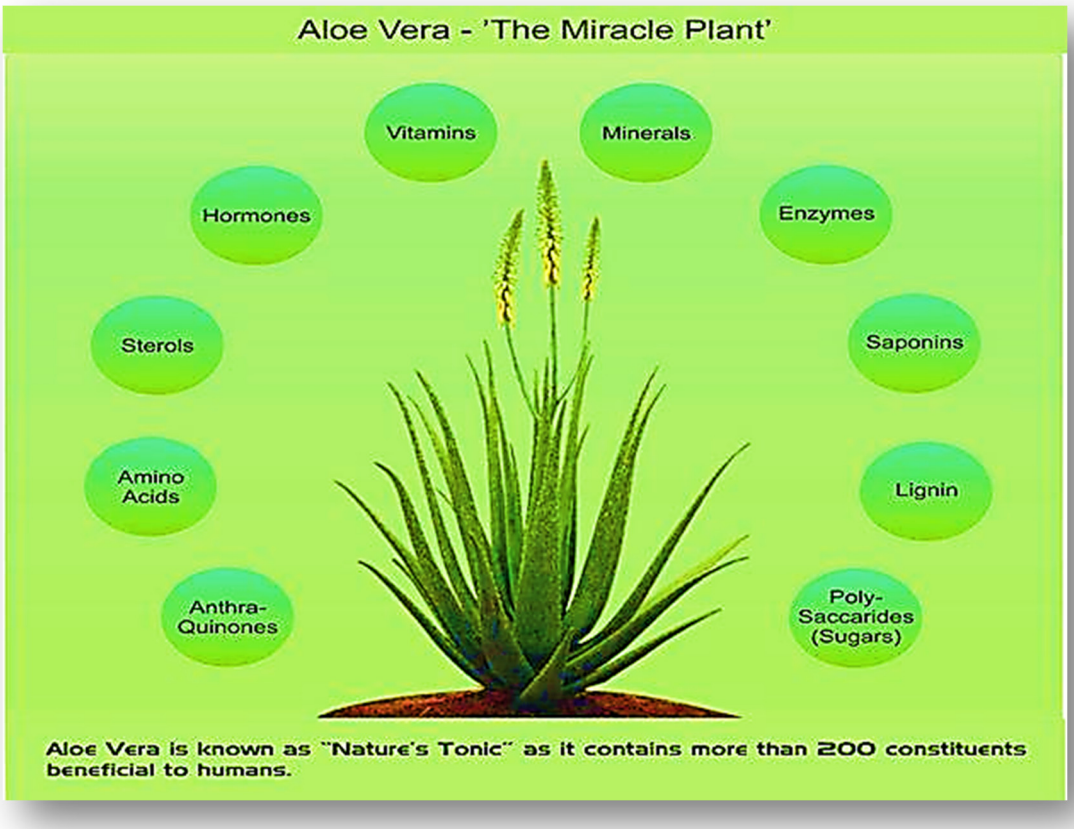
The species has been widely cultivated around the world. It was introduced to China and various parts of southern Europe in the 17th century and it is widely naturalized elsewhere, occurring in temperate and tropical regions of Australia, South America, Mexico, the Caribbean and southeastern United states.

SYNONYMS[60]

1. *Aloe barbadensis Mill*
2. *Aloe barbadensis var. Chinensis Haw*
3. *Aloe chinensis Baker*
4. *Aloe elongata Murray*
5. *Aloe flava pers*
6. *Aloe indica Royle*
7. *Aloe lonzae Tod*
8. *Aloe maculata forssk*
9. *Aloe perfoliata var*
10. *Aloe rubescens DC*
11. *Aloe variegata forssk*
12. *Aloe vera Mill*
13. *Aloe vera var lanzae Baker*
14. *Aloe vera var chinensis*
15. *Aloe vulgaris Lam*

Aloe vera leaves contain phytochemicals such as acetylated mannans, polymannans, anthraquinone C-glycosides, anthrones, other anthraquinones, such as emodin, lectins. It also consists of D-glucose and D-mannose, tannins, steroid, enzymes, plant hormones, amino acids, vitamins and minerals. This may be responsible for their bioactivity.

Figure 23 : Aloe vera- The miracle plant



Health benefits

Aloe vera is a rich source of antioxidants which inhibits the growth of infection spreading bacteria.

- increases collagen production
- improves skin elasticity

- keeps the skin hydrated
- reducing acne and premature ageing.

Topical Aloe vera gel is supposed to be quite effective against burns in particular.

Aloe vera is packed with all the vitamins and minerals (Vitamins – A, C, E, B1, B2, B3, B6, B12, folic acid, calcium, magnesium, zinc, iron and more) for shiny healthy hair. It has a chemical makeup similar to that of keratin, and it rejuvenates the hair with its own nutrients, giving it more elasticity and preventing breakage.

The latex (sticky yellow residue found just under the skin of the leaf) in aloe vera is considered to have strong laxative effects, which helps to relieve constipation. The cooling effects of aloe vera benefit patients with problems of acidity.

It also detoxifies the stomach, curing indigestion, gas and curbs the growth of unhealthy bacterial growth. It is also a

- Good protector of the liver
- Promotes blood circulation
- Combats cholesterol and diabetes
- Improves cellular oxygenation.

Dose

In capsule, it is recommended to take 200-300mg/day. For the juice, 2-3 table spoons (50ml) per day is recommended.

Aloe vera combinations

Aloe vera can combine with

- Spirulina and Chlorella for the detoxification of the organs
- Acai Berry and Goji Berry for the protection of eyes, skin, hair and nail
- Ginkgo Biloba for protection from radiation
- Ginger and turmeric to prevent from cancer, gastrointestinal problems.

ANIMAL MODEL OF EXPERIMENTAL DIABETES MELLITUS

In the 1980's von Mering was working on the absorption of fat from the intestine after removing the pancreas of a dog. The animal developed polyuria, polydipsia and was found to have diabetes mellitus. Many experiments on rabbits and dogs followed, although history has given a special place to Marjorie, one of the dogs used by Banting and Best in their seminal experiments on the isolation and purification of insulin in the 1920's.

There are many animal models which include:

1. Models for insulin dependent diabetes mellitus (IDDM)
2. Models for non insulin dependent diabetes mellitus (NIDDM)
3. Transgenic and knockout animals
4. Invitro methods on isolated organs, cells and membranes
5. Models to study insulin secretion from beta cells
6. Experimental models for diabetic complications

METHODS OF INDUCING DIABETES MELLITUS IN ANIMALS[61]

1. Models for IDDM

- Chemical induced
- Virus induced
- Hormone induced
- Insulin antibodies induced
- Surgical - Pancreatectomy
- Transgenic animals

Chemical induced

Streptozotocin and Alloxan are widely used to induce experimental Diabetes Mellitus in animals but the mechanism of production of Diabetes Mellitus are different in each.

Virus induced

Various human viruses used for inducing diabetes include RNA picornoviruses, coxsackie-B4, reovirus, mengo-2T and lymphocytic choriomeningitis virus. Viruses may produce diabetes mellitus by infecting and destroying of beta cells in pancreas.

Hormone induced

Dexamethasone, a long acting glucocorticoid is used to produce diabetes mellitus. Corticotropin is used to stimulate adrenal cortex that results in hormonal imbalance causing steroid diabetes.

Insulin antibodies induced diabetes:

Giving bovine insulin along with CFA to guinea pigs produce anti-insulin antibodies. Intravenous injection of 0.25 ml-1.0 ml guinea pig anti-insulin serum to rats induce a dose dependent increase in blood gluose levels upto 300 mg/dl.

Surgical induced

In partial pancreactectomy, more than 90 % of the organ must be removed to produce diabetes and total removal of the pancreas results in an insulin dependent form of diabetes. It results in loss of alpha and delta cells in addition to beta cells. This causes loss of counter regulatory hormones, glucagon and somatostatin.

Genetic models

- The NOD mouse
- The BB rat
- WBN/KOB rat
- Cohen diabetic rat

Others

- ✓ Monogenic models in obesity and NIDDM
- ✓ Polygenic models in obesity and NIDDM
- ✓ Transgenic and knockout techniques

Among the chemical methods 'STREPTOZOTOCIN' (STZ) induced diabetes is undertaken in this study.

STZ (2-deoxy-2-(3-methyl-3-nitrosourea) 1-D-glucopyranose) is a broad spectrum antibiotic, which is produced from Streptomyces achromogens. Rakieten et al. first described the diabetogenic property of STZ.

Mechanism of causing beta cell damage by STZ:

- By process of methylation
- Free radical generation
- Nitric oxide generation

STZ induced diabetes in almost all species of animals. Diabetogenic doses varies with species and the optimal dose required in rats are 50-60mg/kg intraperitoneally. The blood glucose level shows the triphasic response with hyperglycemia at 1 hour, followed by hypoglycemia, which lasts for 6 hrs and stable hyperglycemia by 24-48 hrs.

Multiple low dose of STZ also induce diabetes by causing immune mediated pancreatic insulitis in rats. Cyclosporin-A when given with STZ enhances its diabetogenic efficacy. STZ combined with complete Freund's adjuvant: each of CFA, incomplete Freund's adjuvant, Mycobacterium butyricum (component of CFA), Listeria monocytogens, or endotoxin administered 24 hours prior to STZ and then repeated in the three subsequent weeks, all produce hyperglycemia. Neither four administration of CFA nor of STZ alone result in persistent hyperglycemia.

Advantages and disadvantages

STZ has almost completely replaced alloxan for inducing diabetes because of

- Greater selectivity towards beta cells
- Lower mortality rate
- Longer or irreversible diabetes induction
- However, guinea pigs and rabbits are resistant to its diabetogenic action.

The following factors have been considered as possible causes of the selective destruction of beta cells of diabetogenic agents:

- Low concentration of a critical enzyme or co-enzyme (possibly glutathione) in the beta cell.
- Low concentration of an anti-diabetogenic or protective substance (glutathione) in the beta cell.
- Inability or diminished ability of the beta cell to destroy or to detoxify diabetogenic agents.

MATERIALS AND METHODS

MATERIALS AND METHODS

In the present study, the antihyperglycemic activity of aloevera gel was evaluated in adult male albino rats.

The study was done in the central animal house, Institute of Pharmacology, Madurai Medical College, Madurai after getting approval by Institutional Animal Ethical Committee of Madurai Medical College, Madurai, dated 23.03.2017.

STUDY CENTER

Institute of Pharmacology,

Madurai Medical College, Madurai

DURATION OF THE STUDY

This study was done for a period of 6 months since January 2017.

NUMBER OF ANIMALS USED

24 adult male albino rats weighing about 150 –200 grams

Materials used for the study

1. Male Albino rats (Twenty four)
2. Streptozotocin
3. Tab Glibenclamide
4. Aloe vera gel extract
5. Glucometer
6. Glucose strips

7. Oral feeding tube
8. Syringes.

ANIMALS

Inbred adult male albino rats from central animal house, Madurai Medical College were utilized in this study. 24 male albino rats each weighing 150 to 200 grams were included in the study. Animals were allowed standard diet (pellet feed) and tap water ad libitum.

Each group of animals were housed separately with a distinct identity for each animal throughout the study. The diabetic rats were given special care. The floor of cages were filled with thick layer of saw dust and it was changed daily. The diabetic rats were given adequate food pellets and plenty of water by providing two water bottles as the diabetic rats will have polyphagia, polyuria and polydipsia. The bottles were filled with fresh tap water every morning.

STREPTOZOTOCIN

Streptozotocin manufactured by Sisco Research Laboratories Pvt. Ltd was used to induce diabetes mellitus in the albino rats. Streptozotocin was given at the dose of 60mg/kg intraperitoneally as a single dose after overnight fasting.

GLIBENCLAMIDE

Glibenclamide manufactured by Aventis pharma limited (Trade name-Daonil) was used as the standard sulphonyl urea. It is available as 5 mg tablet and the rats were given 1 mg/kg per day orally.

COLLECTION OF BLOOD SAMPLES

The rats were kept in the restrainer. Lateral veins were located and xylol was applied to make the vein prominent. After disinfecting with spirit, 0.2 ml of blood was collected using a 22 gauge needle.

METHOD OF GLUCOSE ESTIMATION

Blood sugar was estimated using glucometer. It uses the glucose oxidize enzyme specific sticks. This method is very reliable, easy and quick to perform. The blood sample was directly placed on the test strip kept in the glucometer. The results will appear in the screen in 15 seconds.

EXTRACTION PROCEDURE

ALOE VERA GEL EXTRACT

The plant was obtained from local areas in Madurai and the species was identified and authenticated by Dr. Stephen, taxonomist, of American College, Madurai and further processing was done by Pharmacognosy department, Madurai Medical College.

Fresh succulent leaves of aloe vera were collected, the inner gel component removed and the leafy exudate homogenized in an electric blender. This was subsequently lyophilized and stored at 4°C. The extract was weighed and reconstituted daily, with distilled water according to the dosage needed (200mg/kg & 400mg/kg) and administered orally, for a period of 14 days.

Glibenclamide was dissolved in distilled water and given in the dose of 1 mg/kg. The standard drug as well as the test extracts of aloe vera gel were given orally using oral feeding tube.

ORAL FEEDING TECHNIQUE

16 Guage feeding tube about 2-3 inches in length which is blunted at the tip with a small ball soldered around the tip was used. The needle was attached to 1 ml syringe containing the drug to be administered. Each rat was grasped gently and secured by the nape of the neck, holding the whole animal with the left hand. After introducing the oral feeding tube laterally through the interdental space, it was advanced into the oesophagus with a gentle rotatory movement. Once it reached the desired level the drug was gently pushed inside.

METHODOLOGY

The study followed the principles of CPCSEA and utmost care was taken while handling the animals and adequate care was provided to them during and after experimentation.

All the twenty four male albino rats selected were starved for 18 hours. Fasting blood glucose level was noted for all the rats by tail venepuncture method. Animals were given feed and water ad libitum. Post prandial blood glucose level for all the rats were screened and found to be within normal limits.

Hypoglycemic effect of aloe vera gel extract in normal rats

Initially 18 animals were selected from the screened group and divided into 3 groups of 6 each. Fasting blood glucose was estimated. The first group received

pellet diet and served as control. Second and third groups received aloe vera gel extract in the dose of 200 and 400 mg/kg respectively. Blood glucose levels of all the three groups were estimated on 1st day, 7th day and 14th day. The results were tabulated.

Hypoglycemic effect of aloe vera gel extract in diabetic rats:

After a washout period of 1 month, all the rats were induced diabetes with Streptozotocin given at a dose of 60 mg/kg intraperitoneally as a single dose after 18 hours of fasting. 72 hours after Streptozotocin administration, the diabetic state was confirmed by blood glucose estimation for all the animals.

The diabetic rats were divided into four groups of six animals each. The first group received pellet diet and water served as control group. The second group received the standard drug Glibenclamide at the dose of 1 mg/kg/day per oral. Third and fourth groups received aloe vera gel extract in the dose of 200 and 400 mg/kg respectively served as Test 1 and Test 2 groups.

GROUP	STUDY	TREATMENT
I	CONTROL	Normal feed and water ad libitum
II	STANDARD	Normal feed and water +Tab Glibenclamide (1 mg/kg) oral
III	TEST -1	Normal feed and water + Aloe vera gel extract (200 mg/kg) oral
IV	TEST -2	Normal feed and water + Aloe vera gel extract (400 mg/kg) oral

The blood glucose level were estimated on 1st day, 7th day and 14th day and the results are tabulated. The data were analysed by using students unpaired‘t’ test to assess the level of significance.

RESULTS

RESULTS

In the present study 24 albino rats were selected and evaluated the antihyperglycemic effect of aloe vera gel extract in normal and streptozotocin induced diabetic rats.

NON - DIABETIC RATS

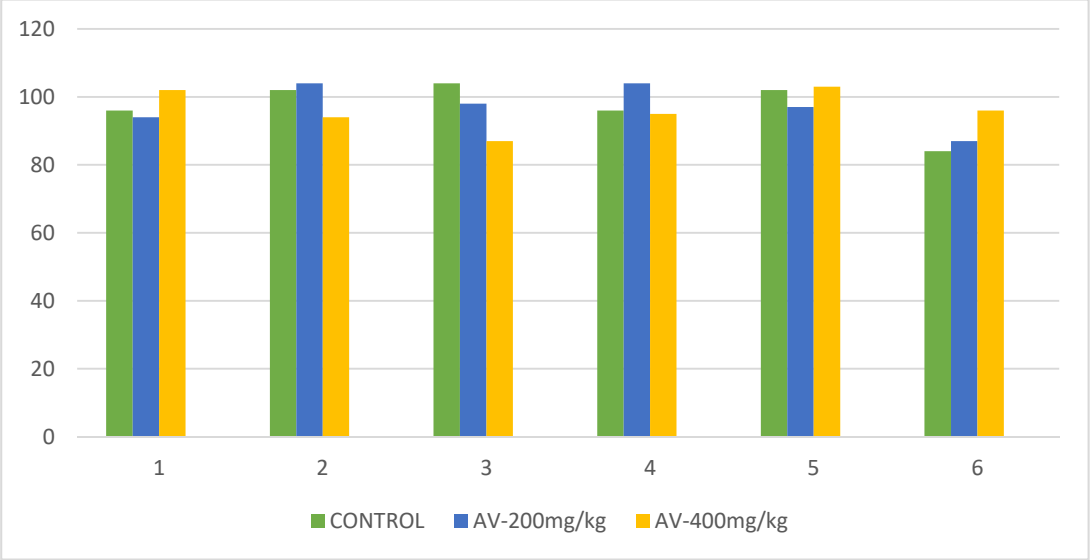
Initially 18 animals with normal fasting and postprandial blood glucose were selected and divided into 3 groups, control, test 1 and test 2. The first group received pellet diet and served as control. The second and the third groups received aloe vera gel extract in the dose of 200 and 400 mg/kg respectively. The blood glucose levels in non-diabetic rats of all the three groups on day 1 were

The blood glucose levels in non-diabetic rats in mg/dl on day 1

S No	Control	AV-200mg/kg	AV-400mg/kg
1	96	94	102
2	102	104	94
3	104	98	87
4	96	104	95
5	102	97	103
6	84	87	96

There was no significant reduction in blood glucose in the aloe vera treated group when compared with control.

The graph showing blood glucose levels in non-diabetic rats of all the three groups on day 1 were:

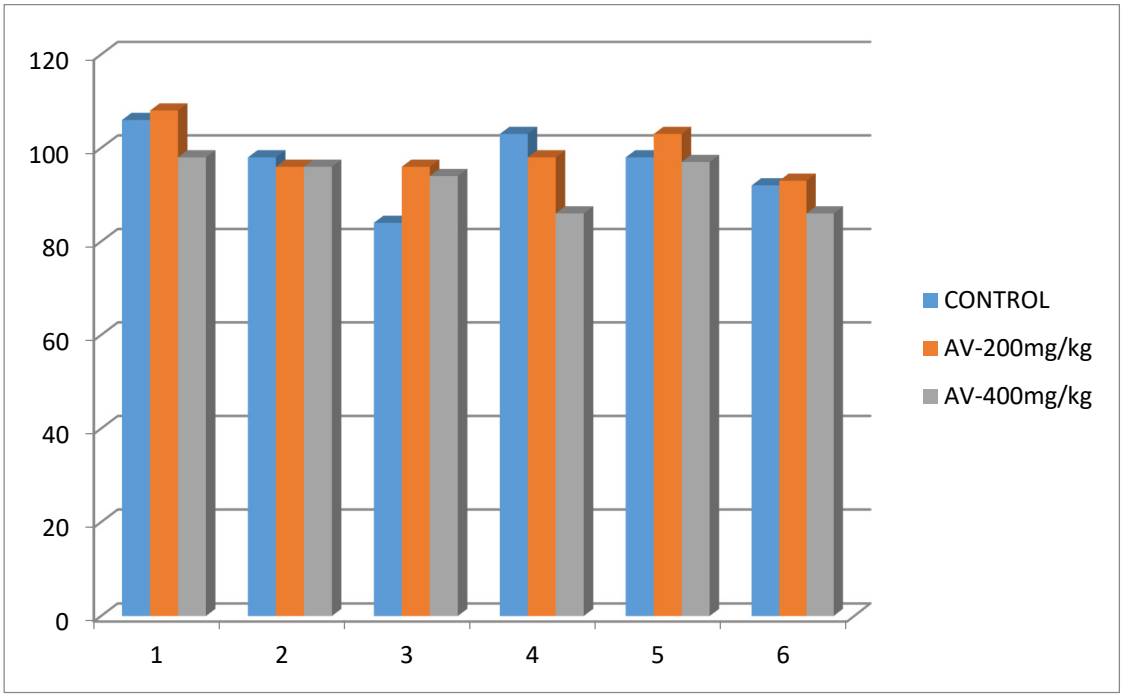


The first group received pellet diet and served as control. In addition to normal pellet diet, the second and the third groups received aloe vera gel extract in the dose of 200 and 400 mg/kg respectively. The blood glucose levels in non-diabetic rats of all the three groups on day 7 were

The blood glucose levels in non-diabetic rats in mg/dl on day 7

S No	Control	AV-200mg/kg	AV-400mg/kg
1	106	108	98
2	98	96	96
3	84	96	94
4	103	98	86
5	98	103	97
6	92	93	86

The graph showing blood glucose levels in non-diabetic rats of all the three groups on day 7 were:

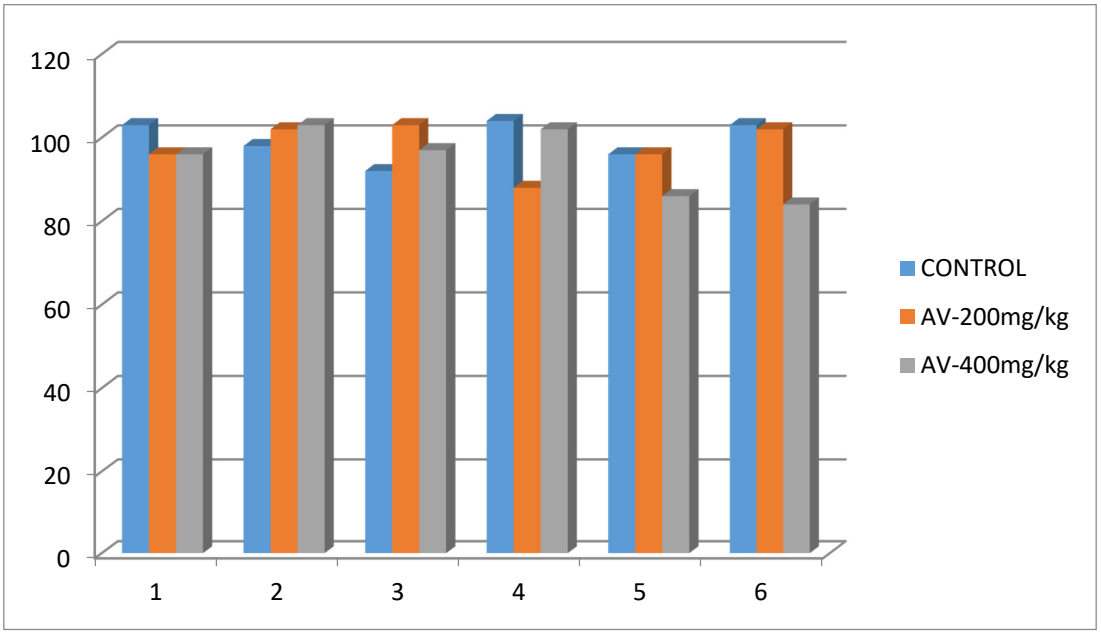


The blood glucose levels in non-diabetic rats of all the three groups on day 14 were:

The blood glucose levels in non-diabetic rats in mg/dl on day 14

S No	Control	AV-200mg/kg	AV-400mg/kg
1	103	96	96
2	98	102	103
3	92	103	97
4	104	88	102
5	96	96	86
6	103	102	84

The graph showing blood glucose levels in non-diabetic rats of all the three groups on day 14 were:



Control Group

The mean blood glucose values of rats on normal pellet diet (non-diabetic rats) on day 1, day 7 and day 14 were 96± 0.1, 96.8± 7.9 and 99.3± 5.2 respectively as shown in Table No.1 ($p > 0.1$).

Test group 1 (Aloe Vera gel extract 200mg/dl)

The mean blood glucose values of rats after administering Aloe vera gel extract of 200mg/kg on day 1, day 7 and day 14 were 97.3± 6.4, 99± 5.5 and 97.3± 5.7 respectively as shown in Table No.1 ($p > 0.1$).

Test group 2 (Aloe Vera gel extract 400mg/dl)

The mean blood glucose values of rats after administering Aloe vera gel extract of 400mg/kg on day 1, day 7 and day 14 were 96.1± 5.8, 92.8± 5.4 and 94.6± 7.9 respectively as shown in table No.1 ($p > 0.1$).

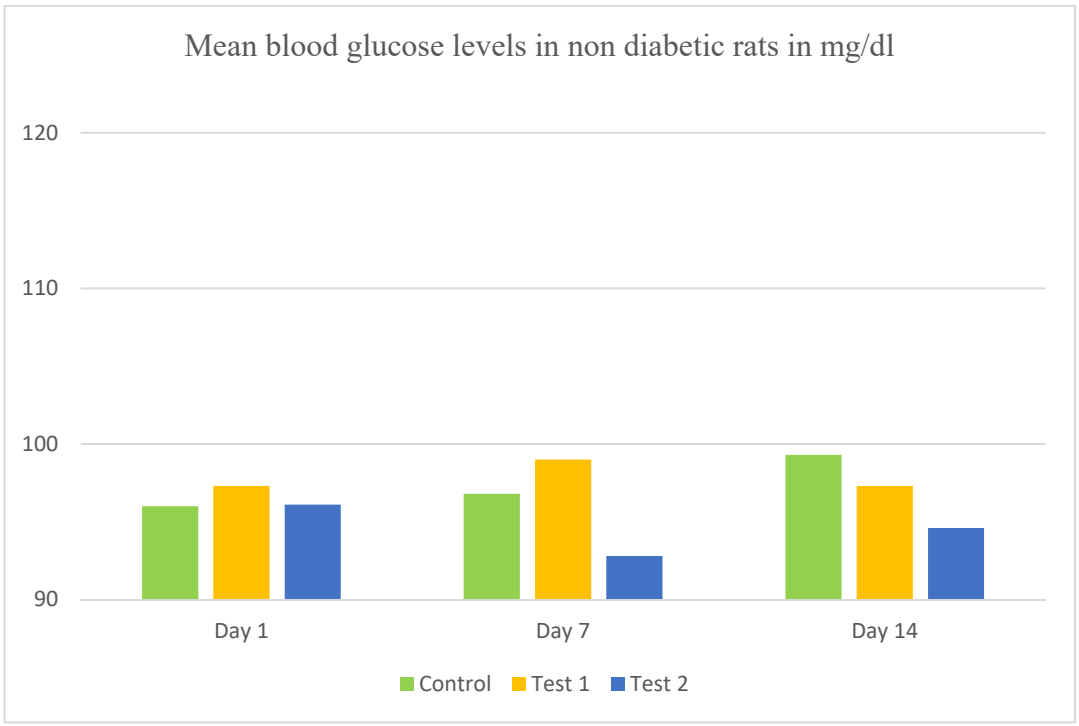
Table -1

Mean blood Glucose Levels in Non-Diabetic Rats in mg/dl

Group	Day 1	Day 7	Day 14
Control Normal pellet diet	96±0.1	96.8±7.9	99.3±5.2
Test 1 AV gel extract 200 mg/dl	97.3±6.4	99±5.5	97.3±5.7
Test 2 AV gel extract 400 mg/dl	96.1±5.8	92.8±5.4	94.6±7.9

($P > 0.1$)

The graph showing mean blood glucose levels in non-diabetic rats in mg/dl



It was noted that from the above data, aloe vera gel extract has no hypoglycemic effect in normal rats. (non-diabetic rats).

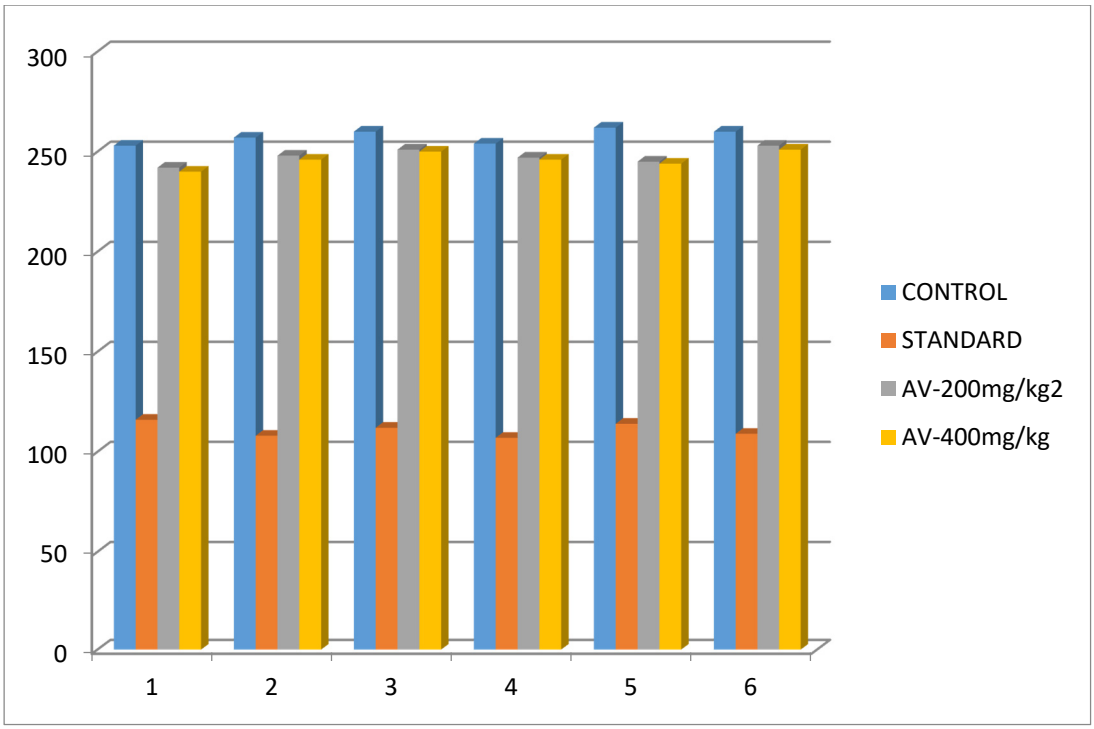
DIABETIC RATS

Diabetes was induced in all the rats by administering streptozotocin intraperitoneally in a dose of 60mg/kg. All the animals were found to be diabetic after 72 hours. The blood glucose values more than 250 mg/dl was considered as as diabetes. The animals were divided into four groups of six animals each. They are control, standard, test 1 and test 2.

The blood glucose levels in diabetic rats in mg/dl on day 1

S No	Control	Standard	AV-200mg/kg	AV-400mg/kg
1	253	116	242	240
2	257	108	248	246
3	260	112	251	250
4	254	107	247	246
5	262	114	245	244
6	260	109	253	251

The graph showing blood glucose levels in diabetic rats of all the three groups on day 1 were:

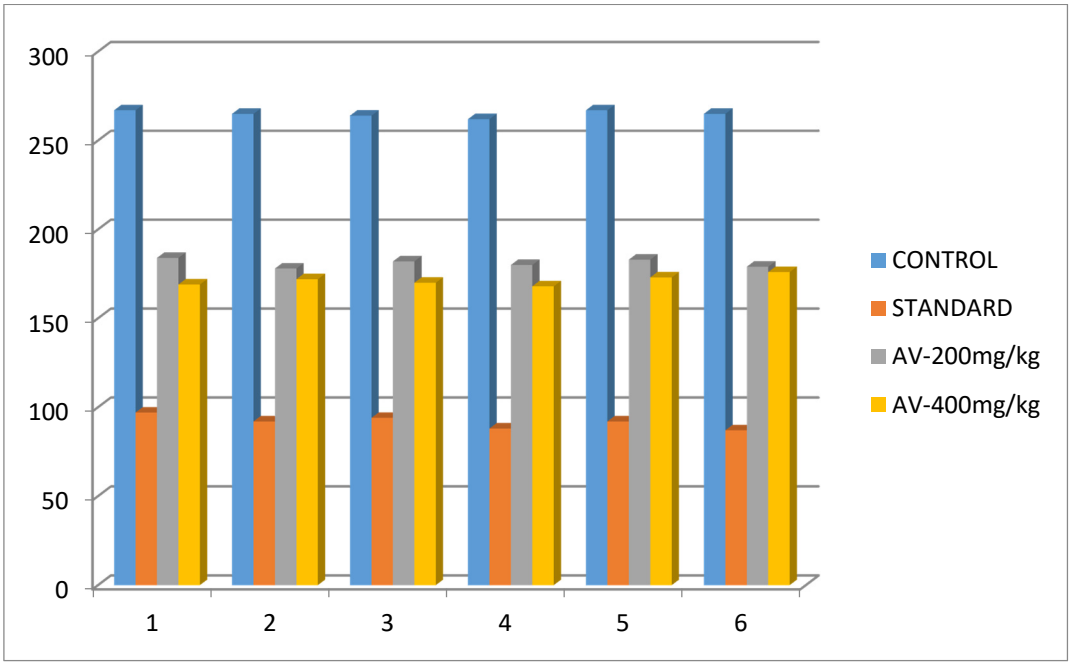


The first group received pellet diet and served as control. The standard group received Tab Glibenclamide. The third and fourth groups received aloe vera gel extract in the dose of 200 and 400 mg/kg respectively. The blood glucose levels in diabetic rats of all the three groups on day 7 were

The blood glucose levels in diabetic rats in mg/dl on day 7

S No	Control	Standard (Glibenclamide)	AV-200mg/kg	AV-400mg/kg
1	267	97	184	169
2	265	92	178	172
3	264	94	182	170
4	262	88	180	168
5	267	92	183	173
6	265	87	179	176

The graph showing blood glucose levels in diabetic rats in all the three groups on day 7 were

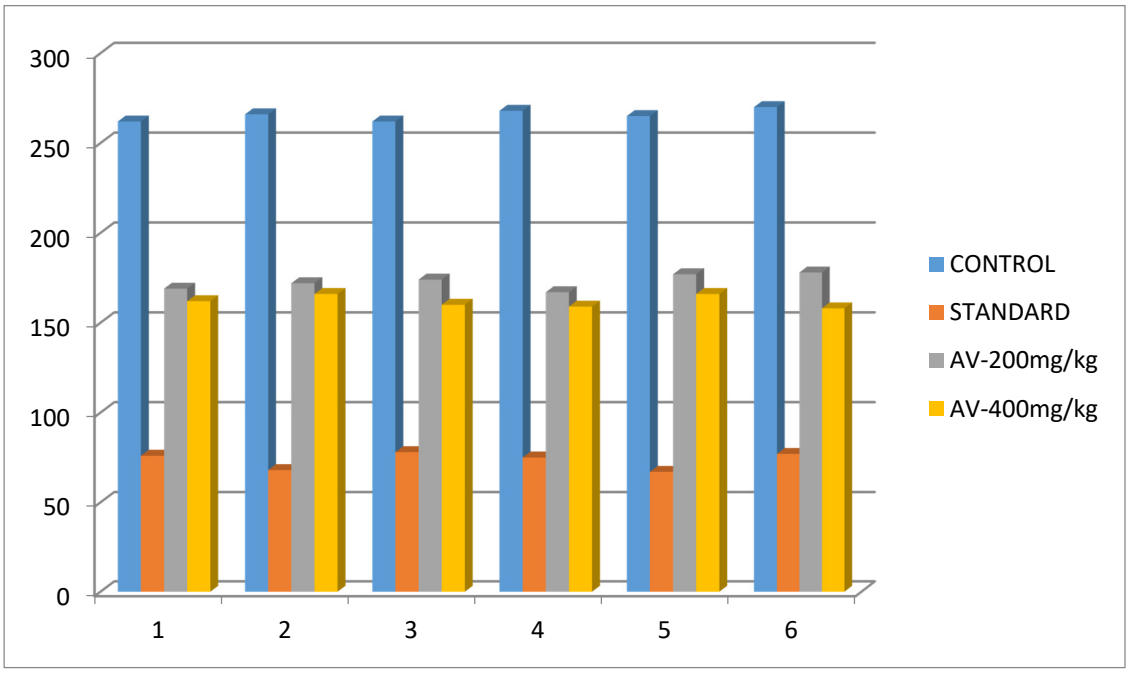


The control group received pellet diet and the standard group received Tab Glibenclamide. The third and fourth groups received aloe vera gel extract in the dose of 200 and 400 mg/kg respectively. The blood glucose levels in diabetic rats of all the three groups on day 14 were

The blood glucose levels in diabetic rats in mg/dl on day 14

S No	Control	Standard	AV-200mg/kg	AV-400mg/kg
1	262	76	169	162
2	266	68	172	166
3	262	78	174	160
4	268	75	167	159
5	265	67	177	166
6	270	77	178	158

The graph showing blood glucose levels in diabetic rats of all the three groups on day 14 were



Control Group

The mean blood glucose values of rats on normal pellet diet (Diabetic rats) on day 1, day 7 and day 14 were 257.6± 3.6, 265± 1.8 and 265.5± 3.2 respectively as shown in Table No.2

Standard group

The mean blood glucose values after the administration of Glibenclamide on day 1, day 7 and day 14 were 111± 3.5, 91.6± 3.7 and 73.5± 4.7 respectively as shown in Table No.2.

Statistical analysis by student's unpaired 'T' test revealed the antihyperglycemic effect of standard drug (Glibenclamide) is highly significant when compared with control group ($p < 0.005$).

Test Group 1 (Aloe Vera gel extract 200mg/dl)

The mean blood glucose values of rats after administering Aloe vera gel extract of 200mg/kg on day 1, day 7 and day 14 were 247.6± 3.9, 181± 2.3, 172± 4.3 respectively as shown in Table No.2.

The aloe vera gel extract of 200mg/kg has shown significant antihyperglycemic effect when compared with control group ($p < 0.05$).

Test Group 2 (Aloe Vera gel extract 400mg/dl)

The mean blood glucose values of rats after administering Aloe vera gel extract of 400mg/kg on day 1, day 7 and day 14 were 246.1± 4.1, 171.3± 2.9, 162.8± 3.2 respectively as shown in Table No.2.

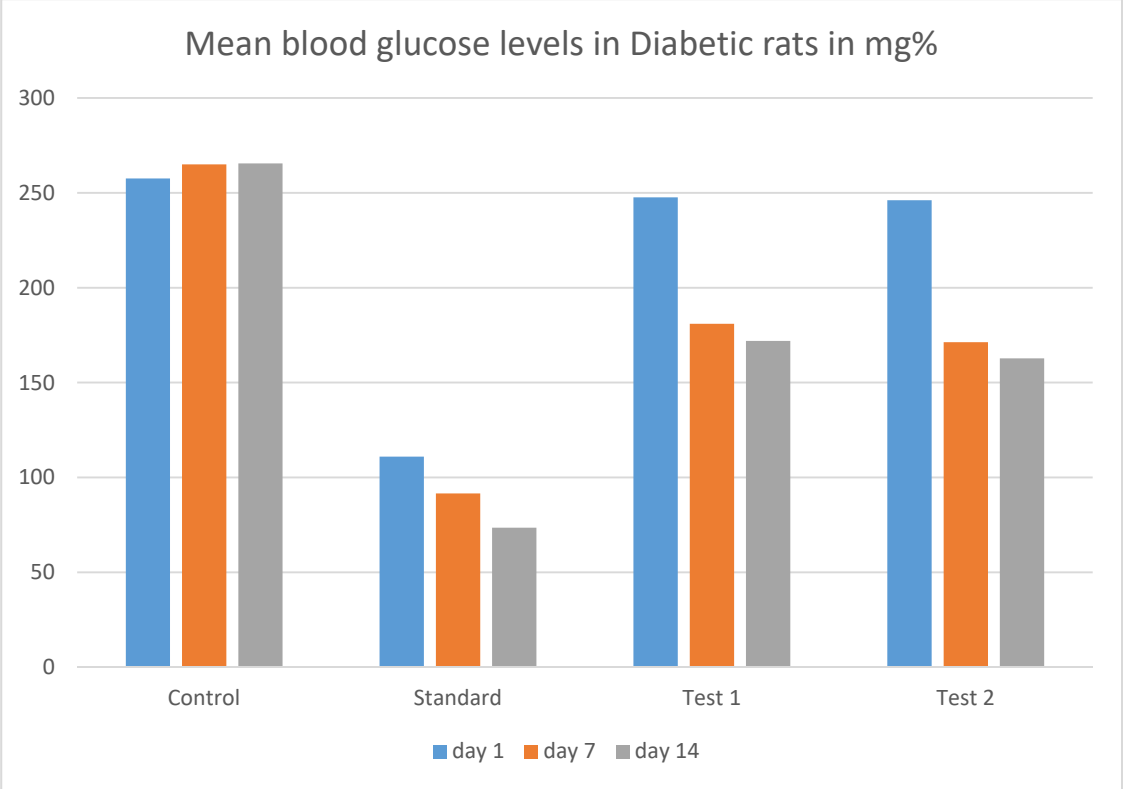
Table -2

Blood Glucose Levels in Diabetic Rats in mg/dl

Group	Day 1	Day 7	Day 14
Control Normal pellet diet	257.6±3.6	265±1.8	265.5±3.2
Standard - Glibenclamide	111±3.5**	91.6±3.7**	73.5±4.7**
Test 1 AV gel extract 200 mg/dl	247.6±3.9	181±2.3	172±4.3
Test 2 AV gel extract 400 mg/dl	246.1±4.1*	171.3±2.9*	162.8±3.2*

*$p < 0.05$

**$p < 0.001$



The aloe vera gel extract of 400mg/kg has shown significant antihyperglycemic effect when compared with control group ($p < 0.05$).

Percentage fall in blood glucose values in diabetic rats

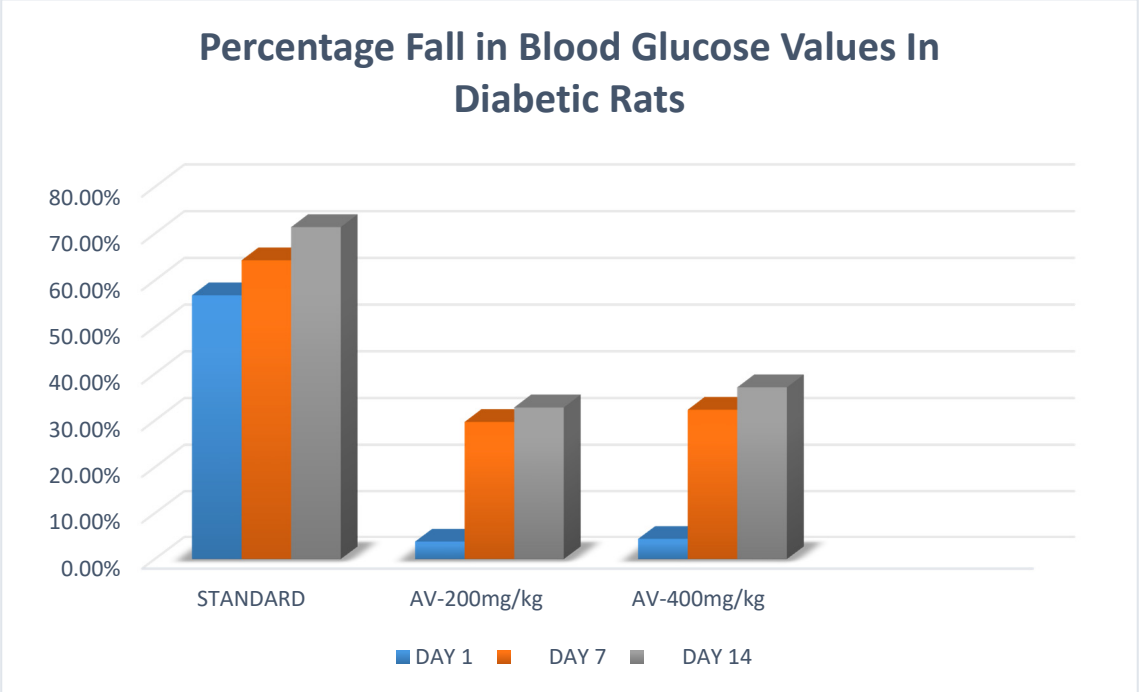
The percentage fall in blood glucose values in the various groups of diabetic rats was shown in Table 3. The percentage of fall in blood glucose levels with standard Glibenclamide in an average was 64.1% and with the aloe vera gel 400mg/kg treated group was 24.6% when compared with control group

Table -3

Percentage Fall in Blood Glucose values in Diabetic Rats

Groups	Day 1	Day 7	Day 14	Average
Standard - Glibenclamide	56.8%	64.3%	71.4%	64.1%
Test 1 AV gel extract 200 mg/kg	3.8%	29.7%	32.8%	22.1%
Test 2 AV gel extract 400 mg/kg	4.4%	32.3%	37.1%	24.6%

The graph showing percentage fall in blood glucose values in Diabetic Rats:



DISCUSSION

DISCUSSION

Diabetes mellitus is a chronic debilitating metabolic disorder charaterised by hyperglycemia and at present, there is a steep rise in the prevalence of DM. It is the major cause of morbidity and mortality worldwide as it may lead to health complications and affect quality of life[62].

Basically, the management of diabetes initiates with a diet modification and exercise. Nonetheless, most diabetic patients eventually require pharmacotherapy, such as oral antidiabetic drugs or injection of insulin. These synthetic drugs are the mainstay of treatment of diabetes and are effective in controlling hyperglycemia but they are not free from harmful side effects.

The limitations, side-effects, and cost of the currently available oral anti-diabetic agents to control blood glucose have stimulated and sparked the researchers to discover and develop novel anti-diabetic agents with fewer side-effects and potential therapeutic outcomes[63].

As an alternative mode of treatment, Ayurvedic medicine has been claimed to be more efficacious and less toxic[64]. An investigation of antihyperglycemic agents of plant origin used in traditional medicine seems important as per the recommendations of World Health Organization (WHO) Expert committee on diabetes mellitus.

Medicinal plants having beneficial effects on diabetes have been reported on several ethnopharmacologial studies. The hypoglycaemic activity of a huge

number of medicinal plants used traditionally has been evaluated and confirmed in different animal models[65].

Emblica officianalis (India gooseberry or amla), Green tea (*Camelia sinensis*), *Momordica Charantia* (Bitter guard), *Cinnamon bark* are proved to be effective antihyperglycemic agents acting through various mechanisms[66]. In this study, one such herbal remedy Aloe Vera gel was evaluated for its antihyperglycemic effect in normal adult male albino rats and Streptozotocin induced diabetic rats.

Initially, antihyperglycemic effect of aloe vera gel extract was evaluated in normal (non-diabetic) adult male albino rats. Three groups of six each includes control, test 1 and test 2 respectively. The blood glucose levels in non-diabetic rats of both the test groups of aloe vera gel extract 200mg/kg and 400mg/kg on day 1, day 7 and day 14 was not much reduced when compared with control group ($p > 0.1$). It is noted that from the above data, aloe vera gel extract did not produce the hypoglycemic effect in normal rats.

After the washout period of one month, antihyperglycemic effect of aloe vera gel extract was evaluated in Streptozotocin induced diabetic rats. Four groups of six each included control, standard, test 1 and test 2 respectively.

On day 1, when compared with the control group, there was an immediate fall in blood glucose level in the standard Glibenclamide group (111 ±3.5) but could not see the immediate fall with the test groups. Later, on day 7 and day 14 there was a significant fall in blood glucose level in the aloe vera gel treated groups.

Statistical analysis by student's unpaired't' test revealed that the antihyperglycemic effect of standard drug (Glibenclamide) was highly significant when compared with control group ($p < 0.001$).

The aloe vera gel extract of 200mg/kg and 400mg/kg has shown significant antihyperglycemic effect when compared with control group ($p < 0.05$).

The percentage fall in blood glucose levels with standard Glibenclamide was 64.1% and with the aloe vera gel 400mg/kg treated group was 24.6% when compared with control group.

Like many chronic disease, chronic hyperglycemia is presumed to cause elevated concentrations of reactive oxygen species and lowered enzymatic as well as nonenzymatic cell antioxidant defences. Reactive oxygen species have been suggested to be involved in beta cell dysfunction and insulin resistance[67]. As explained in an *in vitro* study of the radio protective efficacy of *Aloe vera* gel*, it* is able to scavenge the free radicals 2,2-diphenyl-1-picrylhydrazyl (DPPH), 2,2′-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid), and nitric oxide in a concentration-dependent manner[68].

Jain et al found that *Aloe vera* gel has significant antidiabetic and cardioprotective activity as it significantly reduced oxidative stress in streptozotocin induced diabetic rats and improved antioxidant status.

Previous studies focus on the phytocomponents present in the aloe gel having various biological properties that help to improve health and prevent

disease conditions. An inner clear gel of aloe vera contains 99% water and rest is made of glucomannans, amino acids, lipids, sterols, vitamins, enzymes, minerals, saponins and lignin. It contains 75 potentially active constituents and natural antioxidants (total phenols, total flavonoid, vitamin A (beta-carotene), vitamins C (ascorbic acid) and vitamin E (tocopherol) which are responsible for the antioxidative effect of this plant[69]. It also contains vitamin B12, folic acid, and choline.

In vivo and *in vitro* studies strongly demonstrate that the water soluble fraction of *Aloe* vera and some of its components modulate glucose transporter-4 mRNA expression thus possesses glucose-lowering activities[70]. In a randomized controlled trial, *Aloe vera* gel complex reduced body weight, body fat mass, and insulin resistance in obese pre diabetes and early non treated diabetic patients. One study discussed the efficacy of aloe-emodin-8-*O*-glycoside isolated from *Aloe vera* gel in enhancing glucose transport by modulating the proximal and distal markers involved in glucose uptake and its transformation into glycogen.

Trace elements present in Aloe vera play a very important role in glucose metabolism. The role of inorganic elements like Cr, Zn, Fe, Cu, and Mn improved the impaired glucose tolerance and their indirect role of management of diabetes mellitus[71]. Magnesium is one of the important mineral which takes part in the carbohydrate and fat metabolism. It plays a role in the insulin release and there is a chance of diabetes mellitus in its deficiency. Zn is a versatile element in diabetes as a cofactor for insulin and also enhances the effectiveness

of insulin. Potassium is necessary for the optimal insulin secretion and its depletion can result in reduced glucose tolerance. Thus, trace elements of aloe vera gel extract potentiate the antidiabetic activity of this plant.

So it could be possible that by various mechanisms, like modulating the glucose uptake, antioxidant effect due to the presence of flavonoids, vitamins and potentiation of insulin action by the presence of trace elements, extract of aloe vera gel has antihyperglycemic property with statistically significant fall in blood glucose level.

CONCLUSION

CONCLUSION

Diabetes mellitus is a major public health problem. India has been severely affected by the global diabetes epidemic and there is a significant increase in the burden of the disease. There is clear evidence to show that diabetes prevalence is rapidly increasing, especially in urban India.

Accordingly, diabetes related complications are also on the rise and contribute significantly to overall morbidity and mortality. The low levels of education and poor awareness of the disease in the country are enhancing its impact on health of the population.

Early identification and treatment at grass root level can bring down the morbidity and mortality. Pharmacotherapy is the corner stone in the management of diabetes mellitus and by combining with non-pharmacological therapy, diabetes is almost controllable now.

Even though various groups of medicine are currently available, no drug is free from adverse effects. Tolerability, safety at over doses is also a problem with many antidiabetic drugs. Hence an ideal drug exhibiting better safety and tolerability, which controls diabetes most efficiently is sought for.

Many effective drugs have come from botanical sources. Already natural products like *Allium cepa, Allium sativum* and many are effective in controlling diabetes mellitus. In this present study, aloe vera gel which was used for various illnesses as traditional medicine was evaluated for its antihyperglycemic effect in albino rats.

It was observed that aloe vera gel extract at 200 mg/dl and 400 mg/dl produce statistically significant reduction in blood glucose level in streptozotocin induced diabetic rats when compared with control group but not in normal adult male albino rats.

The study objective has been achieved. Further studies need to be done with more number of animals and different experimental models, to know the exact molecular and biological mechanism behind aloe vera gel as an antihyperglycemic. Identification and separation of the active principle that is responsible for the antihyperglycemic effect has to be further evaluated.

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BIBLIOGRAPHY

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MASTER CHART

MASTER CHART

The blood glucose levels in diabetic rats in mg/dl on day 1

S No	CONTROL	STANDARD	TEST 1	TEST 2
RAT- 1	253	116	242	240
RAT- 2	257	108	248	246
RAT - 3	260	112	251	250
RAT- 4	254	107	247	246
RAT- 5	262	114	245	244
RAT- 6	260	109	253	251

The blood glucose levels in diabetic rats in mg/dl on day 7

S No	CONTROL	STANDARD	TEST 1	TEST 2
RAT- 1	267	97	184	169
RAT- 2	265	92	178	172
RAT- 3	264	94	182	170
RAT- 4	262	88	180	168
RAT- 5	267	92	183	173
RAT- 6	265	87	179	176

The blood glucose levels in diabetic rats in mg/dl on day 14

S No	CONTROL	STANDARD	TEST 1	TEST 2
RAT- 1	262	76	169	162
RAT- 2	266	68	172	166
RAT- 3	262	78	174	160
RAT- 4	268	75	167	159
RAT- 5	265	67	177	166
RAT- 6	270	77	178	158

ABBREVIATION

ABBREVIATION

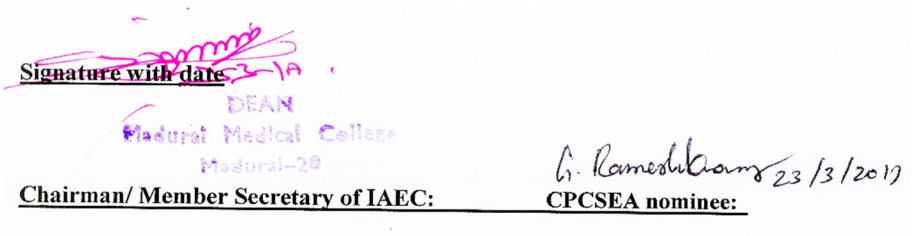
DM	–	DIABETES MELLITUS
ROS	–	REACTIVE OXYGEN SPECIES
JNK/SAPK	–	c-JUN N-TERMINAL KINASE
NF-κB	–	NUCLEAR FACTOR kappa B
MAPK	–	MITOGEN ACTIVATED PROTEIN KINASE
GL	–	GLIBENCLAMIDE
ATP	–	ADENOSINE TRIPHOSPHATE
Ca^{2+}	–	CALCIUM
Cr	–	CHROMIUM
Mn	–	MANGANESE
Zn	–	ZINC
STZ	–	STREPTOZOTOCIN
NAD	–	NICOTINAMIDE ADENINE DINUCLEOTIDE
HLA	–	HUMAN LEUKOCYTE ANTIGEN
TCF7L2	–	TRANSCRIPTION FACTOR 7 LIKE 2
GLUT2	–	GLUCOSE TRANSPORTER 2
GLP	–	GLUCAGON LIKE PEPTIDE
CFA	–	COMPLETE FREUND'S ADJUVANT

ETHICAL
CLEARANCE LETTER

Certificate

This is certify that the project title **EVALUATION OF ANTIHYPERGLYCEMIC EFFECT OF ALOE VERA GEL EXTRACTS IN NORMAL AND STREPTOZOTOCIN INDUCED DIABETIC RATS** has been approved by the IAEC.

Dr.M.R.VAIRAMUTHURAJU MD.,	**SHRI.G.RAMESHKUMAR**
Name of Chairman/Member Secretary IAEC.,	Name of CPCSEA nominee



(Kindly make sure that minutes of meeting duly signed by all the participants are maintained by Office)

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CERTIFICATE

This is to certify that the specimen brought by **DR.S.MEENAMBAL,** postgraduate in MD Pharmacology, Institute of Pharmacology, Madurai Medical College , Madurai is identified as Aloe vera belonging to the family of Liliaceae

26.2.2017

Station : Madurai
Date : 26.2.2017

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CERTIFICATE - II

This is to certify that this dissertation work titled **EVALUATION OF ANTIHYPERGLYCEMIC EFFECT OF ALOE VERA GEL EXTRACT IN NORMAL AND STREPTOZOTOCIN INDUCED DIABETIC RATS** of the candidate **Dr.S.MEENAMBAL** with registration Number **201516101** for the award of **M.D.,** in the branch of **PHARMACOLOGY**. I personally verified the urkund.com website for the purpose of plagiarism Check. I found that the uploaded thesis file contains from introduction to conclusion pages and result shows **3** percentage of plagiarism in the dissertation.

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