EVALUATION OF ANTIHYPERGLYCEMIC EFFECT OF AQUEOUS EXTRACT OF LEAVES OF ANNONA SQUAMOSA IN STREPTOZOTOCIN INDUCED DIABETIC RATS

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THE TAMILNADU

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TAMILNADU

CERTIFICATE

This is to certify that the dissertation entitled **"EVALUATION OF ANTIHYPERGLYCEMIC EFFECT OF AQUEOUS EXTRACT OF LEAVES OF ANNONA SQUAMOSA IN STREPTOZOTOCIN INDUCED DIABETIC RATS"** is a bonafide work done by **Dr.X.A.PRASANNA**, under the guidance and supervision of **Dr.R.SAROJINI, M.D.**, Professor, Institute of Pharmacology, Madurai Medical College, Madurai during the period of her postgraduate study of M.D Pharmacology from 2016-2019.

Dr. K.M.S.SUSILA. M.D.,
Director & Professor,
Institute of Pharmacology,
Madurai Medical College,
Madurai.

Dr.D. MARUTHUPANDIAN
M.S., F.I.C.S., F.A.I.S., FAC
Dean,
Madurai Medical College &
Govt. Rajaji Hospital, Madurai.

Place: Madurai
Date:

CERTIFICATE

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Dr. R.SAROJINI, M.D.,
Professor,
Institute of Pharmacology,
Madurai Medical College,
Madurai.

Place: Madurai
Date:

DECLARATION

I, **Dr.X.A.PRASANNA** solemnly declare that the dissertation titled **"EVALUATION OF ANTIHYPERGLYCEMIC EFFECT OF AQUEOUS EXTRACT OF LEAVES OF ANNONA SQUAMOSA IN STREPTOZOTOCIN INDUCED DIABETIC RATS"** has been prepared by me under the able guidance and supervision of **Dr. K.M.S.SUSILA, 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 May 2019.

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. X.A.PRASANNA**

Date:

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INTRODUCTION

INTRODUCTION

Diabetes mellitus is a metabolic disorder with features of chronic hyperglycemia that occurs due to defective insulin secretion or resistance to insulin action or both. The long term effects include end organ damage especially to the eyes, kidney, heart, blood vessels and nerves[1].

The global prevalence of diabetes among adults is estimated to be 6.4% in 2010 and is extrapolated to increase to 7.7% by 2030. As an emerging epidemic in India, it was estimated that 62.4 million are diabetic in 2011 and it is proposed to increase to 101.2 million by 2030[2]. The importance of this disease arises due to the fact that, many of these patients have an increased risk of atherosclerosis, stroke and peripheral vascular disease.

Dyslipidemia in type 2 diabetes mellitus is prevalent among 72-85% individuals.Dyslipidemia is often associated with diabetes. In type 1 diabetes there is an increased risk of cardiovascular mortality only after the development of nephropathy, whereas, in the case of type 2 diabetes, the risk exists for many years even before the onset of biochemical hyperglycemia[3].

Asian Indians are associated with increased risk of coronary heart disease than Caucasians and 80% of deaths in diabetic individuals is attributed to cardiovascular disease.There are associated lipid

abnormalities due to relative insulin deficiency and chronic hyperglycemia. There is an increase in low density lipoprotein cholesterol, triglyceride levels, and a decrease in high density lipoprotein cholesterol levels[4].

Several theories have been postulated for pathogenesis of diabetes. The glycoxidation hypothesis, proposed that pentosidine and carboxymethyllysine, which are a subclass of advanced glycosolation end products that cause oxidative damage to long lived proteins in diabetes[5].

The free radical theory connotes that the reactive oxygen species leads to cellular damage through stress sensitive pathways such as Nuclear Factor Kappa (NF-KB), Mitogen Activated Protein Kinase (p38 MAPK) and hexosamine pathway which is responsible for onset of late complications of diabetes[6].

Of all the treatment modalities for diabetes, life style intervention is promoted as an effective means to prevent or delay the incidence of diabetes and its complications, thereby reducing the public health burden[7]. Pharmacotherapy of diabetes commonly includes usage of sulfonyl ureas, biguanides and thiazolidindiones. But all these synthetic oral drugs are laden with adverse events on prolonged use[8]. According to the diabetes association there has been an inflation of cost in 2017, since

2012. This may be due to a 13% increase of cost per person with diabetes and an overall 11% increase in prevalence of diabetes[9].

Glibenclamide, a widely used sulfonyl urea causes inhibition of ATP sensitive k+ channels in the beta cells of pancreas, thereby promoting insulin secretion.The additional effects include antiplatelet, antitumor and analgesic activities. Research from prior studies, show that glibenclamide is beneficial in counteracting the oxidative stress mediated cellular damage[10].

The adverse effects of oral hypoglycemic agents include hypoglycemia, nausea, gastro intestinal disturbances, weight gain, hepatotoxicity and rarely cholestatic jaundice, blood dyscrasias. Hence, the advantage of glycemic control is offset with the imbalance in lipidemic control.

This has lead to an exponential growth in the field for research in herbal remedies, that have antihyperglycemic, and anti oxidant potential, with a minimum of adverse effects.

The World Health Organization has enlisted 21000 medicinal plants, of which 2500 species are endemic to India. Around 800 species are reported to have antidiabetic potential, such as *Combretummicranthium*, *Elephantopusscabes, Brassicajuncea* to site a few[11].

*Annona squamosalinn*is one such plant with antidiabetic potential. It is a small, semidecidious tree, which has a very good commercial importance in India. All parts of the plant such as fruits, leaves, root and bark are enriched with nutraceutical values. It is commonly know as "custard apple" or sitaphal[12].

The bioactive components of *Annona squamosa* are alkaloids, glycosides, essential oils, flavanoids, phenolic components, saponins[13]. The other effects of *Annona squamosa* are its antibacterial activity, antigenotoxic activity, antimicrobial and antioxidant, cytotoxic, hepatoprotective, insecticidal, vasorelaxant and anti inflammatory activity[14].

As a novel attempt to cut down the complications and cost of oral hypoglycemic agents and provision of better glycemic control in patients with diabetes, this study has been performed to evaluate the antihyperglycemic effect of the aqueous extract of the leaves of *Annona squamosa (linn)* in diabetic rat models.

AIM

AIM

To study the antihyperglycemic activity of aqueous extract of leaves of *Annona squamosa* in Streptozotocin induced diabetic rats.

REVIEW
OF
LITERATURE

REVIEW OF LITERATURE

“Diabetes is a dreadful affliction, being a meltdown of the flesh and limbs into urine. Life is short, unpleasant and painful, thirst unquenchable, drinking excessive and disproportionate to the large quantity of urine, for yet more urine is passed.” **-Aretaeus of Cappadocia.**

The facets of Diabetes Mellitus are ever changing and challenging, throughout the ages. It was presumably, first described by Physician Hesy Ra in an Egyptian papyrus (1500 B.C), which was discovered by George Ebers. Apollonius of Memphis (250 B.C) used the term ‘Diabetes‘. The sugary nature of urine was detailed by Thomas Willis in 1675 as “Pissing evil” and John Rollo (1797) nomenclature the term “mellitus” - which meant honey in Latin. The contributions to this history were also provided by Indian physicians Charaka and Sushruta around 400-500 B.C, who noted the sweetness of urine in the disease afflicted patients.

Again, the treatment of diabetes was empirical and accomplished with dietary manipulations. A mix of hierde, nardum, mastix, emetics, narcotics, fenugreek, wormseeds were prescribed along with a diet of milk, bread and boiled barley water. Apollinaire Bouchardat, considered by some as the father of diabetology, insisted on reduction of

carbohydrates, intake of green vegetables and intelligent use of exercise in order to bring the disease under control[15].

In the current world, diseases like diabetes, hypertension and coronary vascular diseases have increased several fold, even claiming to be the 'epidemics' of this century, mainly due to changes in lifestyle and nutrition.

Epidemiology

Worldwide estimates projected for the year 2030, show around 366 million people may be affected with diabetes mellitus. The prevalence was assumed to be more in urban areas and the age range in developing countries was between 45 to 64 years. The higher prevalence is most likely due to increasing urbanization and longer life span.

India tops the list of countries with the highest estimates, to project from 31.7 million to 79.4 million in 2030. Indians show a number of peculiar features which make them highly susceptible to diabetes[16]. Adults in urban areas of Tamilnadu have a diabetic prevalence of 13.7% as compared to the rural population (7.8%)[17]. Greater urbanization, growth of middle class and increased life expectancy will further increase the prevalence in near future.

A younger age of onset of diabetes had been noted in Asian Indians. According to the ICMR, 23% of diagnosed youth with diabetes

had type 2 diabetes, which was linked to obesity.The clinical implication is that, given their prolonged life span, they have a greater chance of developing the chronic complications of diabetics[18].

In terms of burden of disease, measured in Disability Adjusted Life Years (DALY), cardiovascular diseases and diabetes mellitus account for 14% of the total disease burden by the year 2030. The economic burden, in terms of accumulated losses in gross domestic product (GDP) between 2006 and 2015 can be as much as $17 billion in India, due to coronary vascular disease and diabetes alone[19].

The prevalence of metabolic syndrome in Tamilnadu population is compared sex wise with U.S population and it was around 37% in males (vs 43% U.S) and 35% in females (vs 38% U.S)[20].

Poor glycemic control increases the cholesterol levels, triglycerides and low density lipoprotein levels, which in turn fostered the development of cardio vascular diseases in diabetic patients. Optimal care, routine self monitoring of blood glucose, lipid profile assessment are key factors, to maintain diabetic dyslipidemia under control[21].

Diabetes is an “iceberg” disease. The increase in prevalence and incidence of Type 2 diabetes has been especially dramatic in developing, newly industrialised countries and countries in economic transition.

Migrant studies indicate that a genetic/ethnic vulnerability of Indians is associated with a higher risk of onset of diabetes and its complications.

The prevalence rises steeply with age especially in the middle years of life. The concordance rate in identical twins who developed Type 2 DM was approximately 90 percent, whereas in Type 1 DM, it was around 50 percent.

Type 1 DM is strongly associated with HLADR3 and DR4. Increasing BMI, weight gain, waist to hip ratio are powerful indicators for subsequent risk of Type 2 DM. Sedentary life style and lack of exercise, along with high saturated fat intake are other important environmental risk factors for development of Type 2 DM. Protein energy malnutrition in early infancy/childhood can lead to partial failure of β cell function, thus causing impaired carbohydrate tolerance. Rubella, mumps and human coxsackie virus B_4 are considered triggers in β cell destruction[22].

Definition

Diabetes mellitus is a metabolic disorder, occurring due to absolute or relative insulin deficiency, resulting in chronic hyperglycemia and attendant long term micro vascular and macro vascular complications such as nephropathy, neuropathy, retinopathy and atherosclerosis. Occasionally, the diagnosis of diabetes may be spotlighted because of

other co-morbidities such as urinary tract infections, furunculosis, coronary artery disease etc.., This complicates the disease further, as the patient may have a long asymptomatic period of 5 to 15 years but present for the first time along with the above mentioned complications[23].

Classification:[24]

1) Type I Diabetes

Idiopathic or immune mediated

2) Type II Diabetes

Insulin resistance with a relative insulin deficiency

3) Other specific types

A) Genetic defects

i. Hepatocyte nuclear transcription factor (HNF)4 alpha (MODY 1)
ii. Glucokinase (MODY 2)
iii. HNF-1 alpha (MODY 3)
iv. Insulin promoter factor (IPF) 1 (MODY 4)
v. HNF-1 Beta (MODY 5)
vi. Neuro D1 (or) Beta 2 (MODY)
vii. Type A insulin resistance
viii. Leprechaunism
ix. Lipodystrophic diabetes

B) Diseases of the exocrine pancreas

i. Trauma / Pancreatectomy
ii. Pancreatitis
iii. Hemochromatosis
iv. Neoplasia
v. Cystic fibrosis

C) Endocrinopathies

i. Cushing's Syndrome
ii. Hyperthyroidism
iii. Acromegaly
iv. Phaeochromocytoma
v. Glucagonoma
vi. Somatostatinoma

D) Drug (or) Chemical Induced

i. β – adrenegic agonists
ii. Glucocorticoids
iii. Thyroid hormone
iv. Nicotinic acid
v. Diazoxide
vi. Thiazides
vii. Second generation antipsychotics
viii. Protease inhibitors

E) Infections

i) Congenital rubella

ii) Cytomegalovirus

F) Rare causes

i) Anti insulin receptor antibodies

ii) Lawrence moon biedel syndrome

iii) Porphyria

iv) Prader-willi syndrome

4) Gestational diabetes mellitus

Homeostatic regulation of glucose[25]

Blood glucose must be well maintained and tightly regulated within a normal range so as to provide an uninterrupted supply of glucose to the nervous system even under extremes of situations. This is due to the fact that the brain depends on the liver for a constant supply of glucose for adenosine triphosphate (ATP) production, since, it has little capacity to store energy.

The glucose levels in blood are maintained on a fine balance between the absorption of glucose from food, and its peripheral utilisation especially by brain and skeletal muscle.

After a carbohydrate meal, the homeostasis is maintained by

1) Suppression of hepatic gluconeogenesis

2) Stimulation of glucose uptake by liver and peripheral tissues

Insulin is the physiological regulator of glucose metabolism. It is released in response to a meal along with a number of factors such as glucagon like peptide and gastrointestinal peptide.The role of these factors is to augment insulin release, a phenomenon known as “incretin effect”.

Insulin stimulates glucose uptake in skeletal muscle and fat through the glucose transporter GLUT-4.Vice versa, when intestinal glucose absorption is reduced, there is excessive hepatic gluconeogenesis and glycogenolysis, to maintain blood glucose levels.

Adipocytes regulate conversion of stored triglycerides to fatty acids. Adipocytes increase the glucose uptake in a fed state through insulin permissive action.

Though type 1 and type 2 diabetes present with similar features their etiology, immunological basis and pathogenesis varies widely, hence posing a challenge in their pharmacotherapeutic options.

Pathogenesis of Type-1 Diabetes

It is an auto immune disease associated with progressive destruction of pancreatic β – cells by T cell mediated immunity. An

interplay of both genetic and environmental factors is involved in the development of susceptibility to this disease. Of major importance are the polymorphic HLA-DRBI and HLA-DQB_1/DQA_1[26].

Candidate gene studies also identified the insulin gene on chromosome 11, as the second most important genetic susceptibility factor accounting for 10% of the cases[27]. The allele for the gene encoding CTLA-4-cyto toxic T lymphocyte antigen, on chromosome 2q33 is the third susceptible locus. Others include a variant of PTPN22, which disinhibits T-cell activation.

The popular candidates for environmental factors include

- Enterovirus
- Rotavirus
- Rubella

The autoantigens implicated in future risk of acquiring type I diabetes are, glutamic acid decarboxylase (GAD65), a protein tyrosine phosphatase like molecule (IA-2) and insulin. People with IA-2 antibodies have a high positive predictive value among the three mentioned above.

Pathogenesis of Type 2 Diabetes

Unlike type 1 diabetes, there is no evidence of an autoimmune basis for this disease. The triad of features include-

- Genetic factors
- Environmental factors, and
- Proinflammatory state.

The risk factors include[28],

i) Family history of diabetes

ii) Obesity

iii) Age >42years

iv) Ethnicity (Asian-American , African-American)

v) Previously diagnosed impaired fasting glucose (or) impaired glucose tolerance

vi) H/O gestational diabetes mellitus (or) delivering of a baby over 4.5kg

vii) Hypertension (BP ≥ 140/90)

viii) High density lipoprotein ≤35 mg %

ix) Triglyceride ≥250mg %

x) Polycystic ovarian syndrome

xi) Stress induced

- Infection
- Myocardial infarction
- Trauma
- Pregnancy
- Stroke

- Emotional stress
- Drugs (Glucocorticoids, estrogens, sympathomimetics , nicotinic acid)

Genetic factors:

First degree relatives have 5 to `10 fold higher risk of developing type 2 diabetes rather than family history naïve patients

The two cardinal features are[29]

i) Insulin resistance

ii) β cell dysfunction

Of these, insulin resistance is the forerunner and it is accompanied by compensatory β cell hyperfunction and hyperinsulinemia in the beginning. Insulin resistance leads to,

i. Failure of inhibition of endogenous hepatic gluconeogenesis
ii. Failure of glucose uptake and glycogen synthesis post prandially
iii. Failure of inhibition of lipoprotein lipase in central adipose tissues free fatty acids are “toxic”. They attenuate insulin receptor signaling.

The functional defect includes reduced phosphorylation of tyrosine of insulin receptor and IRS proteins.This results in reduced GLUT 4 expression on peripheral cells. Furthermore, the levels of adiponectins is reduced in obesity, which contributes to insulin resistance.

The inflammatory component includes secretion of 1L-1β, which mediates further release of proinflammatory cytokines from macrophages and islet cells promoting insulin resistance.

The β cell dysfunction is promoted by several mechanisms such as,

(i) Lipotoxicity

(ii) Glucotoxicity

(iii) Abnormal incretin effect leading to reduced GIP and GLP-1

(iv) Amyloid deposition in the islets.

Clinical features[30]:-

The symptoms of diabetes mellitus include

i. Excessive thirst

ii. Excessive urination

iii. Excessive hunger

iv. Lethargy

v. Sudden weight loss

vi. Drowsiness

vii. Blurring of vision

viii. Dry mouth

Increased urination and thirst are due to hyperglycemia. Osmotic diuresis leads to glycosuria and loss of water and electrolytes in urine.

Increased hunger – The body does not have enough insulin to ensure glucose uptake by the skeletal muscles and organs, hence depleting them of ATP (adenosine triphosphate), leading to hunger.

Weakness of muscles is due to total body potassium loss and catabolism of muscle proteins. Neurotoxicity from sustained hyperglycemia leads to temporary dysfunction of peripheral sensory nerves causing paresthesias.

Prolonged exposure of the ocular lens to hyperosmolar fluids leads to blurred vision. In certain obese individuals the Type 2 diabetes will be occult for some time, detected only with routine laboratory studies.

Chronic skin infections are common. Recurrent furunculosis, urinary tract infections may occur. Some women present with pruritus and symptoms of vaginitis. Balanoposthitis may occur in males.

Centripetal distribution of fat in the abdomen, chest, neck and face might occur. Acanthosis nigricans (hyperpigmented and hyperpkeratotic skin in the axilla, groin and back of neck) is a sure sign of insulin resistance.Cutaneous features of diabetes include Necrobiosis lipoidicadiabeticorum and dermopathy.

Hypertension (50%) and obesity(70%) are common clinical findings in patients with diabetes. Poor glycemic control is indicated by eruptive xanthomas on flexor aspect of limbs. Hyperchylomicronemia

with lipemiaretinalis might occur in uncontrolled Type 2 diabetes with familial hypertriglyceridemia.

Characteristics of Type 1 and Type 2 Diabetes

Features	Type 1	Type 2
1. Onset	Abrupt	Progressive
2. Endogeneous insulin	Low to absent	Normal, elevated or depressed
3. Ketosis	Common	Rare
4. Age at onset	Any age	Vast majority adults
5. Bodymass	Usually non obese	Obese
6. Treatment	Insulin	Diet, oral hypoglycemics, insulin
7. Family history	10% - 50%	30%
8. Twin concordance	30% - 50%	70% - 90%
9. HLA association	HLA–DR, HLA DQ	Unrelated
10. Auto antibodies	Present in >85%	Absent, except in patients with coincidental type 1 diabetes

Diagnostic criteria[31]:-

Blood glucose value	Normal glucose tolerance	Impaired glucose tolerance	Diabetes mellitus
Fasting plasma glucose (mg/dl)	<100	100 – 125	>126
2 hrs after glucose load (75g in 300ml of water) (mg/dl)	<140	>140 -199	>200
HbA_{1c} (%)	<5.7	5.7 – 6.4	>6.5

Additionally, due to increased prevalence of dyslipidemia in diabetes, it is instructive to screen the lipid profile on an annual basis, especially in type 2 diabetes.

Total cholesterol, HDL cholesterol and triglycerides should be checked in fasting state and only after achieving good glycemic control to obviate the false need of lipid lowering agents. The LDL levels can be calculated using the Friedewald's formula.

The ADA guidelines states that the primary goal is to lower the LDL cholesterol levels below 100 mg/dl and in patients with cardiovascular risk, the LDL target levels should be < 70 mg/dl. Raising

HDL values >40 – 50 mg/dl is deemed desirable. A target level of <150 mg/dl is proposed for triglycerides[32].

Complications of Diabetes Mellitus[33]:

Acute complications:

1. Diabetic ketoacidosis (DKA)
2. Hyperglycemic hyperosmolar syndrome (HHS)
3. Iatrogenic hypoglycemia

Underlying mechanism for DKA and HHS includes a reduction in circulating insulin and increase in catecholamines, cortisol, glucagon and growth hormone.

The patients present with

1. Hyperglycemia – Due to increased gluconeogenesis
   - Increased glycogenolysis
   - Reduced glucose utilisation
2. Keto acidosis – due to
   - Increased lipolysis
   - Increased hepatic fattyacid oxidation

Chronic complications

Micro vascular complications

1. Diabetic neuropathy
2. Diabetic retinopathy
3. Diabetic nephropathy

Macro vascular complications

1. Cerebro vascular disease
2. Coronary artery disease
3. Peripheral artery disease
4. Erectile dysfunction
5. Gangrene of lower extremities

Endothelial dysfunction is implicated as the principle cause of micro vascular and macro vascular complications

i. Decreased production of Endothelium derived relaxing factor (EDRF)
ii. Increased inactivation of EDRF
iii. Impaired diffusion of EDRF to smooth muscles
iv. Impaired smooth muscle responsiveness to EDRF

Mechanisms for hyperglycemia induced damage:-

Five major mechanisms result in hyperglycemia induced end organ damage

A.Increased polyol pathway flux

The polyol pathway contains the enzyme aldose reductase which utilizes NADPH as a cofactor to convert glucose to sorbitol. Excessive depletion of NADPH causes reduced regeneration of glutathione, which

requires NADPH as a cofactor. The final outcome is increase in reactive oxygen species due to reduced scavenging by glutathione.

B. Increased intracellular formation of AGEs (Advanced Glycation End products)

These AGE's will alter the function of intracellular proteins and plasma proteins which will in turn lead to induction of ROS, which will activate NF-KB (nuclear factor KB) causing pathological changes in gene expression.

C. Increased expression of the receptors for the AGE's

D. Increased PKC (Protein Kinase) activation

Due to enhanced de novo synthesis of DAG (diacyl glycerol) it depresses the production of NO (nitric oxide by eNOS (endothelial nitric oxide synthase) and increases ET-1 (endothelin) production, thereby causing decrease in renal and retinal blood flow.

E. Overactivity of the hexosaminepalthway

Glucose is shunted into hexosamine pathway. Fructose 6 phosphate is diverted from glycolysis, to provide a substrate for Glutamine Fructose 6 phosphate Amino Transferase (GFAT). This GFAT in turn produces UDP-N acetyl glucosamine which is used for post translational modifications on certain cytoplasmic and nuclear proteins leading to an increased production of TGF-β (Transforming growth factor β) and

PA1-1 (Plasminogen activator inhibitor). The end result being capillary occlusion and vascular occlusion[34].

Treatment modalities of Diabetes Mellitus:-

- Life style modification
- Diet
- Insulin
- Oral anti diabetic agents

Lifestyle modification :-

Patient centered approach and individualised treatment are the pillarstones in maintaining a comprehensive glycemic control. Lifestyle intervention with a focus on increased physical activity must be reinforced as a general diabetes education to all patients. Even a modest weight loss (5-10%) contributes to achieving improved glycemic control and has a positive long term impact on cardiovascular risk factors.

150 minutes/week of moderate aerobic exercises, flexibility training should be advised. Since the high glycemic response that occurs after a meal is implicated in microvascular risk enhancement, it is better to perform exercise an hour after meal[35].

The mechanisms by which exercise regulates glucose levels are,

- Stimulation of GLUT – 4 transporters in muscle and improved glycogen storage.

- Improved overall insulin sensitivity
- Promotion of oxygen delivery to peripheral tissues
- Elevation of 2,3 DPG levels in RBC
- Reduction of HbA1c

Some guidelines to improve glycemic response and avoid hypoglycemia are,

- If glucose level is <100 mg/dl, patient should eat a snack
- If fasting glucose level is >300 mg/dl, exercise is contraindicated
- Monitoring of plasma glucose after exercise must continue up to 6-12 hrs after exercise, because exercise induced hypoglycemia may be protracted.
- Reduction of premeal insulin dose
- Avoid massage or exercising the body part that has received subcutaneous insulin as it might foster rapid absorption and result in hypoglycemia
- Children and adolescent girls have greater variations in blood glucose levels due to play activity and hormonal changes respectively[36].

Dietary Modification[37] :

Diet plays a critical role in diabetes. A few guidelines for dietary management are,

- Regular meals timings
- 8-10 servings of vegetables to be consumed.
- 5-6 servings per day are better than 2-3 large sized meals
- Complex carbohydrates are better than simple sugars.
- Total carbohydrate intake should constitute 50-55% of total calories
- Total protein intake around 15-20% of calories and 25-30% calories should be provided from fat.
- Carbohydrates breakup is a follows:

➢ 25%-in breakfast

➢ 30%-in lunch

➢ 30%-in dinner

➢ 15%-in evening snack

- Fiber intake should be around 14 g /1000 k Cal
- Fat Sources from MUFA (Mono Unsaturated Fatty Acid), PUFA (Poly Unsaturated Fatty Acid) and EPA (EicosaPentanoic Acid) are recommended
- Saturated fat must be < 7% of total calories.

- High biological value foods are preferred
- Salt intake must be reduced to < 1.5g / day
- Allowance for sufficient vitamin C, vitamin B6, vitamin E, zinc, chromium, magnesium, carotenoids and flavanoids must be provided.
- Limitation on alcohol consumption to one drink per day for women and

  2 drinks / day for men.
- Avoidance of foods with high glycemic index such as glucose, sugar, honey, parathas, pastries, mangoes, bananas, sweet potatoes.
- Generous intake of green leafy vegetables, radish, tomatoes and soups, due to their lower glycemic index.

Yoga :-

- Improves glycemic control
- Improves insulin sensitivity
- Relieves stress

Pharmacotherapy :-

Insulin:-

The discovery of insulin in 1921 by Banting and Best and its subsequent utilization on Leonard Thompson in 1922, was a seminal event in the history of diabetes. Insulin is a protein comprising of 2

polypeptide chains: A-with 21 amino acid residues and B-with 30 amino acid residues, both linked by an intra chain disulfide bridge at residues 6 and 11 respectively[38].

Human insulin is now produced by recombinant DNA technology using *E.coli* (or) yeast. Dry human insulin is a microcrystalline powder that precipitates at pH of 5.4 but is soluble at pH 2-3.

The international unit conversion is as follows; 1 I.U. of insulin corresponds to 38.5mg dry substance.

Insulin is recommended for

- Type1 Diabetes mellitus and
- Type2 Diabetes mellitus, who are unresponsive to diet, lifestyle intervention and oral anti diabetic drugs.

Target tissues of insulin include,

- Liver - Increases glucokinase activity
  -Traps glucose in hepatocytes
  - Enhances glycogen synthesis and fatty acid synthesis.
- In skeletal muscle and adipose tissue - Increases translocation of GLUT 4 from intracellular vesicles to cell adipose surface.
- In skeletal muscle - Increases amino acid uptake and promote protein synthesis and promotes glycogen storage.

- In adipose tissue - Increases expression of lipoprotein lipase

  -Deactivates hormone sensitive lipase

  -Net result is increase in triglyceride synthesis.

Mechanism of Action of Insulin:-

Insulin acts through a cell membrane linked tyrosine kinase receptor.

On ligand binding, tyrosine kinases autophosphorylate, thereby leading to phosphorylation of IRS-2(Insulin Receptor Substrates), which are required for further downstream signalling events.

The pancreatic β cells, contain K^+/ATP channel, which is an octamer composed of Kir 6.2 and SUR1 subunits. ATP binds to Kir 6.2 and inhibits it, leading to insulin secretion. This ATP is made available via GLUT2 transporters in the cell membrane which carry glucose inside the cell, after a meal.

Inhibition of K+/ATP channel eventually reduces plasma membrane K^+ conductance, thereby causing membrane depolarisation by opening voltage gated Ca^{2+} channels, Further influx of calcium inside the cell, leads to vesicular exocytosis of insulin[39].

GLP-1, G1P, parasympathetic activation are other factors that inhibit K^+/ATP activity.

Classification of insulin preparations[40]

Rapid acting :

- Insulin glulisine
- Insulin aspart
- Insulin lispro

Short acting :

- Regular (soluble) insulin

Intermediate acing:

- Insulin zinc suspension
- Isophane Insulin

Long acting :

- Insulin glargine
- Insulin determir
- Ultralente insulin

Insulin analogues :-

They are ultra rapid and long acting insulin analogues obtained by recombinant DNA technology with minor substitutions in the amino acids of chain A and/ or chain B.Their advantages include,

- Low incidence of hypoglycemia
- No variation in absorption despite difference in site of injection
- Controls post prandial hyperglycemia

- Rapidly absorbed.
- Longer acting analogue, providing a continous low peakless concentration of insulin.
- Lesser weight gain
- Lesser hypoglycemic episodes between meals

Adverse effects of insulin

1. Hypoglycemia

Type 1 Diabetes mellitus, elderly diabetics, people with diabetic nephropathy and patients on intensive insulin regimen are at high risk for hypoglycemia.

2. Ig E mediated insulin allergy
3. Lipodystrophy
4. Weight gain of 2-4 Kg.
5. Loss of usual accommodation
6. Edema
7. Precipitation of peripheral neuropathy
8. Insulin resistance.

Oral anti-diabetic agents :-

Orally administered anti diabetic agents can be used as monotherapy (or) combined with other classes of oral anti diabetic agents (or) with insulin in certain refractory cases[41]. There has been an exponential burst in the development of newer agents, which are both a

boon and a bane to the physicians, who are loaded with a number of complex medications to choose from.

Classification[42]:-

1) Insulin secretagogues:-

(Bind to SUR) receptor and stimulate insulin secretion)

Sulfonyl ureas

Meglitinides

Dipeptidyl peptidase 4 (DPP 4) inhibitors

2) Insulin sensitisers :-

(Increase peripheral uptake of glucose)

Biguanides

Thiazolidinediones

3) Inhibitors of intestinal absorption of glucose

- Alpha glucosidase inhibitors

4) Incretin Mimmetics :

GLP -1 receptor agonists

5) Newer drugs :

Sodium glucose co transporter (SGLT2) inhibitors

Bromocriptine

PPAR alpha and PPAR gamma inhibitors

Background on Oral hypoglycemic agents

In 1926, Frank and his colleagues synthesized "Synthalin" a guanidine derivative as the first orally active anti diabetic drug. Unfortunately, it was deemed excessively toxic and was withdrawn.

In 1940, August Loubatiere's, afrench pharmacologist, discovered sulfonylurea from a sulfonamide derivative. He noted that an intact (or) a partially functioning pancreas was essential for the activity of sulfonylureas. Carbutamide was the first clinically used derivative but was withdrawn due to bone marrow toxicity. It was followed in 1957 by Tolbutamide.

Phenformin joined the race in 1959, as the first Biguanide. It was followed by metformin, which was not marketed until 1994 in the American market.

Thiazolidinediones were introduced in 1994. The recent ones to join the foray are the GLP -1 agoinst (exenatide) (or) DPP4 inhibitors, by the year 2005. Since then, they have paved way for SGLT 2 inhibitors and the search for an ideal anti diabetic agent is intensive as always[43].

Sulfonylureas :-

They play a role in treatment of non obese type 2 diabetic patients in whom metformin is contraindicated (or) metformin is unable to achieve glycemic control as monotherapy.

First Generation drugs :-

Chlorpropamide, Tolbutamide, Acetohexamide,

Tolazamide.

Second Generation :

Glibenclamide, Gliclazide, Glimepride,

Glipizide.

Mechanism of Action[44] :

They require residual pancreatic beta cell activity. They act by increasing the plasma insulin levels and reduce the hepatic clearance of insulin.

They bind to sulfonyl urea receptors (SUR-1) of the K+/ATP dependant channel present in the cell membrane of beta pancreatic cells. They inhibit the above mentioned channel, thereby blocking K+ conductance via the channel. This leads to depolarization of cell membrane, causing a massive influx of Ca^{2+} into the cytosol, through voltage activated calcium channels.

The inflow of calcium into the cell causes actin myosin filament contraction and extrusion of the intracellular vesicles containing insulin.

The SUR 1 is a member of the ATP binding cassette (ABC) super family and it has a high affinity for glibenclamide. SUR 1 is highly expressed in the pancreatic β cells and in the brain.

The other minor mechanisms of sulfonyl ureas include

- Stimulation of hepatic gluconeogenesis
- Increase in number of insulin receptors
- Increased sensitization of insulin receptors.

The extra pancreatic effects include

- Enhanced insulin action on liver
- Inhibition of triglyceride lipase.
- Inhibition of ketogenesis
- Increased glucose uptake and oxidation.

Unrelated to their antidiabetic activity are

- Effects on water balance
- Inotropism
- Anti -aggregatory effect on platelets
- Adenyl cyclase activation
- Invitro inhibition of catecholamine release
- Inhibition of adenosine monophosphate diesterase.

The clinical implications of the above effects are not clear.

Pharmacokinetics :

Liver metabolism via cytochrome P 450 2C9 is involved in clearance of these drugs. They are renally excreted, so they must be dose adjusted in elderly people or those with impaired renal function.

Chlorpropamide and glyburide have longer half lives, which reflects their higher propensity for causing hypoglycemia. Hence it is better to start at a lower dose for patients with higher risk for protracted hypoglycemia. The sulfonylureas can cause a reduction of HbA1c by around 1.5% to 2%, and a fall in fasting plasma glucose by 60 to 70 mg/dl. Sulfonylurea failure cases have low C peptide levels (or) high (>250mg/dl) fasting plasma glucose levels.

Sulfonylureas show a significant benefit in reduction of risk of micro vascular complications. They have negligible effect on the long term outcome of macro vascular complications.

Adverse effects[45] :-

- Hypoglycemia (5%) is commonest with chlorpropamide.
- Weight gain
- Skin rashes
- Gastro intestinal upset
- Cholestasis
- Hemolytic anemia
- Disulfiram like reaction with 1st generation drugs.

Drug interactions :

Competitors for plasma protein binding	Warfarin ,Salicylates,
Cytochrome P450 inducers and inhibitors	Chloramphenicol, Cimetidine,Rifampicin Monoamine Oxidase inhibitors
Competitors of renal excretion	Allopurinol , Probenecid

Dosing regulations :-

It is better to start with lower doses in elderly (or) renal / hepatic function compromised patients. Dosage is uptitrated every 1-2 weeks until the target glucose level is attained.

Meglitinides :

- Repaglinide
- Nateglinide

These are non sulfonyl urea secretogogues of insulin. They bind at K^+ / ATP channels on the beta cells of pancreas but at an allosteric site than that of sulfonyl ureas. They have a short latency period. Their plasma t1/2 is <1 hr and their duration of action is also shorter.

They preferably reduce the meal time hyperglycemia. It is important to avoid them, if a person has skipped a meal.The drug can be

given before 15 minutes of meal time and started at a low dose of 60 mg. The dose can be up titrated weekly, until the desired glycemic control is achieved (or) a maximum dose of 360mg per day is given, whichever is most tolerable.[46]

Metabolism is via CYP3A4 and so precautions regarding drug interactions is advisable.The advantage of this group, is that, of all the insulin secretagogues, they have the least risk of hypoglycemia.

Biguanides :-

1. Metformin
2. Phenformin
3. Buformin

Metformin is the only biguanide that is currently used widely in a case of type 2 diabetes mellitus. Phenformin and buformin were withdrawn due to severe lactic acidosis. The intracellular target of the drug is the Adenosine Monophosphate activated Protein Kinase system (AMPK)[47].

They cause inhibition of gluconeogenesis and increase the sensitivity for insulin in the target tissues. They require insulin for their action. They reduce absorption of glucose from the intestine. They act as insulin facilitators in adipose tissues and skeletal muscle.The drug is used as monotherapy or combined with other oral antidiabetic agent, especially in obese type 2 diabetic patients.

Adverse events :-

- Nausea, diarrhoea
- Metallic taste
- Lactic acidosis – especially in renal impairment patients, liver failure cases, or patients with cardiogenic / septic shock.

Cautious use of metformin is required if the estimated GFR (glomerular filtration rate) is < 45 ml / min / 1.73 m^2.

Metformin is contraindicated if the eGFR is <30 ml/min/1.73 m^2. It should be withdrawn temporarily before administration of anaesthesia or radiocontrast media. Other uses of metformin may be in polycystic ovarian syndrome.

Thiazolidinediones :-

The glitazones were a serendipitous discovery from ciglitazone, which was a clofibrate analogue, screened as a lipid lowering agent but it accidentally lowered the blood glucose levels. Ciglitazone was withdrawn due to liver toxicity. Rosiglitazone and troglitazone were withdrawn due to hepato toxicity and cardio vascular side effects.Pioglitazone is the only available drug in this group.

Mechanism of action :

Peroxisome proliferator activated receptor gamma is a nuclear receptor, which is complexed with retinoid X receptor.

The PPAR gamma is present in adipose tissue, muscle and liver.

Effects of glitazone are

- Adipocyte differentiation.
- Increased lipogenesis
- Enhanced uptake of fatty acids and glucose.
- Promotion of amiloride sensitive sodium ion reabsortpion in renal tubules.

The thiazolidinediones dimerize the PPAR gamma - RXR complex, and allow it to bind to DNA, thereby promoting insulin signaling such as increased activity of lipoprotein lipase, GLUT -4 translocation, regulation of phosphoenol pyruvate carboxy kinase, malic enzyme activity and others. Therefore, it causes a recalibration of the glucose – fatty acid (Randle) cycle, and hence increases insulin sensitivity[48].

Pharmacokinetics :

Pioglitazone has a shorter half life of < 7 hrs. Metabolism is by CYP2C and CYP3A4 isoenzymes. Elimination is through bile.

Current role of thiazolidinediones :

As an additive drug with metformin. The advantage is that, it retards the progression of impaired glucose tolerance to frank diabetes.

After disease onset, it reduces the requirement of insulin in a type 2 diabetic patient.

Adverse events :

- Liver dysfunction that requires frequent liver function test monitoring
- Weight gain
- Edema
- Doubled risk of fractures
- Risk of bladder cancer
- Headache, fatigue
- Gastro intestinal upset

The drug is contraindicated in children and women of child bearing age.

Alpha-glucosidase inhibitors

- Acarbose
- Miglitol
- Voglibose

Mechanism of Action:

The alpha glucoside enzyme functions, in the brush border of the intestine, to breakdown the complex carbohydrates into simple sugars, which are absorbed into circulation.The role of alpha-glucosidase

inhibitors in type 2 diabetes, is to bind reversibly to the alpha-glucosidase enzyme, thereby delaying the absorption of carbohydrates that are taken in a meal. This results in lesser post prandial hyperglycemia.

Advantage:

As mono therapy, these drugs do not cause hypoglycemia. The drugs are taken with the start of the meal.

Adverse effects:

- Flatulence
- Abdominal cramps
- Diarrhoea

Contraindications:

- Colon ulcers
- Intestinal obstruction
- Inflammatory bowel disease[49]

Incretin mimmetics:-

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

Dipeptidyl peptidase 4 inhibitors :

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

Sodium glucose co transporter 2 inhibitors:

1. Canagliflozin
2. Empagliflozin
3. Dapagliflozin

Other drugs :

1. Amylin analogue – Pramlintide
2. Bromocriptine
3. Colesevelam hydrochloride
4. Dual PPAR alpha and PPAR gamma agonists

Insulin mimetics :-

GLP -1 receptors are present in beta cells, vessel walls, central nervous system heart, lung, kidney and GIT mucosa.

The binding of insulin mimetics to the GLP-1 receptor will lead to activation of cAMP – PKA pathway and activation of guanine nucleotide exchange factors (GEFs). Down streamsignalling via protein kinase C

and phosphatidyl inositol 3 kinase also occurs. These eventually lead to a glucose dependent exocytosis of insulin and an increased bio synthesis of insulin[50].

The GLP-1 analogues are resistant to DPP 4, hence having a longer duration of action.They cause a 1% decrease in HbA1C levels and a modest weight loss of 2.5 –4 kg.They are administered as subcutaneous injections either daily or weekly depending on their plasma half lives.

Adverse effects :-

- Nausea, vomiting
- Pancreatitis
- Spurious association with medullary carcinoma of thyroid.

Contraindications -Creatinine clearance < 30 ml / minute

Limitations :-

- Cost
- Injection site reactions
- Hypoglycemic episodes

Drug interactions :-

Delays gastric emptying

Alters pharmacokinetics of antibiotics and oral contraceptives.

Dipeptidyl peptidase inhibitors:-

DPP-4 is a transmembrane glycoprotein, whose function is to degrade glucagon like peptide 1. The role of GLP -1 is to enhance insulin secretion via the "incretin" effect. Hence the DPP -4 inhibitors by preventing the degradation of GLP -1 increase the time of action of the peptide.

The DPP -4 inhibitors act in a glucose dependent manner, hence, they pose a negligible risk of hypoglycemia, when used as monotherapy in type 2 diabetes patients. But the risk of hypoglycemia is present if they are combined with insulin / sulfonylureas.

The medications are taken once (or) twice daily as oral formulations. They have a renal mode of elimination, except linagliptin, which has a predominant hepatobiliary clearance. Saxagliptin has strong CYP 3A4 interactions, hence caution is advised while administering it with CYP 3A4inhibitors such as clarithomycin, ketoconazole[51].

Adverse effects :-

- Severe joint pain
- Angioedema
- Anaphylaxis
- Increased risk of infections
- Pancreatitis

The drug has a good safety profile in cardio vascular patients.

Sodium glucose co transport 2 inhibitors[52]:-

Normal (90%) reabsorption of glucose in proximal renal tubules is aided by Sodium glucose co transporter 2. These transporters were targeted and inhibited, and this led to increased excretion of glucose in urine and thereby reduced the levels of blood glucose in type 2 diabetic patients.These drugs are approved as oral formulations for clinical use, their activity centers on decreasing the plasma glucose threshold by about 90-100 mg/dl.

Advantages:-

- Reduction of HbA1c levels
- Modest weight loss
- Oral formulations
- Lower cardiovascular mortality.
- Reduction of blood pressure

They are contra indicated if estimated GFR is < 45-60 ml/ mt / body surface area.

Adverse events :-

- Genital infections
- Urinary tract infections
- Reduction in bone mineral density
- Increased LDL cholesterol levels
- Higher rates of breast and bladder cancer.

Pramlintide :-

It is approved for use in both type 1 and type 2 diabetes. It is given before meals.

Its effects are via

- Reduction in glucagon secretion
- Reduction in appetite
- Prolonged gastric emptying time

Limitations :

- Hypoglycemic risk
- Subcutaneous mode of delivery
- Duration of action is only 150 minutes
- Cannot be mixed in an insulin syringe.

The dose ranges from 15-120 mg and is preferably uptitrated from the lowest dose[53].

Bromocriptine :

Reduces HbA1c levels by 0.5% as a combination therapy with life style intervention or other anti diabetic drugs. Action is presumed to be through regulation of a dopamine induced hepatic – hypothalamic circuit, that reduces hepatic gluconeogenesis.

Adverse events :

Nausea

Dizziness

Headache

Vomiting

1-2 kg weight gain

Dual PPAR alpha and PPAR gamma receptor agonist [54] :-

- Saroglitazar
- Aleglitazar

These drugs improve peripheral glucose sensitivity and also have beneficial effects on lipid levels. They cause reduction of HbA1c levels. And are available as oral formulations in the dose of 2-4 mg per day.

Adverse effects :-

- Weight gain
- Bone fractures
- Precipitation of heart failure

Goals of therapy[55] :

The holistic approach to the treatment of either type 1 (or) type 2 diabetes is to

- Provide symptom relief.
- Retard the development of long term micro vascular or macro vascular complications.
- Enable the patient to have an improved quality of life.

Central to the success of these goals is the integrated participation of the patient himself, herself his/her family members, the primary health care provider, the endocrinologist and (or) a cardiologist / nephrologist /podiatrist / neurologist if essential.

The treatment goals for adults with diabetes are as follows :-

HbA1C	< 7%
Fasting plasma glucose	< 70-130 mg/dl
Post prandial plasma glucose	< 180 mg/dl
Blood Pressure	<130/80 mm of Hg
Low density lipoprotein	< 100 mg/dl
High density lipoprotein	> 40-50 mg/dl
Triglycerides	< 150 mg/dl

Achievement of the goal comes with a package of life style intervention, pharmacotherapy, counselling and patient education, foot care and self monitoring of glucose levels.

Self Monitoring :-

The advantages of self monitoring include[56]

- Accurate records of glucose fluctuations.
- Prior alert regarding acute hypoglycemic episodes.
- Incentive for patients to attain glycemic control

The time interval between self check-up depends on the severity of the disease, the type of diabetes and individual motivation. Glucometers with self applicable strips are available for easy use, especially after proper guidance and education. A continuous glucose monitoring system with a cable, a glucose sensor, a com–station and a monitor is also available for managing unstable cases, pregnancy fluctuations and for patients on insulin pumps.

The umpteen number of oral antidiabetics that flood the pharmaceutical markets come with their own catches. They are ineffective in about one fifth of diabetic cases and even the drugs which initially produced an effect might later lead to a secondary failure. Further, they are laden with adverse effects, the most common being hypoglycemic risk. None of the above drugs can simulate a physiological insulin secretion nor provide a tight glycemic control.Thebiguanides have an increased risk of renal and hepatotoxicity. The thiazolidinediones are associated with cardiovascular risk and fractures.Even the latest SGLT 2 inhibitors have an increased risk of infections and the DPP4 inhibitors, an increased risk of gastrointestinal complications[57].

Hence, a search for a natural drug with improved effectiveness and lesser adverse effects for diabetes is consistently in the forum. With the dawning knowledge of the adverse effects associated with allopathy, we

find more individuals drawn towards the alternative forms of medications especially the ‘herbal substances’, to heal them of ailments.

From time immemorial, plants have been considered a valuable and powerful source of natural products that can maintain human health and many studies have been funded in the direction of natural therapeutics.According to the World Health Organization, medicinal plants are considered to be rich in lead compounds that can be developed into newer drugs[58].

The indigenous system of Ayurvedha, Siddha and Unani has catered to the needs of about 70% of the rural population. India, being the home ground for these medicinal plants, naturally, more focus is laid on developing drug from these plants. Herbal medications are preferred especially in chronic ailments such as diabetes, asthma, arthritis, chronic myalgia, wherein man has not yet mastered the disease by pharmacotherapy. This is further reinforced by the “Phobia” associated with long term use of synthetic drugs.

All the above points has led to an increase in the trend of using natural products in chronic diseases. The downside of this rampant usage of natural products, is that, if they are not properly quality controlled, they might lead to contamination, inefficacy and adverse effects.

***Annona squamosa*[59]:-**

Annona squamosa linn, of the family Annonaceae, is commonly cultured in Thailand and its origin is from South America. However, it is commonly found in India and is grown in gardens for its ornamental values and fruits.Tribal use of *Annona squamosa* has been recorded in a variety of common ailments.

It is a small ever green tree (or) a shrub of 7 meters in height, that bears edible fruits called “sugar apple”. It is also known as custard apple, sharifa or sitaphalam.

The simple alternate leaves occur singly, about 5- 17cm in length and they have a rounded base and a pointed tip. They are pale green on both surfaces. The leaf stalks are 0.4 – 2cms in length, green in colour.

Taxonomic classification:-

Kingdom	:	Plantae
Division	:	Mognoliophyta
Class	:	Magnolipsida
Order	:	Magnoliales
Family	:	Annonaceae
Genus	:	Annona L.
Species	:	Annona squamosa

Phytochemical constituents:-

The plant is reported to contain glycosides, alkaloids, flavanoids, tannins, phytosterols, anonaine, aporphine, glaucine, isocorydine, norcorydine, coryeline. The leaves are rich in anonine, borneol, camphene, β-caryphyllene, eugenol, farnesol, geraniol, hexacontanol, isocorydine, higemamine, limonine, linalool acetate, menthone, methyl salicylate, β-sitosterol, thymol, rutin, n-triacontanol. The leaf extract does not contain aminoacids or alkaloids[60].

Parts used:-

- Fruits
- Bark
- Leaves
- Root

Pharmacological properties:-

The presence of acetogennins were considered responsible for

- Antimalarial
- Immunomodulatoy
- Cytotoxic activities

The Presence of diterpenes was found to possess,

Anti HIV activity

Anti plateletaggregatory activity

Flavanoids were reported to have,

Anti microbial

Pesticidal activities

Folkloric use of this plant was reported in instances like tumor and inflammation. The crushed leaves were inhaled to cure a fainting spell. They acted as soothers on ulcers and wounds. A decoction of the leaves was drunk in case of dysentery.

The aqueous extract of leaves was reported to attenuate hyperthyroidism. It was used by tribes to manage diabetes. They have astringent properties.

Various parts of the plant were proposed to have mollusicidal activity against Schistosoma species. The flavanoids in the leaf extract have antioxidant activity which at times, confers a hepatoprotective effect.

They possess larvicidal activity against Anopheles stephensiand they are also active against helminthes. The acetogenins were considered responsible for the above mentioned effects.

Experimental animal models for diabetes mellitus[61]:-

In 1980, Von Meringpancreatectomised a dog to study fat absorption from the intestine, and found that the animal developed

polyuria and polydipsia, which were the cardinal features of diabetes. Marjorie was a famous experimental canine that was used by Banting and Best , for insulin purification.

Models for IDDM:-

1. Chemically induced diabetes
2. Hormone induced diabetes mellitus
3. Insulin antibodies induced diabetes
4. Viral agent induced diabetes.
5. Surgically induced diabetes

Genetic models:-

1. Non obese diabetic mice.
2. BB wistar rats.
3. Wistar Bonn / kobori rats
4. Cohen diabetic rat

Chemically induced diabetes:-

The chemicals commonly used are alloxan / streptozotocin. It is the most common animal model for type I diabetes. The chemicals act by one of the following 3 mechanisms.

1. β cell damage.
2. Temporary inhibition of insulin production and / or secretion.
3. Reduction of tissue sensitivity to insulin

Beta cell damage is preferred as it closely resembles the natural disease.

Hormone induced diabetes mellitus:-

Dexamethasone is injected at a dose of 2-5 mg/kg, intraperitoneally in a twice daily manner, over a number of days. Rats are preferred for this model, whereas guinea pigs and rabbits can also be used. Corticotropin can be used for adrenal cortex stimulation leading to steroid diabetes.

Insulin antibodies induced diabetes:-

Anti insulin antibodies are extracted from guinea pigs which are treated with bovine insulin along with CFA. These antibodies are injected as 0.25-1ml intravenous injection to rats, to obtain a diabetic cut off level of 300 mg %. Large doses can cause ketonemia, ketonuria, acidosis and fatality.

Viral agent induced diabetes:-

Viruses may destroy the β cells of pancreas or they may elicit an autoimmune response in the body to destroy the β cells. The viruses used for this purpose are,

1. RNA picornovirus
2. Coxsaxckie B4
3. Encephalomylocarditis virus
4. Mengo 2t

5. Reo virus
6. Lymphocytic choriomeningitis virus.

They are inoculated into suckling mice, or cultured in mice β cells.

Surgically induced diabetes:-

It can be attained by partial (or) total removal of the pancreas by surgery. Even in partial pancreatectomy >10% of residual tissue can allow for normal functioning of the gland. The results might be temporary for a few days to months, depending on the amount of functional cells. Total pancreatectomy results in an insulin dependent state.

Limitations:-

- ✓ Loss of α cells and δ cells of pancreas, lead to a decline in counter regulatory hormones such as glucagon and somatostatin.
- ✓ Indigestion due to loss of exocrine part of pancreas
- ✓ Technical difficulty in rats.

Hence, a combination of surgery and chemical induced diabetes is preferred for increasing the reliability of the model.

Models for NIDDM:-

Chemicals for NIDDM induction:-

1. Streptozotocin
2. Adrenaline, EDTA (Ethylene diamine tetra acetic acid)
3. Diazoxide
4. Thiazides

5. Furosemide
6. Alloxan

Genetic models for NIDDM :-

A) Monogenic models of obesity and NIDDM

i. Yellow mouse / agouti mouse
ii. Obese and diabetic mouse with mutation in C57BL/KSJ inbred strain.
iii. Tubby mouse – mutation in the single C57BL / 6J male strain
iv. Fat mouse – CPE fat mutation
v. Zucker Diabetic fatty rat
vi. WDF / Ta –Fa rat.
vii. Koletsky and JCR ; LA – Corpulent rats.

Polygenic models of obesity and NIDDM

(i) New zealand obese mouse.
(ii) Diabetic db /db mice.
(iii) Nagoya-Shibata Yasuda mouse.
(iv) Chinese hamster
(v) Djungarian hamster
(vi) Goto – Kakisaki rat

Genetically manipulated animal models:-

Genes modified for insulin resistance code for

- Insulin receptor, IRS 1 and 2, GLUT, Hexokinase, TNF α, RAS associated with diabetes, Fatty acid binding protein 2

Genes modified for reduced secretion of insulin code for

- GLUT 2, Glucokinase, Hepatic nuclear factors

Of all the above theoretical models, the most feasible, reliable method is chemical induction of diabetes.

Streptozotocin(STZ)[62] :-

Streptozotocin is an antimicrobial agent and has historically been used as an alkylating agent. Rakieten first described the diabetogenic potential of STZ. It is a 2 deoxy 2 (methyl nitrosoamino) carbonyl – amino) D- glucopyranose. The drug is hydrophilic, relatively stable at pH7.4 and at 37˚C.

It acts as a nitric oxide donor and causes DNA alkylation. Its β cell specificity is due to its selective cellular uptake and accumulation. Streptozotocin is a less lipophilic nitrosourea analogue, getting inside the β cells via GLUT 2 glucose transporter. The methyl nitrosoureamoiety, causes transfer of the methyl group of streptozotocin to the DNA of host cell, resulting in fragmentation of DNA. Additional protein glycosylation

and overstimulation of poly ADP – ribose polymerase causes cellular depletion of ATP and NAD, ultimately resulting in necrosis of the host cells.

A minor mechanism of action may be due to generation of superoxide and hydroxyl radicals during the hypozanthine metabolism thereby accelerating the process of cellular necrosis.

Effects of STZ[63]:-

- Inhibition of insulin biosynthesis
- Inhibition of insulin secretion
- Disruption of glucose metabolism
- Dysfunctional glucose transport
- Dysregulation of glucokinase activity

Limitation:-

- GLUT2 expression in liver and kidney, causes STZ uptake in these tissues, resulting in toxicity.
- Variations in sensitivity to STZ with regard to species and strains.
- Male rodents were more sensitive whereas female rodents were protected by their estradiol levels.
- Circadian variation in STZ susceptibility.

Dose:-

A single intraperitoneal dose of 40-70 mg /kg is commonly used to induce diabetes in Wistar rats.The doses vary with strains. Multiple low doses or a single large dose of STZ can also be given as intraperitoneal or intravenous injections.

Preparation -The stability is best maintained at a pHof 4-4.5. Hence the STZ is better reconstituted in a citrate buffer and ice cold saline preparation, freshly prepared and used within 30 minutes.

Advantages of STZ over alloxan:-

1. Greater uptake into β cells and hence greater selectivity
2. Lesser systemic toxicity or mortality
3. More complete suppression of β cells
4. Better chemical stability.

MATERIALS AND METHODS

METHODOLOGY

The aqueous extract of leaves of *Annona squamosa* were evaluated for their antihyperglycemic effect, in adult male albino rats.

The study was done in the Central animal house, Institute of Pharmacology, Madurai Medical College, Madurai. The Institutional Animal Ethical Committee of Madurai Medical College, approved the study on 31.05.2018.

Study Center

Institute of Pharmacology,

Madurai Medical College, Madurai

Duration of the Study

The study period was 6 months and it was done since March 2018.

Number of Animals used

24 adult male albino rats weighing about 150 –200 grams

Materials

1. Male albino rats
2. Streptozotocin
3. Tab Glibenclamide
4. Aqueous extract of leaves of Annona squamosa
5. Glucometer and glucose strips

7. Oral feeding tube

8. Syringes.

Animals

Twenty four, inbred adult male albino rats of 150 – 200 g, from Central animal house, Madurai MedicalCollege were utilized in this study. The rats were equally split into four groups.Each group had six animals. One group was kept as control, one as standard. The remaining two groups were treated with the plant extract.The animals were given pelletfeed and water according to their needs.

Each group of animals were housed separately.They were individually and distinctly marked with picric acid. The diabetic rats were given special care. The bottles were filled with fresh tap water every morning.The cages were cleaned daily and the bottom layer was refilled with sawdust.This was done to maintain a hygienic environment and prevent spread of infection in the diabetic animals.

Streptozotocin

Streptozotocin was procured by HiMedia Research Laboratories Pvt. Ltd.

All the groups of rats received injection streptozotocin intraperitoneally as a single dose of 50mg/kg.

Glibenclamide

Glibenclamide, a second generation sulphonyl urea was given to the standard group at an oral dose of 1 mg/kg/day.

Collection of Blood Samples

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

Method of Glucose Estimation

Glucometer was used to detect the blood sugar levels. It uses the glucose oxidaseenzyme specific sticks. The glucose test strip was inserted into the glucometer. A drop of blood, withdrawn by tail venipuncture method, was directly placed on the strip.The results were displayed on screen within 15 seconds.

Extraction Procedure

Annona Squamosa Leaf Aqueous Extract

Dr. Stephen, taxonomist, of American College, Madurai identified and certified the species as Annona leaves, which were obtained from a nearby farm. The crude extract was prepared in the Pharmacognosy department of Madurai Medical College.

Leaves were washed well with water.The fresh /air-dried leaves (25^{o}C for 5 days in the absence of sunlight) were extracted in 1 litre of boiling water for 2 hours. The concentrate obtained was dark brown. It was cooled and filtered using Whatman No.1 filter paper. The filtrate was centrifuged and the sediment was discarded.The supernatant extract was concentrated and used for the study. Each day, the necessary amount of extract was dissolved in distilled water and administered orally.

Glibenclamide (1mg/kg) was similarly dissolved in distilled water and given daily. The standard drug as well as the test extracts of annonasquamosa were givenorally using oral feeding tube and the treatment duration was for 14 days.

Oral Feeding Technique

A 16 Gauge feeding tube with a blunted tip was used. The tube was attached to 1 ml syringe which contained the drug to be given. The animals were hadled with utmost care and held by the nape of their neck. The oral feeding tube was inserted laterally through the interdental space, and by gentle rotations it was placed in the oesophagus. After ascertaining the desired level, the drug was gently pushed inside.

Methodology

The study followed the guidelines of CPCSEA.After overnight fasting, the blood glucose level was observed for all the rats by tail

venipuncture method. The baseline values were normal. All the rats were injected intraperitoneally with 50 mg/kg of Streptozotocin. Diabetic status of the rats were estimated after 3 days of streptozotocin injection, by repeat measurement of blood glucose levels.The animal was proclaimed diabetic if the blood sugar was > 250 mg/dl. The rats were split into groups of six and housed as control, standard, test 1 and test 2 groups. The control group received pellet diet and water. The standard group received the drug Glibenclamide at the dose of 1 mg/kg/day orally. Test 1 and test 2 groups received Annona squamosa leaf extract in the dose of 300 mg/kg and 600 mg/kg respectively.

Group Study Treatment

GROUP	STUDY	TREATMENT
I	CONTROL	Normal feed and water
II	STANDARD	Normal feed and water +Tab Glibenclamide (1 mg/kg) oral
III	TEST -1	Normal feed and water + aqueous extract of leaves of Annona squamosa (300 mg/kg) oral
IV	TEST -2	Normal feed and water + aqueous extract of leaves of Annona squamosa (600 mg/kg) oral

The blood glucose level were estimated at baseline and on Day 1, Day 7 and Day 14 and the results are tabulated. ANOVA was used as the statistical test to detect any significant difference between the groups.

RESULTS

RESULTS

In the present study, the antihyperglycemic effect of leaves of *Annona squamosa* aqueous extract was assessed using 24 albino rats.The animals were equally split into four groups. They served as the control, standard, test 1 and test 2 groups.

The blood glucose levels in nondiabetic rats in mg/dl - baseline

S. No.	Control	Standard	T1	T2
1	130	111	112	113
2	115	124	114	108
3	117	134	118	130
4	108	106	128	104
5	152	113	100	107
6	81	129	108	113

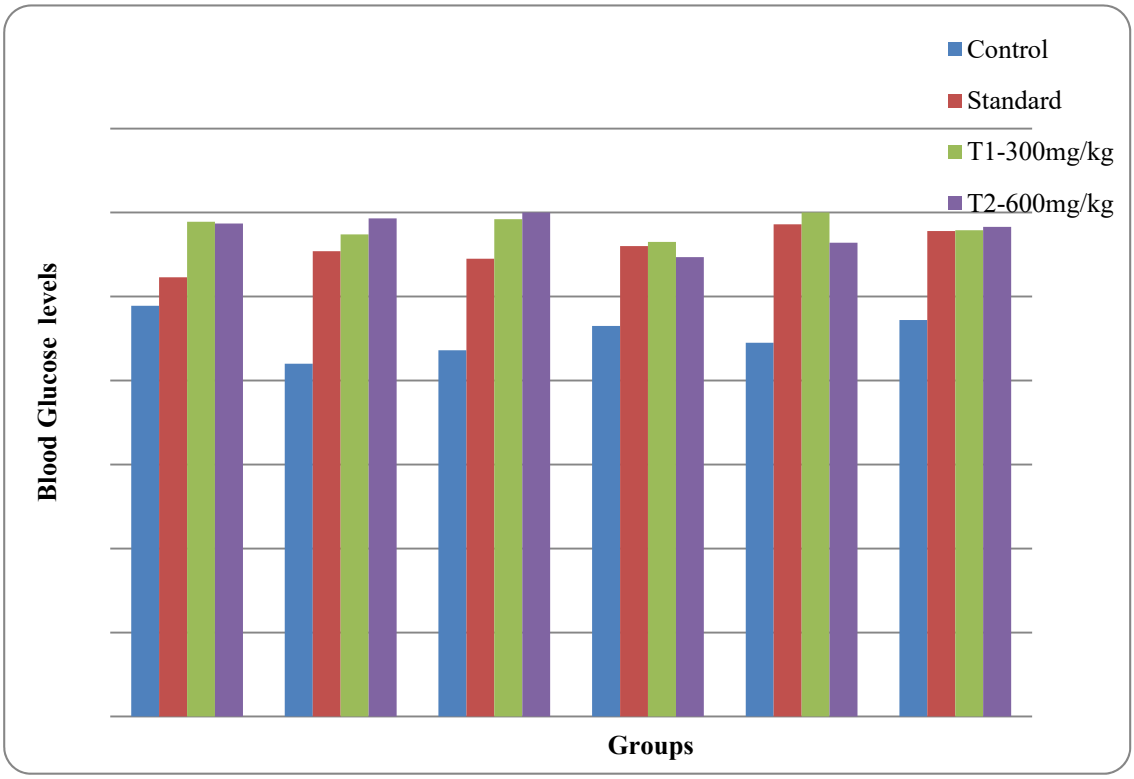
Streptozotocin intraperitoneally in a dose of 50mg/kg was given to all rats.

After 72 hours, all the animals had blood glucose values >250 mg/dl. The first group received pellet diet and served as control. The standard group received Tab Glibenclamide 1mg/kg orally. The third and fourth groups received aqueous extract of the leaves of *Annona squamosa* in graded doses of 300 and 600 mg/kg respectively.On Day 1, the blood glucose levels in diabetic rats of all the groups were

Day one blood glucose levels in diabetic rats (mg/dl)

S. No.	Control	Standard	T1-300mg/kg	T2-600mg/kg
1	489	523	589	587
2	420	554	574	593
3	436	545	592	589
4	465	560	565	547
5	445	586	594	564
6	472	578	579	583

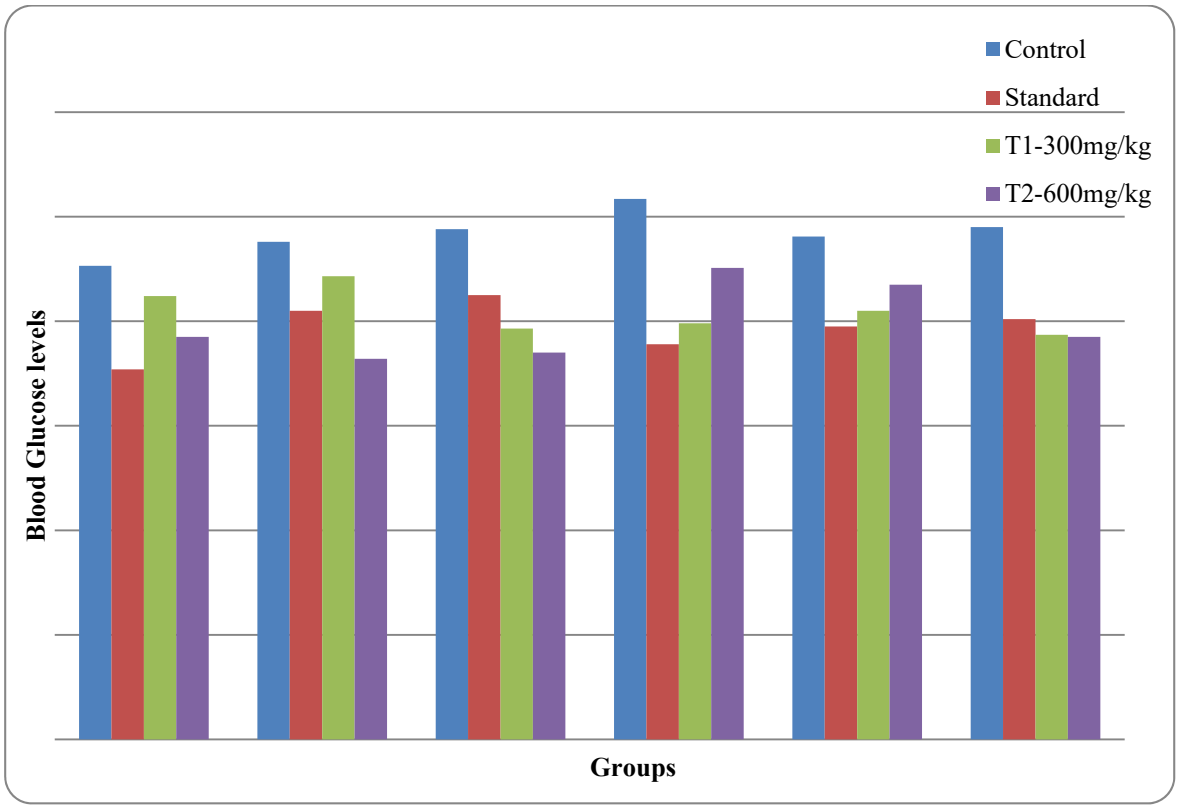
The following graph was formulated from Day 1 blood glucose levels



Day 7 blood glucose levels in diabetic rats (mg/dl)

S. No.	Control	Standard	T1-300mg/kg	T2-600mg/kg
1	453	354	424	385
2	476	410	443	364
3	488	425	393	370
4	517	378	398	451
5	481	395	410	435
6	490	402	387	385

The following graph was formulated from day 7 blood glucose values

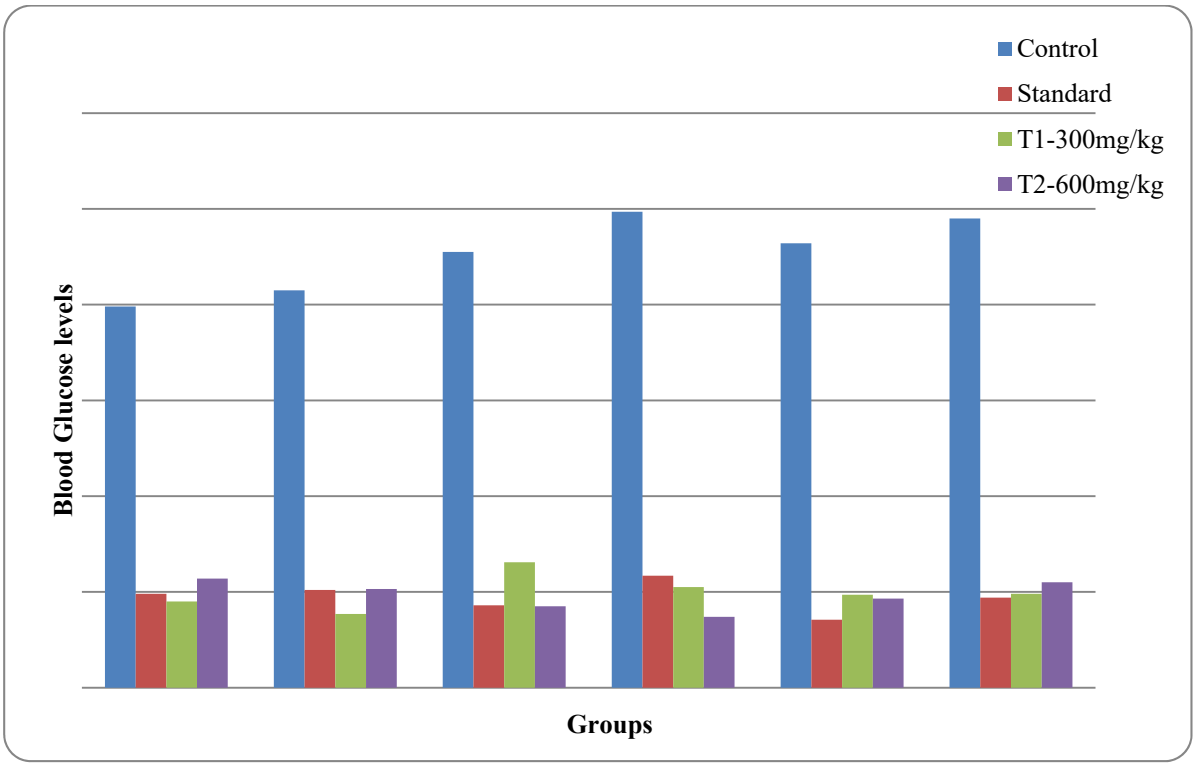


The day 14 values of blood glucose were

Day 14 blood glucose levels in diabetic rats (mg/dl)

S. No.	Control	Standard	T1-300mg/kg	T2- 600mg/kg
1	435	98	90	114
2	446	102	77	103
3	459	86	131	85
4	447	117	105	74
5	464	71	97	93
6	468	94	98	110

The graph shows the day 14 blood glucose levels



Control Group

The mean blood glucose values of diabetic rats of the control group were 454.55±25 on day 1, 484.1±20 on day 7 and 453.1± 12.57 on day 14, as shown in Table No.2

Standard group

The mean blood glucose values of diabetic rats in the standard group after the administration of Glibenclamide were 557.6±22 on day 1, 394±25 on day 7 and 94.6±15.4 on day 14 as shown in Table No.2.

Test Group 1 (Annona squamosa extract 300mg/kg)

The mean blood glucose values of rats that received Annona squamosa extract of 300mg/kg were 582.1±11 on day 1, 409±21 on day 7 and 99.6±18 on day 14 as shown in Table No.2.

Test Group 2 (Annona squamosa extract 600mg/kg)

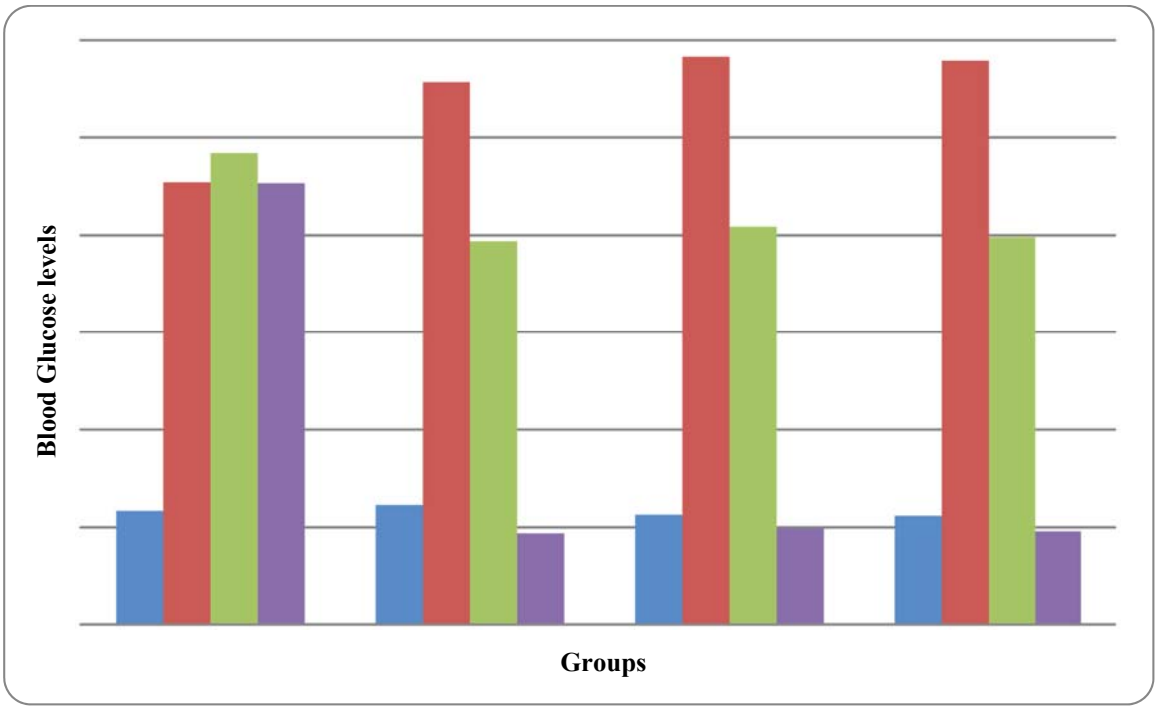
The mean blood glucose values of rats after administering Annona squamosa extract of 600mg/kg were 577.1± 17 on day 1 , 398.3±35 on day 7 and 96.5±15 on day 14 as shown in Table No.2.

Table -2

Blood Glucose Levels in Diabetic Rats in mg/dl

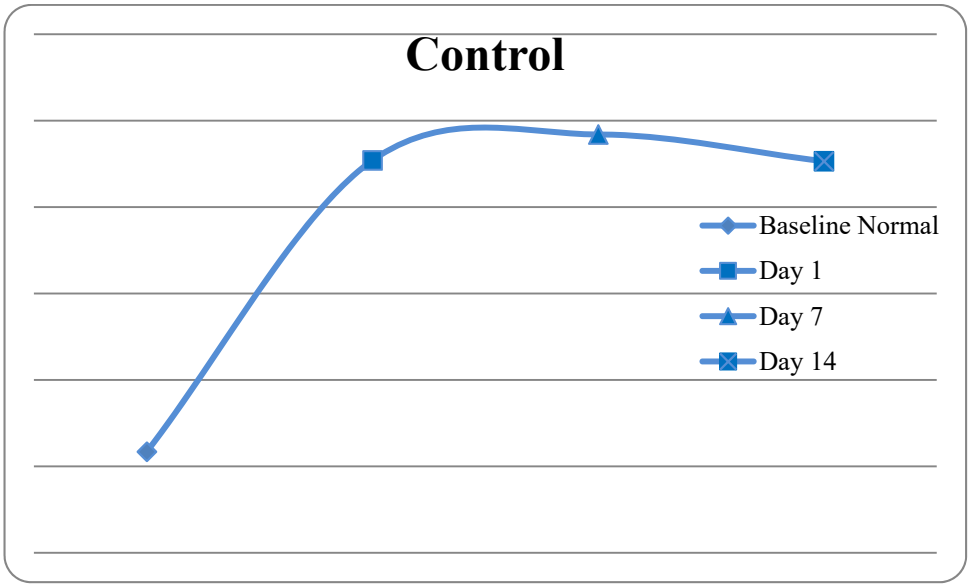
Group	Day 1	Day 7	Day 14
Control Normal pellet diet	454.55±25	484.1±20	453.1±12.57
Standard - Glibenclamide	557.6±22	394±25	94.6±15.4
Test 1 Annona squamosa extract 300 mg/kg	582.1±11	409±21	99.6±18
Test 2 Annona squamosa extract 600 mg/kg	577.1±17	398.3±35	96.5±15

The following graph shows the normal baseline value, Day 1, 7 and 14 values of all the four groups as mean average respectively.

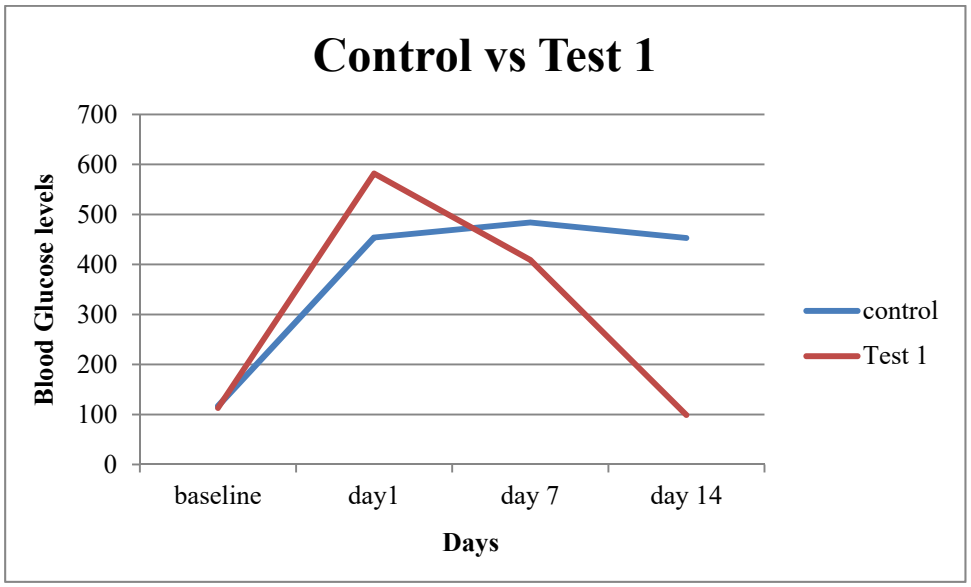


ANOVA was calculated between the groups and it showed no significant difference at their baseline values.The analysis of variance conducted after 14 days of treatment showed a significant difference ($p < 0.001$) between the groups.

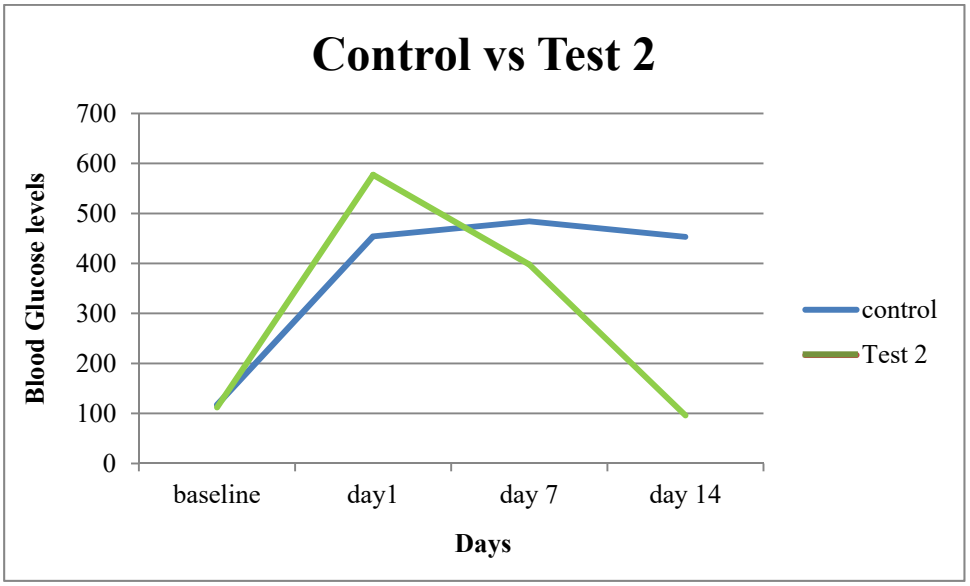
The following line graph shows the blood glucose levels of the control group of diabetic rats during the study duration.



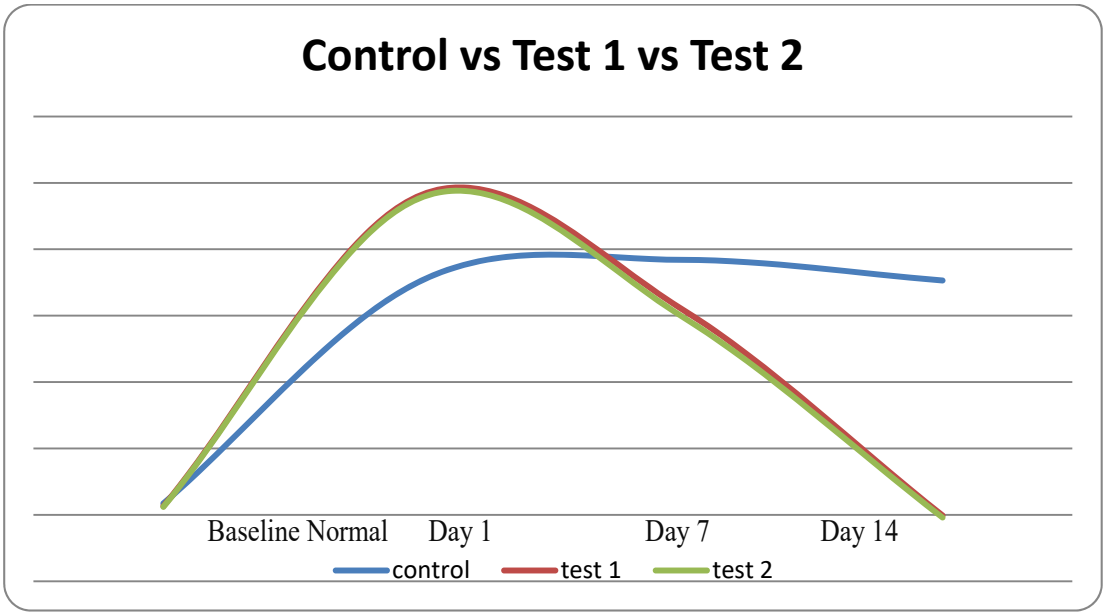
The following graph compares the blood glucose levels of the test 1 group with the control.



The following graph compares the blood glucose levels of the test 2 group with the control group of diabetic rats.



The following graph compares the blood glucose levels of the treatment groups with the control group of diabetic rats.



Day	Control	Std	T1	T2
Baseline	117.1±23.5	119.5±11.11	113.3±9.4	112±9.2
Day 1	458±6.1	554.1±3.7	583.3±5.0	580.8±5.1
Day 7	486.1±6.1	391.8±5.0	403.3±5.3	389.8±1.3
Day 14	453.1±39.7	94.6±15.4	99.6±18	96.5±15

A post hoc test conducted on day 14 showed the difference was significant in the standard group >annona 600mg/kg group >annona 300 mg/kg group, when compared with the control.

DISCUSSION

DISCUSSION

Diabetes is a global endocrine disorder, which is rapidly emerging as an “epidemic” in developing countries like India, which is being converted into a world capital for this disease. The initial launch pads of this disease are deficiency of insulin (or) insulin resistance, resulting in hyperglycemia. Their long term effects include increased oxidative stress, dysfunctional lipid levels and end organ damage of the renal, retinal, nervous and cardiovascular system[64].

In the long run, this disease affects the quality of life and curtails life span. The comprehensive management of diabetes includes diet, life style intervention, along with pharmacotherapy, like parenteral insulin (or) usage of oral anti diabetic agents. Until now, the pharmacotherapeutic field is ever expanding and effective in maintaining a tight glycemic control. The downside is their harmful side effects.

Hence, alternative forms of treatment are being tried with indigenous medicinal plants. More that 400 plant species have been indentified to have hypoglycemic activety with supporting literature. Most of the plants contain additional beneficial properties and lack the adverse effects associated with the drugs[6]. *Artemisia pallens, Bidenspilosa, Allium Cepa, Aloe babadensis* are a few plants with proven anti diabetic potential[66]. One such plant is *Annona squamosa* (Custard

apple), and the leaves of this plant, were taken in graded does and evaluated for its anti hyperglycemic effect in streptozotocin induced diabetic rats.

Initially, a group of 24 adult male albino rats were housed and divided into 4 groups of six each, as the control, standard, test 1 and test 2 groups respectively. The animals were made diabetic by streptozotocin intraperitoneal injection.

In comparison with the untreated group, Annona treated groups showed a remarkable fall in blood glucose levels on day 7 and day 14. The values of test 2, were comparable with that of standard group.

ANOVA was calculated between the groups and it showed no significant difference at their baseline values. The analysis conducted at the end of the study, showed a significant difference between the groups at a p value of < 0.001.

A post hoc analysis was conducted, which showed the statistical difference was more with standard group > Test group 2 > Test group 1, when compared with the control group.

Kaleem et al (2008), reported that the activity of the leaves of *Annona squamosa* might be through an enhancement of insulin release from beta cells of pancreas or it may be due to increased GLUT4 uptake of glucose from plasma to peripheral tissue. An increase in the levels of

insulin and C-peptide were also noticed in the groups treated with plant extract, when compared with the sham group. It further showed an increase in the serum total protein and albumin levels, because of incorporation of the amino acids into proteins, by insulin[67].

Gupta et al, further suggested that the aqueous extract of leaves of *Annona squamosa*, increases insulin release even from partially destroyed pancreatic cells, by probably regenerating the beta cells (or) release of stored insulin from granules[68]. It was suggested to improve glucose tolerance and the extract reduced the levels of total cholesterol, LDL, VLDL in diabetic animals and also increased the levels HDL cholesterol.

In vitro studies from aqueous extract was shown to inhibit theglucose 6 phosphatase enzyme activity, thereby preventing glycogenolysis. The aqueous extract also slightly inhibited (18.1%) the intestinal absorption of glucose in rats.

Annona squamosa contains acetogenins, squamosin B, flavonoids, quercetin, reticulatain[69], as its phytochemical constituents, which may be causing the anti diabetic effect.

Similar to many chronic diseases, diabetes mellitus is the cause and effect of elevated levels of reactive oxygen species (ROS). The ROS in low levels are important to maintain immunity, cause cellular maturation

and cell signaling. Its harmful effects include oxidative stress and metabolic derangement.

Chronic hyperglycemia stimulates ROS formation through oxidative phosphorylation, NAD(P)H oxidase, lipooxygenase, cytochrome P450 monooxygenase, glucose auto oxidation. The normal pancreatic beta cells contain minimal amounts of antioxidants only and hence their partial destruction (or) dysfunction in diabetes, makes them more susceptible to damage, than other tissues[70]. Hence, a drug with antioxidant property may have a dual effect on the outcome of diabetes management.

In vitro studies, proved that the leaves of *Annona squamosa*, have a free radical scavenging activity of 1, 1, - Diphenyl -2- Picryl Hydrazyl (DPPH) radical. The Oxygen Radical Absorbance Capacity (ORAC) assay, also quantitated the inhibition percentage of free radicals and the length of inhibition time in *Annona* extracts, which were proven significant[71]. In vitro studies on hexane extract of *Annona* leaves, showed inhibition of PTP1B (Protein Tyrosine Phosphatase 1B) in a dose dependant manner. The normal function of PTP1B is negative regulation of signaling of insulin. This is achieved through dephosphorylation of IR-beta (Insulin receptor beta) and IRS-1 (Insulin Receptor Substrate 1). The inhibition of PTP1B causes disinhibition of insulin secretion, thereby

activating downstream cellular events and increasing P13 kinase dependant increase in glucose uptake into the cells. The in vitro study was followed by treatment of ob/ob mice with the herbal extract, which improved glucose tolerance and lowered the triglyceride levels[72]. A further fractionation of potential bio active molecules might be needed to further elucidate the possible links in mechanism of action of *Annona*.

Diabetes mellitus inevitably leads to chronic glomeruloscelerosis and renal failure. So, provision of a drug, that is renoprotective in diabetes can be an added benefit. In a study, the aqueous extract of *Annona squamosa*, was found to lower the plasma urea, uric acid and creatinine levels in diabetic rats with impaired renal function. This was postulated to be the effect of the leaf extract on Super Oxide Dismutase (SOD)[73].

In a 10 days study, it was found to increase the levels of renal SOD activity, which is an antioxidant, hence protecting the kidney from ischemia and renal failure.

In summary, increased mRNA expression of GLUT4 in peripheral tissues, the insulin releasing property, free radical scavenging property, inhibition of intestinal absorption of glucose and inhibition of PTP1B, might be responsible for the antihyperglycemic activity of Annona, leading to a statistically significant fall in the fasting blood glucose levels.

CONCLUSION

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Diabetes mellitus is an economic and social burden in India. Urbanisation and lack of physical activity are important contributory factors in pushing diabetes into the verge of becoming an epidemic.Diabetic complications have also increased manifold despite advances in the medical field and they contribute significantly to overall morbidity and mortality. The early age of onset and co occurrence of obesity has further complicated the disease outcome.

Sensitive screening procedures and awareness regarding the disease and its complications will go a long way in curtailing diabetes. Pharmacotherapy is one of the pillars for treatment of diabetes.

Even though umpteen antidiabetic drugs are in the market, no drug is free from adverse effects. Patient compliance is decreased due to their various side effects. Hence search for a “near ideal”drug exhibiting better safety and tolerability, which provides good glycemic control continues.

Many effective drugs have come from botanical sources.Various parts of *Allium cepa, Allium sativum* and many other are considered to have antihyperglycemic activity. In this study, *Annona squamosa* leaf extract which was widely used in folklore medicine for several ailments was evaluated for its antihyperglycemic effect in albino rats.

-92-It was observed that annona leaf extract at 300 mg/kg and 600 mg/kg led to a statistically significant fall in blood glucose level in diabetic rats when compared with the control group.

Fractionation of the active principle and further studies on animals of higher phylogenetic scale is needed in the near future, to elucidate the mechanism of action and confirm the antihyperglycemic activity of *Annona squamosa.*

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MASTER CHART

MASTER CHART

The blood glucose levels in nondiabetic rats in mg/dl - baseline

S. No.	Control	Standard	T1	T2
1	130	111	112	113
2	115	124	114	108
3	117	134	118	130
4	108	106	128	104
5	152	113	100	107
6	81	129	108	113

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

S. No.	Control	Standard	T1- 300mg/kg	T2- 600mg/kg
RAT- 1	489	523	589	587
RAT- 2	420	554	574	593
RAT -3	436	545	592	589
RAT -4	465	560	565	547
RAT -5	445	586	594	564
RAT -6	472	578	579	583

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

S. No.	Control	Standard	T1- 300mg/kg	T2- 600mg/kg
RAT -1	453	354	424	385
RAT -2	476	410	443	364
RAT -3	488	425	393	370
RAT -4	517	378	398	451
RAT -5	481	395	410	435
RAT -6	490	402	387	385

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

S. No.	Control	Standard	T1-300mg/kg	T2- 600mg/kg
RAT - 1	435	98	90	114
RAT- 2	446	102	77	103
RAT- 3	459	86	131	85
RAT -4	447	117	105	74
RAT-5	464	71	97	93
RAT-6	468	94	98	110

ABBREVIATION

ABBREVIATION

DM	–	DIABETES MELLITUS
ROS	–	REACTIVE OXYGEN SPECIES
JNK/SAPK	–	c-JUN N-TERMINAL KINASE
NF-Kb	–	NUCLEAR FACTOR kappa B
MAPK	–	MITOGEN ACTIVATED PROTEIN KINASE
GL	–	GLIBENCLAMIDE
ATP	–	ADENOSINE TRIPHOSPHATE
Ca^{2+}	–	CALCIUM
STZ	–	STREPTOZOTOCIN
NAD	–	NICOTINAMIDE ADENINE DINUCLEOTIDE
HLA	–	HUMAN LEUKOCYTE ANTIGEN
GLUT2	–	GLUCOSE TRANSPORTER 2
GLP	–	GLUCAGON LIKE PEPTIDE

ETHICAL CLEARANCE LETTER

Certificate

This is to certify the project title **EVALUATION OF ANTIHYPERGLYCEMIC EFFECT OF AQUEOUS EXTRACT OF LEAVES OF ANNONA SQUAMOSA IN STREPTOZOTOCIN INDUCED DIABETIC RATS**

has been approved by the IAEC.

31.5.18

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Name of Chairman/ Member Secretary IAEC:

DEAN
Madural Medical College
Madurai-20

Signature with date

31/5/18

Prof. Dr. P.Vasan Ph.D
Name of CPCSEA nominee:

Professor
Department of Animal Nutrition
Veterinary College & Research Institute
Namakkal

Chairman/ Member Secretary of IAEC: **CPCSEA nominee:**

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

Dr. D.STEPHEN, M.Sc.,Ph.D.,
ASSISTANT PROFESSOR
MADURAI-625002

DEPARTMENT OF BOTANY
THE AMERICAN COLLEGE

CERTIFICATE

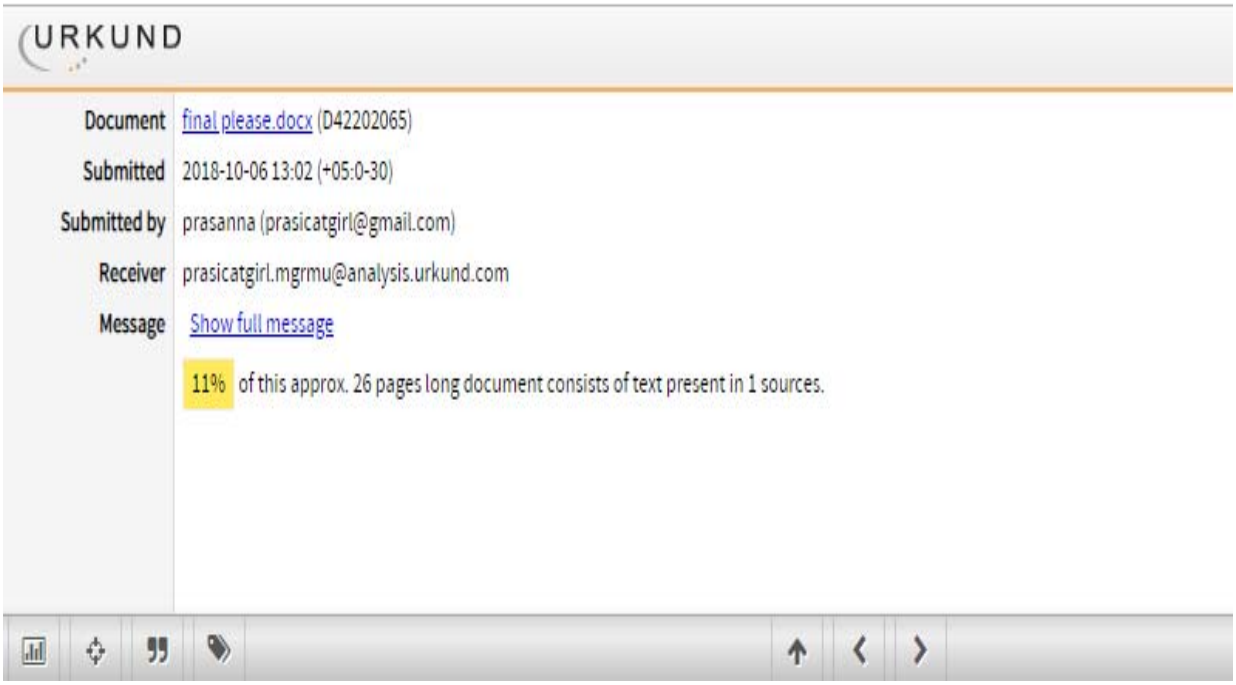
This is to certify that the specimen brought by Dr. X.A. PRASANNA , postgraduate in MD Pharmacology, Institute of Pharmacology, Madurai Medical College, Madurai is identified as *Annona squamosa* , belonging to the family Annonaceae.

Station : Madurai. **(Dr.D.STEPHEN)**

Date :

Dr. D. STEPHEN, Ph.D.,
ASST. PROFESSOR IN BOTANY
THE AMERICAN COLLEGE
MADURAI - 625 002
TAMILNADU-INDIA

ANTI PLAGIARISM CERTIFICATE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Dr. R.SAROJINI.,
PROFESSOR,
Institute of Pharmacology,
Madurai Medical College,
Madurai - 625 020