EVALUATION OF *IN VITRO* AND *IN VIVO* ANTIDIABETIC ACTIVITY OF ETHANOLIC ROOT EXTRACT OF *Cassia fistula* L. ON STREPTOZOTOCIN INDUCED DIABETIC RATS

A dissertation submitted to

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CHENNAI - 600 032.

In the partial fulfillment of the requirements

for the award of the degree of

MASTER OF PHARMACY

IN

PHARMACOLOGY

Submitted by

Reg. No: 261426069

Under the guidance of

Mrs. R. INDUMATHY, M. Pharm.,

INSTITUTE OF PHARMACOLOGY

MADRAS MEDICAL COLLEGE

CHENNAI - 600 003

APRIL – 2016

CERTIFICATE

This is to certify that the dissertation entitled **"EVALUATION OF *IN VITRO* AND *IN VIVO* ANTI-DIABETIC ACTIVITY OF ETHANOLIC ROOT EXTRACT OF *Cassia fistula* Linn. ON STREPTOZOTOCIN INDUCED DIABETES IN RATS"** submitted by Registration No. 261426069 in partial fulfillment of the requirements for the award of Degree of Master of Pharmacy in Pharmacology by the Tamil Nadu Dr.M.G.R. Medical University, Chennai is a bonafide work done by her in the institute of Pharmacology, Madras Medical College, Chennai during the academic year 2015-2016 under the guidance of Mrs. R. Indumathy, M. Pharm., Assistant professor in Pharmacy, Institute of Pharmacology, Madras Medical College, Chennai-600 003.

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INDEX

ABBREVIATIONS

DM	Diabetes Mellitus
WHO	World Health Organization
CF	Cassia fistula
GOD-POD	Glucose Oxidase-Peroxidase
HMP	Hexose Mono Phosphate
G6P	Glucose-6-Phosphate
LDH	Lactate dehydrogenase
G6PD	Glucose-6-phosphate dehydrogenase
NADP	Nicotinamide adenine dinucleotide phosphate
GLP-1	Glucagon-likepeptide-1
GI	Gastro intestinal
SU	Sulfonyl Urea
PPAR	Peroxisome Proliferator Activated receptor
DPP-4	Dipeptidyl peptidase inhibitors
STZ	Streptozotocin
PPHG	Post-prandial hyperglycemia
FDA	Food and Drug Administration
Rtd	Retired
CSIR	Council for Scientific and Industrial Research

EDTA	Ethylene diamine tetra acetic acid
ATP	Adenine Tri Phosphate
HDL	High Density Lipoprotein
ANOVA	Analysis of variance
SD	Standard Deviation
GD	Glucose Diffusion
p. o	Oral route
b. w	Body weight
rpm	Revolution per minute
GDM	Gestational Diabetes Mellitus
MODY	Maturity Onset Diabetes in Young
NHC	Nonketotic Hyperosmolar Coma

1. INTRODUCTION

The worldwide prevalence of diabetes have increased from around 60 million in 1980 to about 118 million in 1995 and are set to increase to 220 million by the year 2010 (Amoes *et al*.,1997). According to the International Diabetes Federation (IDF), Diabetes affects at least 285 million people worldwide and that number will be expected to reach 471 million by the year 2035 [(1)].

Traditional use of herbal medicine is the basis and integral part of various cultures, which was developed within an ethnic group before the developed and spread of modern science. Herbal drugs constitute a major part in all the traditional systems of medicine. These have made a great contribution in maintaining human health. A majority of the world's population still rely on herbal medicines to meet its health needs. The practice continues today because of its biomedical benefits and its place in culture beliefs in many part of world.

India, china and several other nations have an ancient tradition of herbal remedies. The written records in Ayurveda, the ancient system of medicine in India, contain more than 800 herbal remedies. The Charaka Samhita and Sushruta Samhita are two treasure troves containing knowledge of plant based drugs and are even today, held in the highest esteemed the world over [(2)].

However, the name Diabetes was given by the two Roman physicians Celsius and Aretaeus in 1st A.D in 1921. Banting and Best solved the problem of diabetes to a great extent by discovered Insulin as a therapeutic agent in Insulin Dependent Diabetes Mellitus. The first oral hypoglycemic agents suitable for clinical use were the sulfonylureas developed by Auguste Loubatieres in the year 1940[(3)].Conventionally, insulin dependent diabetes mellitus is treated with exogenous

insulin and non-insulin-dependent diabetes mellitus with synthetic oral hypoglycemic agents like sulphonylureas and biguanides[4]. However they fail to prevent complications of diabetes and also produce adverse health effects [5]. Therefore, different medicinal systems are using the active plant constituents, which discovered as natural hypoglycemic medicine came from the virtue of traditional knowledge [6]. Asia's large population and rapid economic development have made it an epicenter of the epidemic. Asian populations tend to develop diabetes at younger ages and lower BMI levels. Several factors contribute to accelerated diabetic epidemic in Asians, including the "normal-weight metabolically obese" phenotype, high prevalence of smoking and heavy alcohol use; high intake of refined carbohydrates (e.g. white rice) and dramatically decreased physical activity levels.

DIABETES MELLITUS

The word "Diabetes" is derived from "Greek" word "Diabainein" which means "to pass through". It is characterized by an excess of glucose in blood and urine, hunger, thirst and gradual loss of weight. Insulin is a hormone which regulates the carbohydrates and triglyceride metabolism through its action at several sites and facilitates the entry of glucose into the cell. Insulin also stimulates the synthesis of glucokinase and moderates the degree of gluconeogenesis. In the diabetic patient, there is an aberration in the function of insulin.

CLASSIFICATION OF DIABETES MELLITUS

1) Type 1 Diabetes Mellitus [10]

The hallmark also known as Insulin Dependent Diabetes Mellitus (IDDM). It is not associated with obesity and may be associated with acidosis or ketosis.

2) Type 2 Diabetes Mellitus

Type 2 diabetes is characterized by tissue resistance to the action of insulin combined with a relative deficiency in insulin secretion. It is also known as adult onset diabetes or Non-Insulin Dependent Diabetes Mellitus (NIDDM).

3) Gestational Diabetes Mellitus (GDM) [11]

It is defined as any abnormality in glucose levels noted for the first time during pregnancy and resolves after delivery.

4) Maturity Onset Diabetes in Young (MODY)

MODY is defined as hyperglycemia diagnosed before the age of 25 years and treatable for more than 5 year without insulin.

5) Genetics [12]

Susceptibility to both IDDM and NIDDM is determined to a substantial extent by genetic factor.

SIGNS AND SYMPTOMS OF DIABETES METTILUS [13]

These are mainly due to hyperglycemia with decreased utilization of glucose by cells: as a result, there is an extracellular glucose excess and intracellular glucose deficiency, a situation called starvation in the midst of plenty.

1. Hyperglycemia (raised blood glucose), it predisposes to infection like boils and urinary tract infection.
2. Glycosuria (presence of glucose in the urine)
3. Polyurea (excess urine production)
4. Dehydration
5. Polydipsia (increase thirst) is a result of the dehydration that results from the osmotic dieresis.

6. Polyphagia (excess eating) low glucose utilization by glucose cells of ventromedial nucleus in hypothalamus (satiety center) result in no inhibition of lateral nucleus in hypothalamus (feeding center) which eventually produces increased hunger.
7. Loss of weigh.
8. Ketonuria.
9. Poor resistance to infections due to protein depletion.
10. Hyperlipedemia (abnormally high serum lipid levels). Insulin deficiency decreases LDL receptor availability, which decreases serum cholesterol clearance. This decreased clearance produces hypercholesterolemia or high blood chlosterol.
11. Electrolyte depletion.

COMPLICATIONS OF DIABETES MELLITUS [(15)]

- Diabetic ketoacidosis
- Nonketotic hyperosmolar coma (NHC)
- Insulin shock

LONG TERM SEQUELAE OF DM

Long-term problems associated with DM include neuropathies, nephropathies, microangiopathies, microangiopathies and retinopathies.

CHEMICAL AGENTS CAPABLE OF INDUCING DIABETES [(16)]

- Alloxan
- Streptozotocin

Alloxan Induced Diabetes

Alloxan, a cyclic urea analogue, was the first agent producing permanent diabetes in animals. It is a highly reactive molecule readily reduced to diuleric acid, then auto-oxidized back to Alloxan resulting in the production of free radicals. These free radicals damage the DNA of β-cells and cause cell death. Second mechanism proposed for Alloxan is its ability to react with protein SH groups, especially the membrane proteins like glucokinase on the β-cells, finally resulting in cell necrosis.

Drawbacks

- High mortality in rats.
- Causes ketosis due to free fatty acid generation.
- Diabetes induced is reversible.
- Some species like guinea pigs are resistant to its diabetogenic action.

Streptozotocin Induced Diabetes

STZ [2-deoxy-2-(3-methyl-3-nitrosourea) 1-D-glucopyranose] is a broad-spectrum antibiotic, which is produced from *streptomyces achromogens*. STZ causes β-cell damage by process of methylation, free radical generation and nitric oxide production.

Advantages

STZ has almost completely replaced Alloxan for inducing diabetes because of

- Greater selectivity towards β-cells
- Lower mortality rate
- Longer or irreversible diabetes induction.

Disadvantage

Guinea pigs and rabbits are resistant to its diabetogenic action.

OTHER DIABETES MELLITUS INDUCED MODELS

1. Hormone-Induced Diabetes Mellitus

Dexomethasone, a long acting glucocorticoid, is used to produce NIDDM at a dose of 2-5mg/kg, i.p, twice daily over a number of days in rats.

2. Insulin Antibodies-Induced Diabetes

Giving bovine insulin along with CFA to guinea pigs produces anti-insulin antibodies.

3. Viral Agents –Induced Diabetes

Viruses are thought to be one of the etiologic agents for IDDM. Viruses may produce diabetes mellitus by

- Infecting and destroying of β-cells in pancreas.
- A less infecting or cytologic variant producing a comparable damage by eliciting immune auto reactivity to the β-cells.
- Viruses producing systemic effect, not directly affecting the β-cells.

4. Surgically Induced Diabetes

Surgical removal of all or part of the pancreas can induce Diabetes Mellitus. In partial pancreactomy more than 90% of the organ must be removed to produce diabetes.

MANAGEMENT OF DIABETES MELLITUS [17]

The goals of treatment for diabetes are to reduced and control blood glucose levels, relive the symptoms of the disease and prevent complications. Intensive treatment and careful control of blood glucose levels can reduce the risk of complications of diabetes [18].

I.NON PHARMACOLOGICAL INTERVENTIONS

The major environmental factors that increase the risk of type 2 diabetes, presumably in the setting of genetic risk are nutrition and sedentary lifestyle with consequent over weight and obesity. Medical nutrition therapy (i.e. diet) and exercise are important aspects of non-pharmacologic treatment for diabetes. Weight loss is a vital part of treatment for type 2 diabetes because it can help improve the sensitivity of cells to insulin and the uptake of glucose by cells.

II. PHARMACOLOGICAL INTERVENTIONS

A) Injectable anti-diabetic agents [19]

1) Insulin

All patients with type 2 diabetes require insulin injections. Patients with type 2 disease who have multiple symptoms of hyperglycemia are pregnant or have ketosis also should use insulin injections. Currently, insulin used for treatment is derived from beef and pork pancreas as well as recombinant (human) DNA technology.

2) Glucagon like peptide-1 (glp-1) agonist

The glucogan like peptide-1 (GLP-1) is an important incretin that is released from gut in response to oral glucose. It is difficult to use clinically because of

rapid degradation by the enzyme dipeptidyl peptidase-4 and it is injected subcutaneously twice daily one hour before meals acts for 6-10 hours.

3) Amylin agonists

This synthetic amylin (a polypeptide produced by pancreatic β-cells which reduces glucagon secretion from α-cells and delays gastric emptying) analogue attenuates postprandial hyperglycemia when injected subcutaneously just before meal and exerts a centrally mediated anorectic action.

B) Oral hypoglycemic drugs:

Oral hypoglycemic drugs include sulfonyl ureas, biguanides, meglitinide/ phenylalanine analogue, thiazolidinediones, α-glucosidase inhibitors, dipeptidyl peptidase inhibitors and incretin mimetics.

1) Sulfonyl ureas [e.g. Glibenclamide, Glimepride, Glipizide, etc.]

2).Biguanides [e.g. Metformin]

3) Meglitinide / D-Phenylalanine analogues

[E.g. Repaglinide, Nateglinide]

4) Thiazolidinediones [e.g. Rosiglitazone, Pioglitazone]

5) $\alpha -$ Glucosidase Inhibitors (e.g. Acarbose, Miglitol, Voglibose)

6) Dipeptidyl Peptidase Inhibitors: (e.g. Sitagliptin)

7) Incretin Mimetics: (e.g.Liraglutide)

All oral antidiabetic agents have side effects. Sulfonylureas are associated with weight gain and hypoglycemia. The major side effect of α-glucosidase inhibitors is flatulence. The major problems with the thiazolidinediones are those

of fluid retention, dilutional anemia. Metformin increased risk of gastrointestinal problems [20]. In India it is proving to be a major health problem, especially in the urban areas. Though there are various approaches to reduce the ill effects of diabetes and its secondary complication, herbal formulations are preferred due to lesser side effect and low cost [7].

More than 400 different plants and plants extracts have been described as reputedly beneficial for the diabetic patient. Most of these plants have been claimed to possess hypoglycemic properties but most claims are anecdotal and few have received adequate medical or scientific evaluation. Those that have been evaluated may be grouped into three categories:

1. Plants from which a reputedly hypoglycemic compound or partially characterized hypoglycemic fraction has been prepared.
2. Plants reported to exert a hypoglycemic effect, but the nature of the active principle is unestabilised.
3. Plants that reputedly exert a hypoglycemic effect, but the scientific evidence is equivocal. These categories exclude the numerous traditional plants for which an independent scientific or medical has not been published.

Cassia fistula L. found throughout the tropical parts of India. It is said to be useful in the treatment of hematemesis, pruritis, intestinal disorder, leucoderma, diabetes, antipyretic, analgesic & laxative [8]. It has various pharmacological activities like antifungal, antioxidant, antimicrobial, anti-inflammatory, anti-tumor, hypoglycemic activities [9]. However, till date there has been no investigation supporting the anti-diabetic properties of root of this plant. Hence, this study has been

taken with an aim to evaluate the *in vitro* and *in vivo* anti-diabetic potential of root bark of *Cassia fistula* L. on Streptozotocin induced diabetic model.

2. AIM AND OBJECTIVES

- Successive extraction of root bark of *Cassia fistula* L. by Soxhlet extraction apparatus using various solvents n-hexane, ethyl acetate and ethanol.

- Phytochemical evaluation of root bark of ethanolic extract of *Cassia fistula* L.

- To determine the inhibition of alpha-amylase enzyme and glucose diffusion property of the prepared extract of the *Cassia fistula* L. using dialysis tube and GOD-POD kit.

- To evaluate the safety of the effective extract of *Cassia fistula* L. by acute toxicity study in Wistar rats.

- To investigate the *in vivo* anti diabetic effect of active extract of *Cassia fistula* L. on Streptozotocin induced diabetic wistar rats.

3. REVIEW OF LITERATURE

Review of literature related to antidiabetic activity

Kumari *et al*., 1995 [21]

Allium cepa (Onion) is an essential plant cultivated throughout India, belongs to the family Liliaceae. Various parts such as seedling, callus, bulb etc., are known to possess anti-diabetic activity. Investigation revealed the presence of sulfur containing amino acid, which when administered orally to Alloxan induced diabetic rats (200mg/kg for 45 days) significantly controlled blood glucose and lipid in serum and normalized the activity of liver Hexokinase, glucose-6-phosphatase and HMG-CoA reductase. The effect was in accordance with that of Glibenclamide and Insulin.

Zacharias *et al*., 1980 [22]

Allium sativum (Garlic) belongs to the family of Alliaceae. Aqueous extract of garlic increased hepatic glycogen and free amino acid content when given orally to sucrose fed rabbits (10ml/kg/day). Garlic is known to decreased fasting blood sugar, triglyceride level in serum liver and aorta and protein levels when compared with sucrose controls.

Faiyaz Ahmed *et al.*, 2008 [23] was evaluated the antihyperglycemic activity of the bark powder and aqueous extract of *Ficus glomerata* (Moraceae) in STZ induced diabetic rats. Oral administration of *Ficus glomerata* bark powder (FGB) and *Ficus glomerata* aqueous extract (FGAE) at 500mg/kg caused 21% and 52% reduction in fasting blood glucose respectively and also decreased glycosuria significantly. Histology of pancreas suggested normalized of islets of langerhans and β-cells with respect to their number and cellular architecture. The results suggested that the bark of *Ficus glomerata* has significant anti-hyperglycemic activity in

experimental animals and has potential to be used as an adjunct in the management of diabetes mellitus.

M.R.M. Rafiullah *et al*., 2006 [24]

The effect of aqueous extract of *Syzygium cumini* Linn. *Gymnema sylvestre* (Retz) Schult and *Portulaca olearcea* Linn. were investigated in fasting normal and Streptozotocin (STZ) induced diabetic rats. The effects of extract on oral glucose tolerance in normal fasting rats were also studied. The aqueous extract of *S.cumini* (200mg/kg) and *G.sylvestre* (200mg/kg) decreased the blood glucose in normal rats significantly at 2 and 4 hours of extracts administration ($p<0.05$, $p<0.001$). The *S.cumini* and *G.sylvestre* extracts decreased the increase of glucose levels significantly ($p<0.05$) at 90 and 180 minutes after the glucose load in glucose tolerance test. In STZ induced diabetic animals, the aqueous extracts of *S.cumini* and *G.sylvestre* decreased the blood glucose significantly ($p<0.05$) at 4 hours. The aqueous extract of *P.olearancea* did not show any hypoglycemic activity.

Rajasekaran *et al.*, 2005 [25]

Aloe barbabensis commonly called as Aloe vera is a medicinal herb; belongs to the family Liliaceae; Leaf gel, Leaf pulp and dried sap are known to possess antidiabetic and antioxidant activity by oral administration of ethanolic extract at a concentration of 300mg/kg body weight for 21 days. It was proved more effective in controlling oxidative stress found in diabetes.

Anderson and Polansky, 2002 [26]

Camellia sinensis belongs to the family Theaceae, commonly known as Tea. The blood glucose level lowering activity has been extensively investigated. Antihyperglycemic activity of hot water extract of green tea in STZ induced diabetic

rats were studied by Gomes *et al.*(1995), these findings have been supported by Anderson and Polansky (2002). Tea polyphenols possess antioxidant capacity; have also been reported to inhibit α-amylase.

Pradeep Goyal *et al*., 2015 [27]

Borassus flabellifer is used extensively in the indigenous system of medicine as an antidiabetic agent. The investigation focus on the antihyperglycemic and antihyperlipidemic property of acetone insoluble ethanolic fraction of *Borassus flabellifer* extract on streptozotocin induced diabetic rats. The diabetic induced animals were fed with extracts of *Borassus flabellifer* at the increasing doses of 150mg, 300mg, 600mg/kg body weight administrated animals revealed a significant ($P<0.01$) decreased blood glucose level and higher reduction in hyperlipedemia when compared to the diabetic controls rats($P<0.01$). The histopathological studies of the endocrine region of pancreas of diabetic animals revealed that shrinkage of β-cells of islets of langerhans. The extracts treated animal revealed restoration of β-cells. The restoration of β cells was evident at higher dose level i.e. 600mg/kg body weight extracts fed group.

Review of literature related to Cassia *fistula* Linn.

ANTIOXIDANT ACTIVITY

Manonmani G *et al*., 2005 [28]

Aqueous extract of *Cassia fistula* (Linn) flowers (ACF) was screened for its antioxidant effect in alloxan induced diabetic rats. An appreciable decreased in peroxidation products viz thiobarbituric acid reactive substances, conjugated dienes, hydro peroxides was observed in heart tissues of ACF treated diabetic rats. The decreased activities of key antioxidants enzyme such as superoxide dismutase,

catalase, glutathione peroxidase, glutathione reductase and glutathione in diabetic rats were brought back to near normal range upon ACF treatment. These results suggested that ACF has got promising antioxidative activity in alloxan induced diabetic rats.

Raju Ilavarasan *et al.*, 2005 [29]

Antiinflammatory and antioxidant activities of the aqueous (CFA) and methanolic extracts (CFM) of the *Cassia fistula* Linn. bark were assayed in wistar albino rats. The extracts were found to possessed significant anti-inflammatory effect in both acute and chronic models. *Cassia fistula* bark extracts showed significant radical scavenging by inhibiting lipid peroxidation initiated by CCl_4 and $FeSO_4$ in rat liver and kidney homogenates. Further, the acute toxicity study with the extracts showed no sign of toxicity up to a dose level of 2000mg/ p.o. Thus it could be concluded that *Cassia fistula* bark extracts possessed significant anti-inflammatory and antioxidant properties.

ANTIFUNGAL ACTIVITY

Souwalak phongpaichit *et al*., 2004 [30]

Crude methanol extracts from leaves of *Cassia alata*, *Cassia fistula* and *Cassia tora* were investigated for their antifungal activities on three pathogenic fungi (*Microsporum gypseum, Trichophyton rubrum and M.gypseum* with the 50% inhibition concentration (IC_{50}) of hyphal growth at 0.5 and 0.8mg/ml, respectively, whereas the extract of *C. fistula* was the most potent inhibitor of *P. marneffei* with the IC_{50} of 0.9mg/ml.

ANTIPARASITIC ACTIVITY

Patricia sartorelli *et al*., 2008 [31]

The fractionation through bioguided antileishmanial activity of the dichloromethane extract of *Cassia fistula* fruits led to the isolation of the active isoflavone biochanin A, identified by spectroscopic methods. This compound showed 50% effective concentration (EC_{50}) value of 18.96µg/mL against promastigotes of *Leishmania* (L.) *chagasi.* The cytotoxicity of this substance against peritoneal macrophages resulted in an EC_{50} value of 42.58µg/ml. Additionally biochanin A presented an antitrypanosomacruzi activity, resulting in an EC_{50} value of 18.32µg/ml more effectiveness than benzimidazole. These results contribute with novel antiprotozoal compounds for future drug design studies.

CNS ACTIVITY

U.K.mazumder *et al*., 1998 [32]

The methanolic extract of the seed of *Cassia fistula* was tested for different pharmacological actions in mice. The methanolic extract (ME) of the seed significantly potentiated the sedative actions of sodium pentobarbitone, diazepam, meprobamate and chlorpromazine. A depressant action of ME was also evident from the behavioural studies on mice.

HYPOLIPIDEMIC ACTIVITY

Gupta *et al.*, 2009 [33]

The effect of 50% ethanolic extract of *Cassia fistula* Linn. legume was assessed on serum lipid metabolism in cholesterol fed rats. Oral feeding of cholesterol (500mg/kg b.w/day) dissolved in coconut oil (0.5ml/day) for 90 days caused a significant ($p<0.001$) elevation in total and LDL cholesterol, triglycerides and

phospholipids in serum of rats. Administration of *C. fistula* legume extract at the doses 100, 250, 500mg/kg b.w/day along with cholesterol significantly prevented the rise in the serum total and LDL cholesterol, triglycerides and phospholipids in a dose dependent manner. The ratio of HDL cholesterol /total cholesterol ratio were elevated in serum of *C. fistula* extract treated groups as compared to cholesterol alone fed control rats.

LAXATIVE AND ANTIMICROBIAL ACTIVITY

KA Abo *et al*., 1999 [34]

Colorimetric estimation of anthroquinones content, antimicrobial and laxative effects of leaves and pods of *Cassia fistula Linn*, *C. specatabilis* DC and *C. podocarpa* Guill are described because of the popular uses of these species by herbalists in Ibadan. The pods of the *Cassia* species exhibited potent antifungal activity than the leaf samples. Pods of *C. fistula* showed significant antibacterial activity when compared to that of ampicillin. This study justifies the use of the *cassia* species in traditional medicine.

ANTIULCER ACTIVITY

S. Karthikeyan *et al*., 2010 [35]

The ethanolic leaf extract (ELE) of *cassia fistula* Linn was evaluated for antiulcer activity against pylorus ligation-induced gastric ulcer. Ranitidine (30mg/kg b.w) and ELE at doses of 250, 500 and 750mg/kg/b.w.were administered orally in different groups of rats (n=6), 1h prio to pyloric ligation. ELE pre-treatment significantly attenuated the fall in status of sialic acid and fucose accompanied by an increase in hexose, hexosamine, total non-amino polysaccharide, total carbohydrate and C: P ratio in the gastric juice of pylorus ligated rats and this effect could be due to

protective of the mucosal barrier system. This protective ability of ELE against pylorus ligation-induced gastric ulcer could be attributed the radical scavenging and antioxidant properties. Higher doses of ELE (750mg/kg b.w) produced maximum antiulcer activity comparable to ranitidine treatment.

ANTI-ITCHING ACTIVITY

Manisha Talekar *et al*., 2015 [36]

Leaves of *Cassia fistula* Linn is known as a rich source of tannins, flavonoids and glycosides. Might be medicinally important and/or nutritionally valuable and used in many skin diseases. The therapeutic effect of paste of leaves of *Cassia fistula* Linn in management of eczema was evaluated. Total 30 patients of eczema fulfilling the inclusion criteria were randomly selected from the O.P.D and I.P.D. of national institute of Ayurveda, Jaipur. The informed consent from each patient was taken and treated with paste of leaves of *Cassia fistula* Linn. for 4 weeks. Leaves of *Cassis fistula* Linn was found to be more effective to control discoloration, itching, oozing, pain, burning, lines/thickening of skin and eruption.

PROTEASE-INHIBITORY ACTIVITY

Ratna wijaya *et al*., 2000 [37]

A novel trypsin inhibitor was extracted from the seeds of *Cassia fistula* by a process successively involving soaking seeds in water, extraction of seeds in methanol and extraction of the cell wall material at high ionic strength. The protease inhibitor (PI) was subsequently purified by chromatography on carboxy methylcellulose, gel filtration and reversed phase HPLC (RP-HPLC). The *C. fistula* seed PI is homologous to the family of plant defensins (Ý-thionins), which have four disulfide linkages at highly conserved locations. The *C. fistula* PI inhibits trypsin (IC_{50} 2 µM) and is the

first known example of a plant defensins with protease inhibitor activity, suggesting a possible additional function for some members of this class of plant defensive proteins.

HEPATOPROTECTIVE ACTIVITY

Sagar dawada *et al*., 2012 [38]

The protective effects of the alcoholic extract of *Cassia fistula* root against CCl_4 induced hepatic failure in male albino rats (Wistar strain) were investigated. The administration of alcoholic root extract (200mg/kg and 100mg/kg of body weight) for 7 days, elicited protective action since the elevated levels of marker enzymes (SGOT, SGPT, ALP) of liver functions were found to be decreasing progressively in a dose dependent manner. The results found in alcoholic extract 200mg/kg treated rat were quite promising and were comparable with a standard drug silymarin. In the alcoholic extract 200mg/kg treated rat group all the marker enzymes were analyzed to be decreasing significantly. The alcoholic root extract of *Cassia fistula* root (200mg/kg and 100mg/kg) was found to be possessed dose dependent, significant protective activity against CCl_4 induced hepatotoxicity.

PLANT PROFILE [39]

Name : *Cassia fistula L*

Synonym : *Bactyrilobium fistula* Willd.

Cassia bonplandiana DC.

Taxonomy

Kingdom : plantae

Class : Magnoliopsida

Subclass : Rosidae

Order : Fabales

Genus : Cassia

Family : Caesalpiniaceae

Vernacular name

English : Indian Laburnum, Golden shower

Tamil : Sarakonrai, Sarak konnai

Sanskrit : Aragvadha, Krtamala

Telugu : Rela

Malayalam: Konna, Kritamalam

Distribution

Subtropical, common in tropical India and Pakistan, Myanmar, Thailand and Sri Lanka.

Description

Plant

The tree is a medium sized tree, growing to 10 – 20m (33 -66ft) tall with fast growth.

Leaves

The leaves are deciduous, 15 – 60cm long and pinnate with three to eight pairs of leaflets, each leaflet 7 – 21cm long and 4 – 9cm broad.

Flowers

The flowers are produced in pendulous racemes 20 -40cm long, each flower 4- 7cm diameter with five yellow petals of equal size and shape.

Fruits

The fruit is a legume, 30 -60cm long and 1.5- 2.5cm broad, with a pungent odor and containing several seeds.

Chemical composition

Glycosides, cardiac glycosides,

Anthroquinones, flavonoids

Parts used

Roots, leaves, fruits, barks are used.

Properties and uses

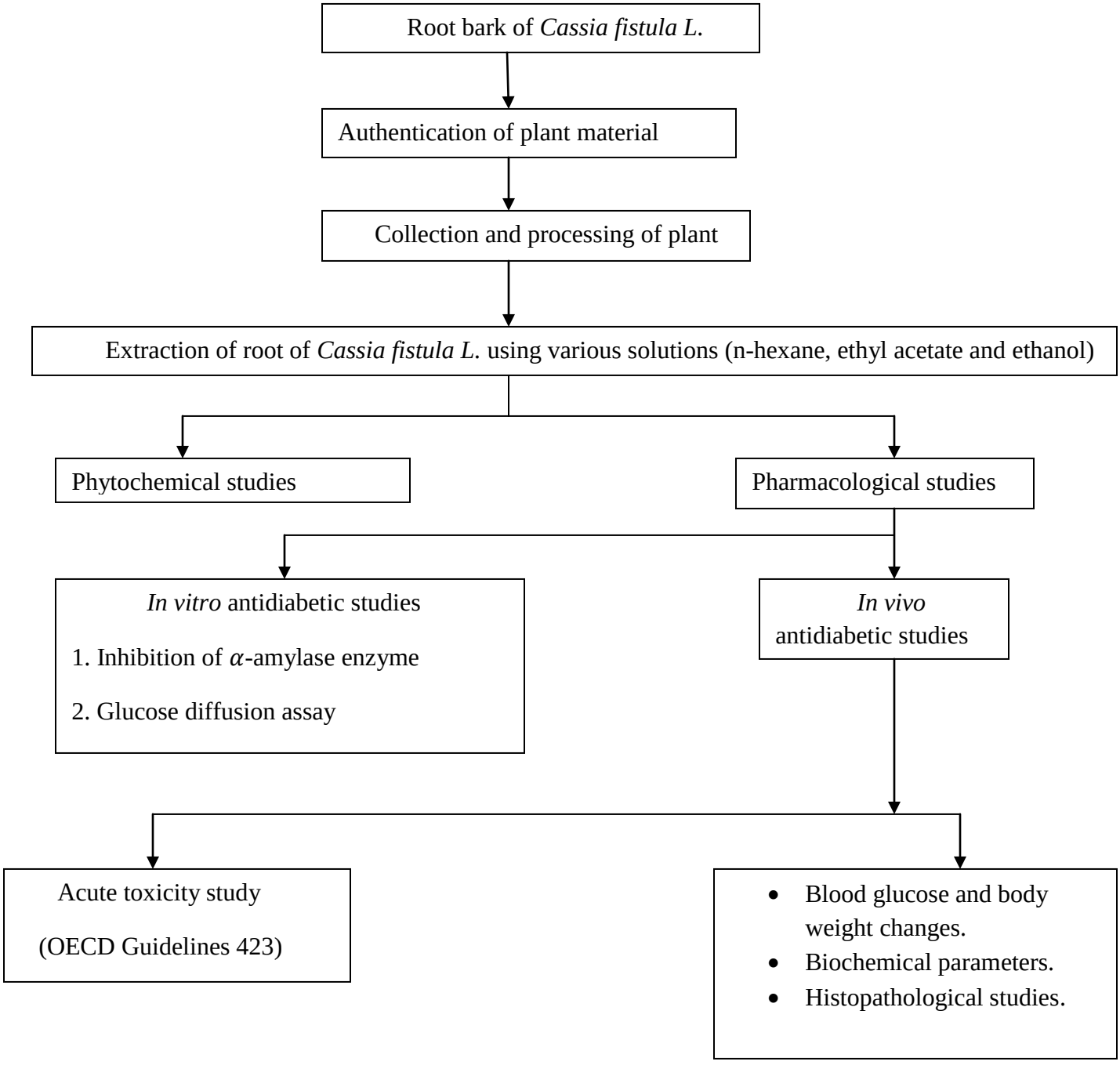
- The fruit pulp is considered a purgative.
- Roots are used as a hepatoprotective and antibacterial activity [38].

- Leaves and seed have various pharmacological activities like anti fungal, antioxidant, antimicrobial, anti-inflammatory, anti-tumor, hypoglycemic activities [40].
- It is used in the treatment of hematemesis, pruritis, intestinal disorder, leucoderma, diabetes, antipyretic, analgesic & laxative [8].

***Cassia fistula* L. - WHOLE PLANT**

***Cassia fistula* L. -root bark**

4. PLAN OF WORK



5. MATERIALS AND METHODS

PLANT COLLECTION AND IDENTIFICATION

Dried entire plant of *Cassia fistula L.* was collected from forest around Komaneri, Tirunelveli District, Tamil Nadu (India), in the month of July. It was authenticated by prof. V. Chelladurai, Research officer-Botany (Scientist-C) (Rtd), Central Council for Research in Ayurveda & Siddha, Govt. of India.

PREPARATION OF PLANT EXTRACTION

The plant was shade dried at room temperature and was subjected to size reduction to a coarse power by using dry grinder. 50grams of this coarse power was packed into Soxhlet apparatus and was subjected to extraction sequentially with 500ml of n-hexane, ethyl acetate and ethanol. The extraction was continued until the colour of the solvent in the siphon tube became colorless. Extraction procedure was carried out in Institute of Pharmacology, Madras Medical College, Chennai. Extracts of ethyl acetate and ethanol were subjected to evaporation by using Rotary evaporator at 60ºC.

VARIOUS EXTRACT	CONTENT(g)	PERCENTAGE YIELD
n-Hexane	5	10
Ethyl acetate	17	34
Ethanol	24	48

5.1 QUALITATIVE PHYTOCHEMICAL STUDIES [41-43]

The freshly prepared extracts of n-hexane, ethyl acetate and ethanol were subjected to phytochemical screening for the presence or absence of active phytochemical constituents by following methods.

Test for Alkaloids

Crude extract was treated with few drops of dilute hydrochloric acid and filtered. The filtrate was tested with various alkaloid reagents such as

Mayer's reagent : Cream precipitate

Dragendroff's reagent : Orange brown precipitate

Wagner's reagent : Reddish brown precipitate

Test for Steroids

Salkowskis test: Crude extract was mixed with 2ml of chloroform. Then 2ml of conc. Sulphuric acid was added carefully and shaken gently. Appearance of reddish brown colour ring indicated the presence of steroids.

Test for Flavonoids

Lead acetate test: Crude extract was treated with few drops of lead acetate solution. Appearances of yellow colour precipitate indicate the presence of flavonoids.

Alkaline reagent test: Crude extract was treated with few drops of sodium hydroxide solution. Formation of intense yellow color, which becomes colorless on addition of dilute acid, indicates the presence of flavonoids.

Shinoda test: Crude extract was treated with 5ml of 95% ethanol, few drops of concentrated hydrochloric acid and 5grams of magnesium turnings, appearance of pink colour indicated the presence of steroids.

Test for Phenols and Tannins

Crude extract was mixed with 2ml of 2% solution of ferric chloride. Appearance of violet colour indicates the presence of phenolic compounds and tannins.

Crude extracts was dissolved in water and treated with 10% of lead acetate solution, appearance of white precipitate indicate the presence of tannins and phenolic compounds.

Test for Saponins

Foam test: A small amount of extract was extracted with petroleum ether. To the insoluble residue left after extraction, a few ml of water was added and shaken vigorously for 15minutes and was observed for the formation of honeycomb froth that persisted for at least 30minutes.

Test for Terpenoids

Noller's test: The extract was warmed with tin and thionyl chloride. Pink coloration indicates the presence of terpenoids.

Test for Glycosides:

Borntrager's test: A small amount of extract was hydrolyzed with hydrochloric acid for few hours on a water bath and the hydrolysate was extracted with benzene. The benzene layer was treated with dilute solution and was observed for formation of reddish pink colour.

Legal test: The extract was dissolved in pyridine and was made alkaline with few drops of 10% sodium hydroxide and then freshly prepared sodium nitroprusside was added and observed for the formation of blue colour.

Libermann-burchard test: 1mg of the extract was dissolved in few drops of chloroform, 3ml of glacial acetic acid. Warmed, cooled under tap water and drops of concentrated sulphuric acid was added along the side of the test tube, formation of bluish green colour indicates the presence of steroids.

Test for Carbohydrate

A small quantity of various extracts were dissolved separately in 5ml of distilled water and filtered. The filters were subjected for the following tests:

Molisch test: The filtrate was treated with few drops of alpha-naphthol. Then about 1ml of concentrated H_2SO_4 was added along the sides of the test tube. Violet colour ring formation at the interface indicates the presence of carbohydrates.

DRUGS AND CHEMICALS

Sodium chloride, D-glucose, Ethanol, Sodium citrate tribasic-dihydrate, Citric acid monohydrate, Streptozotocin, Glibenclamide.

5.2 *IN VITRO* ANTIDIABETIC EVALUATION

Physical Method

Ability of the plant materials to retards the movement of glucose from the intestine into the blood was evaluated by physical methods *in vitro*. The following are the convenient models for assessing the materials which affect the absorption of glucose *in vitro*.

A. Glucose Diffusion Assay [44]

Plant extracts were mixed with glucose and placed in the sealed dialysis membrane and kept in the orbit shaker bath at 37ºC, at 150rpm. The movement of glucose across the membrane into the external solution was measured at periodic intervals using commercial GOD-POD kit.

Requirements

- Dialysis membrane
- 0.15M sodium chloride solution
- D- Glucose (25Mm in sodium chloride solution)
- Orbit shaker
- GOD - POD kit

Procedure

Dialysis membrane containing 2ml of 25mM glucose solution was mixed with 1ml of different plants extracts and was placed in the centrifuge tube containing 45ml 0.15M NaCl and then kept in orbit shaker bath at 37ºC at 150rpm. The movement of glucose into the external solution was monitored at set of time intervals using GOD-POD kit. Glucose concentration in the external solution was expected as mg/dl/hr.

B. Inhibition of Alpha-Amylase Enzyme [45]

Requirements

- 1% α −Amylase
- 1% Starch
- Iodine reagent
- 0.02M Sodium Phosphate buffer and HCl

Procedure

A starch solution (1%) was obtained by stirring 0.1g of potato starch in 100ml of 16Mm of sodium acetate buffer. The enzyme solution was prepared by mixing 27.5mg of alpha-amylase in 100ml of distilled water. The colorimetric reagents is prepared by mixing sodium potassium tartarate solution and 3, 5 di nitro salicylic acid solution 96Mm. Both control and plant extracts (200, 400, 600, 800, 1000 and 1200µg/ml) were added with starch solution and left to react with alpha-amylase solution under alkaline conditions at 25ºC. The reaction was measured over 3 minutes. The generation of maltose was quantified by the reduction of 3, 5 dinitro salicylic acid to 3-amino-5-nitro salicylic acid. This reaction is detectable at 540nm.

Calculation

Percentage inhibition (I %) = (Ac- As) / Ac × 100

Where Ac is the absorbance of the control and As is the absorbance of the sample.

***IN VIVO* ANTI DIABETIC STUDIES [46]**

Experimental animals

The present study was conducted after obtaining approval from the Institutional Animal Ethics Committee and this protocol met the requirements of national guidelines of CPCSEA (PROPOSAL NO: 08/43/CPCSEA dated: 10/08/2015). The wistar rats (150-200 gm) used for this study were procured from Animal house, Madras Medical College, Chennai, India.

Quarantine and Acclimatization

Quarantine is the separation of newly received animals from those already in the facility until the health and possibly the microbial status of the newly received animals have been determined. The newly procured Wistar rats were quarantined for the period of 10 days to minimize the chance of introduction of pathogens into established animals and allowed to develop the psychological, physiological and nutritional stabilization before their use.

Housing

The animals were housed in well ventilated animal house which was maintained at a constant temperature and relative humidity of 55 to 60%. The animals were housed in spacious polypropylene cages and paddy husk was utilized as bedding material.

Diet and water

The animals were maintained on standard pellet diet and purified water. The animals were provided with food and water ad libitum except during fasting. The bed material was changed twice a week.

Animal identification

All animal cages used in the study had a proper identification i.e., labels. Each animal in the cage was marked either on head or body or tail with picric acid for their appropriate identification.

TOXICITY STUDIES

Acute toxicity was designed as per the OECD guidelines (423). [38], [47]

5.3 ACUTE TOXICITY STUDY

Principles and purpose

The main purpose of acute toxicity is to evaluate the degree of toxicity in a quantitative and qualitative manner with the purpose of comparing it with other drug substances (e.g. other drug candidates for the same indication).

Experimental Animals

Three healthy adult wistar albino rats weighing between 150-250g were selected for the study. For all the three animals food, but not water was withheld overnight prior to dosing.

Selection of dose levels and administration of doses

Being a traditional herbal medicine, the mortality was unlikely at the highest starting dose level (2000mg/kg/b.w). Hence a limit test at one dose level of 2000mg/kg/b.w was conducted in all the three animals as per the OECD guidelines (423).

5.4 *INVIVO* ANTIDIABETIC EVALUATION

The antidiabetic activity of *Cassia fistula* Linn. was evaluated in diabetic Wistar rats. Diabetes was induced by intra-peritoneal injection of Streptozotocin 45mg/kg/b.w for 7 days. The antidiabetic effect of plant extract was compared with standard drug Glibenclamide.

Preparation of Streptozotocin Solution

Preparation of 0.1M citrate buffer solution pH 4.5. An accurately weighed quantity of trisodium citrate dehydrate (2.941g) is dissolved in 100ml distilled water and accurately weighed quantity of citric acid (2.101g) is dissolved in 100ml distilled water. Mix 44.5ml of 0.1M citric acid monohydrate and 55.5ml of 0.1M trisodium citrate dehydrate (pH 4.5).

Induction of diabetes in rats

After 10 days of acclimatization, the rats were subjected to overnight fasting. Diabetes was induced by intraperitoneal injection of Streptozotocin, freshly dissolved in citrate buffer pH 4.5. The animals were allowed to drink water 5% glucose solution overnight to overcome the drug induced hypoglycemic due to massive release of insulin from β-cells. After the induction, on 3rd day the blood glucose levels were measured and the animals with a blood concentration of more than 250mg/dl were considered as diabetic and taken for the experiment. Administration of the plant extract was started on the 4th day after streptozotocin injection and this was considered as the 1st day of treatment, which was continued for 21 days.

Experimental design

The fasting glucose and body weight of all animals were recorded at the beginning of the study. The blood glucose was checked by one touch glucometer throughout the study, in the experiments, 30 rats were divided into 5 groups of six rats each.

Group 1	Normal control rats, received distilled water.
Group 2	Streptozotocin induced diabetic rats received distilled water and served as diabetic control for 21 days.
Group 3	STZ induced diabetic rats received standard drug Glibenclamide (5mg/kg/BW, p.o) for 21 days.
Group 4	STZ induced diabetic rats received the plant extract (200 mg/kg/BW, p.o) for 21 days.
Group 5	STZ induced diabetic rats received the plant extract (400 mg/kg/BW, p.o) for 21 days.

For all rats, body weight was measured before and after the induction of diabetes. Blood glucose level was measured on 1, 7, 14 and 21st day throughout the study period by tail tip cutting method. At the end of the experiment, sufficient blood was collected by retro-orbital bleeding from all the animals under mild anaesthesia for estimation of haematological and biochemical parameters.

Biochemical parameters

The blood samples were centrifuged at 3000rpm for 5 minutes using REMI (412LIG) cooling centrifuge. The serum was kept at -80ºC until analyzed. Levels of total cholesterol, triglycerides (TGL), high density lipoprotein (HDL) were determined with an automatic analytical instrument (Hitachi 911, Japan).

A) ESTIMATION OF LIPID PROFILE [48]

Principle

The cholesterol esters and cholesterol present in the sample are acted upon by Cholesterol Esterase to release Cholesterol and Fatty acids. The Cholesterol is oxidized by Cholesterol Oxidase to yield 4-Cholesterol 3-one and hydrogen peroxide as by product. Hydrogen peroxide together with 4-aminoantipyrin and phenolic compound in the presence of peroxidase gives the colored complex. The intensity of the colour is proportional to the total cholesterol in the sample and is measured at 550nm or with Green filter.

Cholesterol ester + H_2O $\xrightarrow{\text{cholesterol esterase}}$ **Cholesterol + Fatty acids**

Cholesterol + O_2 $\xrightarrow{\text{cholesterol oxidase}}$ **4-Cholesterol-3-one- H_2O_2**

$2H_2O_2$ + Phenol + 4-aminoantipyrine $\xrightarrow{\text{peroxidase}}$ **Red quinine + $4H_2O$**

Requirements

- Enzyme reagents
- HDL precipitate reagent

Procedure

1ml of enzyme reagent and 10µl of test or standard were mixed well and incubated at 37ºC for 5mins. The absorbance of test and standard were measured at 505nm or using Green filter.

Calculation

$$\text{Cholesterol conc.mg/dl} = \frac{\text{Absorbance of test}}{\text{Absorbance of standard}} \times \text{conc. of standard (200)}$$

HDL Cholesterol [49]

Procedure

Step 1

200µl of serum and 300µl of HDL ppt reagent were mixed well and allowed to for 10mins. Then the mixture was centrifuged at 3000rpm for 10mins and the supernatant was separated.

Step 2

1ml of enzyme reagent and 100µl of supernatant from step A were mixed together and incubated for 5mins at 37ºC. The absorbance is read at 505nm.

Calculation

$$\text{HDL Cholesterol conc.mg/dl} = \frac{\text{Absorbance of test}}{\text{Absorbance of standard}} \times \text{conc. of standard (50)}$$

TRIGLYCERIDE [50]

In human nutrition, triglyceride is the most prevalent glycerol esters encountered. They constitute 95% of tissue storage fat are the predominant form of

glycerol ester found in plasma. The investigation of triglyceride is part of the overall evaluation of lipids disorders.

Principle

$$\text{Triglyceride} + H_2O \xrightarrow{\text{LPL}} \text{Glycerol} + \text{free fatty acid}$$

$$\text{Glycerol} + \text{ATP} \xrightarrow{\text{GK}} \text{Glycerol -3- phosphate} + \text{ADP}$$

$$\text{Glycerol-3-phosphate} + O_2 \xrightarrow{\text{GPO}} \text{DAP} + H_2O_2$$

$$H_2O_2 + \text{4AAP} + \text{3, 5-DHBS} \xrightarrow{\text{peroxidase}} \text{Quinoeimine Dye} + 2H_2O_2$$

Requirements

- Pipes buffer pH 7.0
- 4-AAP 0.4mmol/l
- Magnesium 2mmol/l
- ATP 2mmol/l
- GK
- POD
- LPL
- GPO
- Surfactants

Procedure

1ml of reagent mixed with 10µl of sample and incubated for 5min at 37ºC. Then the absorbance is measured at 505nm.

Calculation

Triglyceride mg/dl = $A_X/A_S \times$ Concentration of standard

Where, A_X- Absorbance of sample, A_S- Absorbance of standard

HISTOPATHOLOGICAL STUDIES

At the end of 21st day, all the animals were sacrificed to collect the pancreas and liver. The organs were rinsed in ice cold 0.9% saline and were fixed in 10% formalin embedded in paraffin and cut into 5 µm thick sections in a microtome. Sections were mounted on glass slides using standard techniques. After staining with Hematoxylin-Eosin, the section were examined under 100X magnification and photographed under a light microscope equipped for photography (Olympus CK 40).

STATISTICAL ANALYSIS

The results were expressed as Mean ± SD. All the parameters were analysed by one way ANOVA. The probability values $p<0.01$, $p<0.001$ are considered as statistically significant. The statistical analysis was performed by using vasarstats one way ANOVA online software (htttp..//vasarstats.net/anova1u.html).

6. RESULTS

6.1 PRELIMIARY PHYTOCHEMICAL ANALYSIS

Table 1: Preliminary phytochemical analysis of ethanolic extract of *Cassia fistula* L.

TEST	RESULTS
Test for Flavonoids	
a) Shinado's test	Present
b) Sodium hydroxide test	Present
Test for Alkaloids	
a) Dragendroff's test	Absent
b) Mayer's test	Absent
c) Wagner's test	Absent
Test for Steroids	
Salkowskis test	Absent
Test for Tannins	
With lead acetate	Absent
Test for Saponins:	
Foam test	Absent
Test for Terpenoids	
With Tin and thionyl chloride	Absent
Test for Glycosides	
a) Borntrager's test	Present
b) Liermann-burchard's test	Present
c) Legal's test	Present
Test for Carbohydrates	
Molish's test	Absent

6. 2 ACUTE TOXICITY STUDY

Table 2: Acute toxicity study of ethanolic root extract of *Cassia fistula L*.

S.NO	PARAMETERS	RESULTS
1.	Toxic signs	Absent
2.	Pre-terminal deaths	Nil
3.	Body weight	No specific change
4.	Motor activity	Normal
5.	Tremors	Absent
6.	Convulsions	Absent
7.	Straub reaction	Absent
8.	Righting reflex	Present
9.	Lacrimation and salivation	Normal
10.	Unusual vocalization	Absent
11.	Sedation	Absent
12.	Body temperature	Normal
13.	Analgesia	Absent
14.	Ptosis	Absent
15.	Diarrhea	Absent
16.	Skin colour	Normal
18.	Respiration	Normal
19.	Scratching	Absent
20.	Grooming	Absent
21.	Aggressiveness and restlessness	Absent

Animals were observed for behavioral signs of toxicity like motor activity, tremor, etc., and no significant toxic signs were observed during 14 days. The results of the acute toxicological studies revealed that the administration of ethanolic extract of *Cassia fistula L.* by oral route upto 2000mg/kg/b.w did not produce any mortality and it was tolerated.

6.3 *IN VITRO* ANTIDIABETIC STUDIES

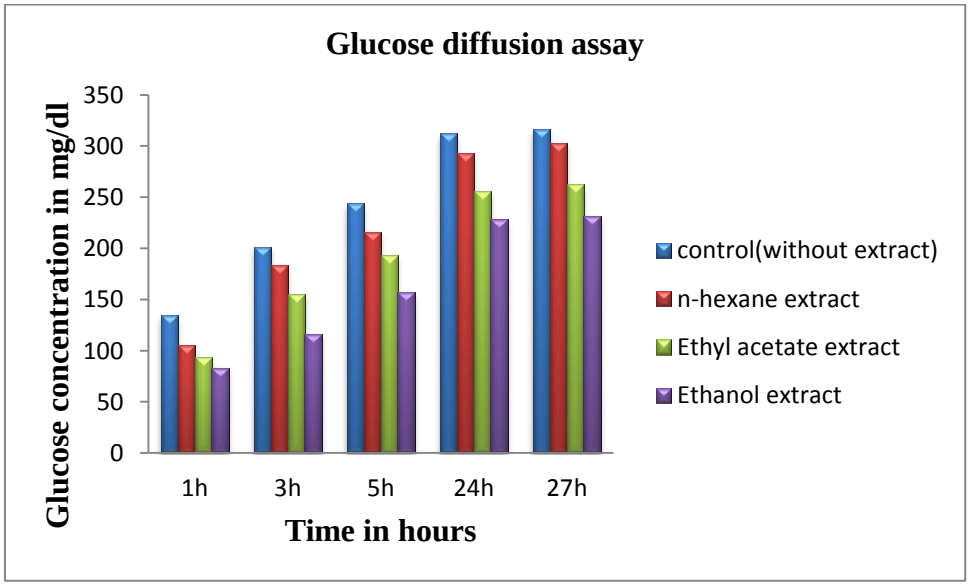
GLUCOSE DIFFUSION ASSAY

Table 3: Mean glucose intensity in the external solution of various extracts of *Cassia fistula* L. at different time intervals

Time in hours	Control (in the absence of extract)	n-Hexane extract (50mg/ml)	Ethyl acetate extract (50mg/ml)	Ethanol extract (50mg/ml)
1h	134.33±1.20	105.33±1.20[a]	94±1.15[a]	83.33±0.88[a]
3h	201.33±1.76	184±1.15[a]	155.33±0.88[a]	116.33±0.88[a]
5h	244.33±1.76	216±1.15[a]	193.66±0.88[a]	157.33±1.20[a]
24h	312.33±2.02	293±1.15[a]	256.33±1.45[a]	228.66±1.76[a]
27h	316.66±1.76	303.33±1.20[a]	262.33±0.88[a]	232±2.18[a]

Glucose values are expressed as mean ±SD
a -p<0.001- compared to control

Figure 1: Mean glucose intensity in the external solution of various extracts of Cassia *fistula* L.



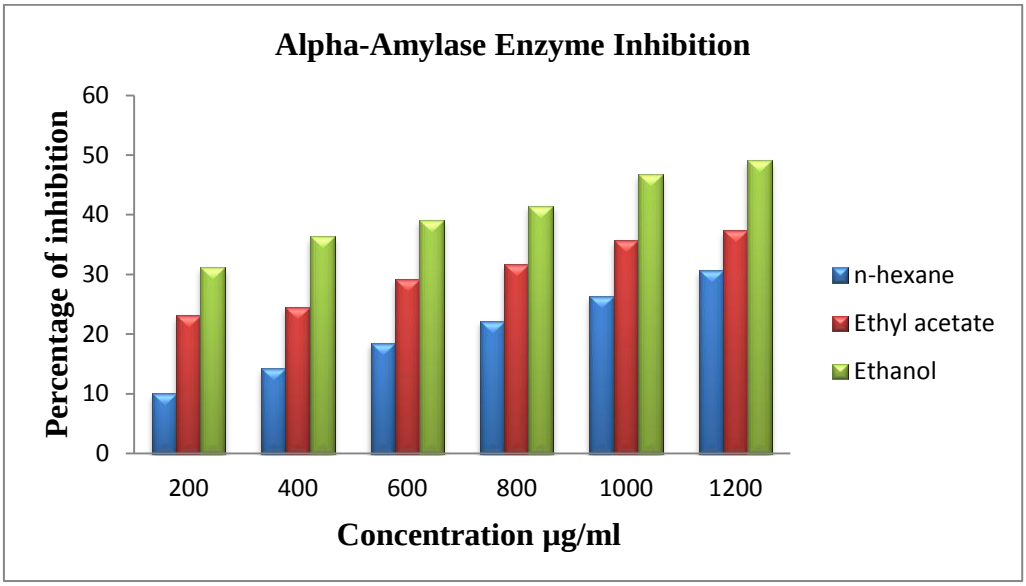
Cassia fistula L. extracts the concentration of glucose into the external solution by retarding its diffusion through membrane. After 27 hrs the control showed 316.66mg/dl of glucose in the external solution whereas the *Cassia fistula* ethanolic extract after 27 hrs showed 232mg/dl of glucose. *Cassia fistula L.* ethanolic extract possessed a good effect in glucose diffusion and was showed highest activity.

INHIBITION OF ALPHA-AMYLASE ENZYME

Table 4: *Invitro* anti diabetic activity of root of *Cassia fistula* L. in alpha amylase enzyme inhibition method

S.No	Concentration of sample (µg/ml)	% Inhibition		
		n-hexane	Ethyl acetate	Ethanol
1	200	10.02	23.09	31.10
2	400	14.21	25.49	36.34
3	600	18.50	29.06	39.09
4	800	22.19	31.67	41.48
5	1000	26.34	35.64	46.71
6	1200	30.63	37.39	49.14

Figure 2: % inhibition of alpha amylase Enzyme



Cassia fistula L. ethanolic extract was a dose dependent increase in percentage inhibitory activity against alpha-amylase enzyme. At a concentration of 200µg/ml of plant extract showed a percentage inhibition 31.10% and for 1200µg/ml of plant extract showed inhibition of 49.14%. It showed highest activity at concentration of 1200µg/ml.

6.4 *IN VIVO* ANTIDIABETIC ACTIVITY

Table 5: Effect of ethanolic extract of *Cassia fistula* L. on body weight in STZ induced diabetic rats.

GROUP	TREATMENT	AVERAGE BODY WEIGHT IN DAYS (gm)			
		1st Day	7th Day	14th Day	21st Day
1.	Normal control	145.83±3.6	149.83±0.75	150.5±2.58	151.66±3.01
2.	Diabetic control STZ (45mg/kg)	153.66±2.80[a]	147.5±2.42[c]	145±1.41[a]	134.66±3.32[a]
3.	Glibenclamide (5mg/kg)	162.66±3.93[a]	159±1.78[a]	155.83±2.32[a]	151.33±1.21[a]
4.	*Cassia fistula L* extract 200mg/kg	149.83±1.60[b]	143.8±1.21[c]	142.83±1.16[b]	137.83±1.17[c]
5.	*Cassia fistula L* extract 400mg/kg	157.16±2.31[c]	145±1.41[c]	143±1.67[c]	138.5±1.04[c]

Data were expressed as mean ± SD, n=6

[c] **p<0.05 significant**
[b] **p<0.01 most significant**
[a] **p<0.001 highly significant**

Diabetic control was compared with normal, Experimental groups were compared with diabetic control. **(Table 5**) shows the mean body weight during the study period of all groups. The mean body weight was decrease in G2, G3 and G4 & G5 on 21st day when comparing with 1st day of treatment.

G2 showed significant reduction in body weight p< 0.05. When compared within all group.

Figure 3: Body weight of STZ induced diabetic Wistar rats after 3 weeks of treatment with ethanolic extract of *Cassia fistula* L.

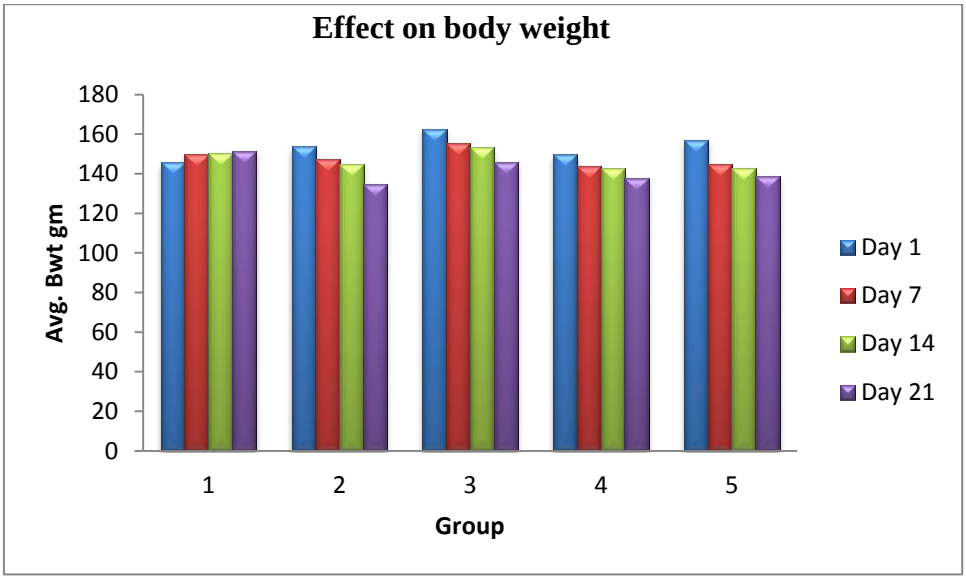


Table 6: Effect of ethanolic extract of *Cassia fistula* L. on whole blood glucose in STZ induced diabetic rats.

GROUP	TREATMENT	WHOLE BLOOD GLUCOSE (mg/dl)			
		1st Day	7th Day	14th Day	21st Day
1.	Normal control	77.66±1.37	86.33±0.81	92.66±2.16	98.17±2.13
2.	Diabetic control STZ (45mg/kg)	382.66±1.75[a]	398.16±1.72[a]	415.01±1.41[a]	430.66±0.81[a]
3.	Glibenclamide (5mg/kg)	369.16±0.76[b]	232.5±2.16[b]	216.16±1.16[a]	154.83±0.75[a]
4.	*Cassia fistula L* extract (200mg/kg)	434.16±0.75[a]	220.33±1.86[a]	156±1.14[a]	95.83±1.73[a]
5.	*Cassia fistula L* extract (400mg/kg)	372.16±1.47[a]	234.16±1.94[a]	155±1.18[a]	95.16±1.5[a]

Values are expressed as mean ±SD, n=6.
[c] p<0.05 significant,
[b] p<0.01 most significant
[a] p<0.001 highly significant

Diabetic control was compared with normal, Experimental groups were compared with diabetic control.

Within group analysis

The mean blood glucose level showed a significant reduction in G3, G4, and G5 on 21st day when compared with 1st day of treatment.

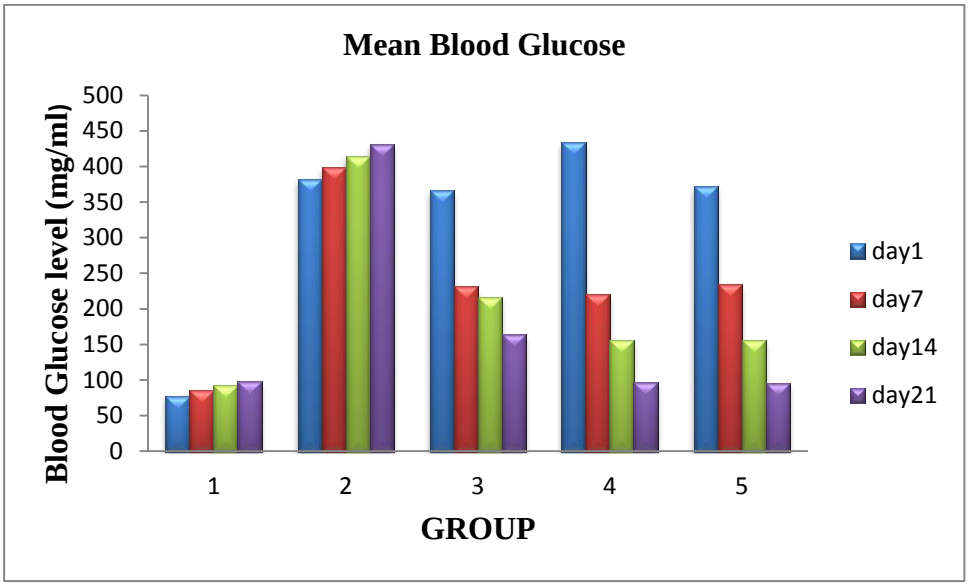
Between group analysis

When comparing with G2, the mean blood glucose was lowered in G3, G4 and G5 on day 21st day of treatment.

On comparing control group G1 with G2, G3, G4 and G5 a statistically significant ($p<0.001$) difference was observed in G2.

On comparing G2 with G3, G4 and G5 a statistically significant ($p< 0.001$) difference was seen in both the groups of the animals.

Figure 4: Mean blood glucose level



LIPID PROFILE

Table 7: Effect of ethanolic root extract of *Cassia fistula* L. on lipid profile

Group	Treatment	Cholesterol	Triglycerides	HDL
1.	**Normal Control**	118.8±1.16	100.16±1.17	23±0.89
2.	**Diabetic control STZ (45mg/kg)**	237.5±1.87[a]	238±1.67[a]	18.83±0.75[a]
3.	**Glibenclamide (5mg/kg)**	119.83±1.47[a]	97.83±1.47[a]	29.16±1.72[a]
4.	***Cassia fistula* extract (200mg/kg)**	123.33±1.36[a]	98.83±1.47[a]	23.5±1.04[a]
5.	***Cassia fistula* Extract (400mg/kg)**	120.33±1.03[a]	97.9±1.5[a]	24.66±1.03[a]

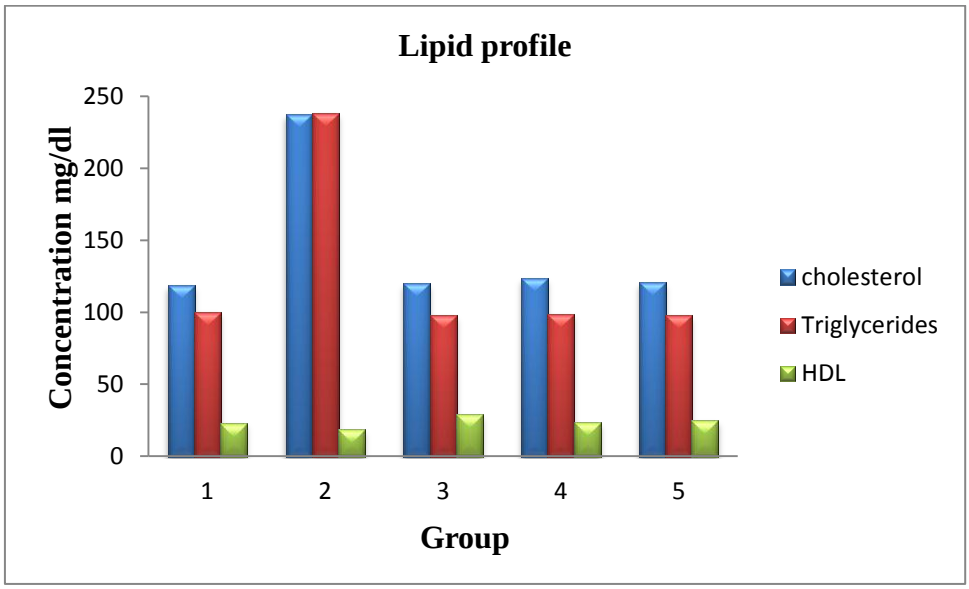
Values are expressed as mean ±SD, n=6.
[c] p<0.05 significant
[b] p<0.01 most significant
[a] p<0.001 highly significant

Diabetic control was compared with normal, Experimental groups were compared with diabetic control.

The mean cholesterol and triglyceride levels were higher in GP2, than the group with GP3, GP4 & GP5.

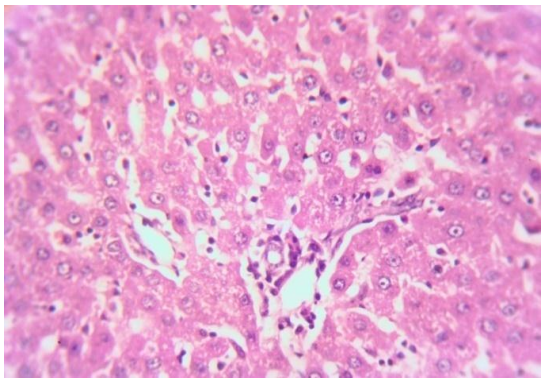
A significant ($p < 0.001$) decrease in Triglyceride & cholesterol level was observed in G2 Vs G3, G4 and G5.

Figure 5: Effect of ethanolic root extract of *Cassia fistula* L. on lipid profile level

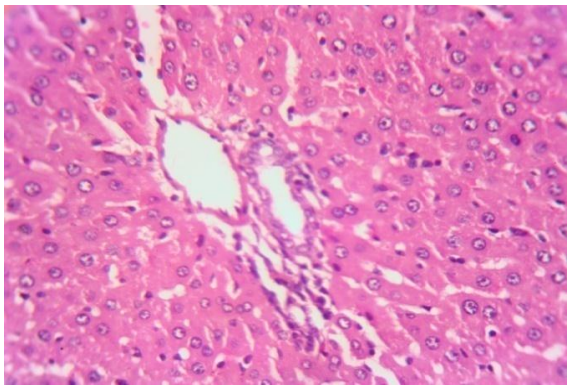


HISTOPATHOLOGICAL STUDY OF LIVER

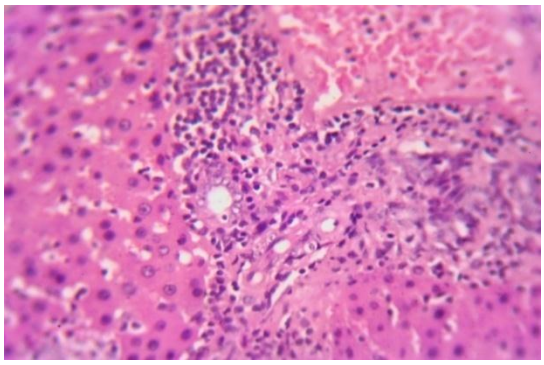
(PORTAL TRAID)



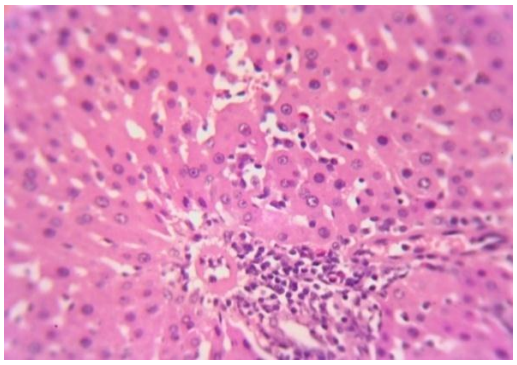
1. NORMAL CONTROL



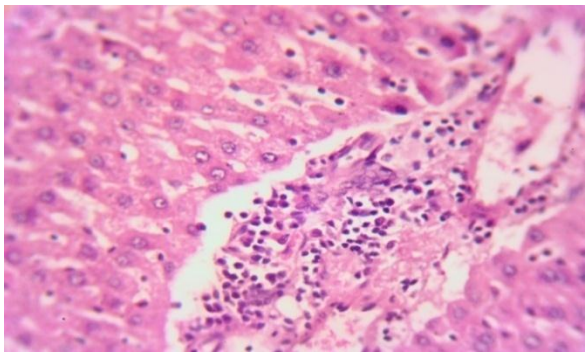
2. DISEASE CONTROL



3. GLIBENCLAMIDE



4. *Cassia fistula* 200mg/kg

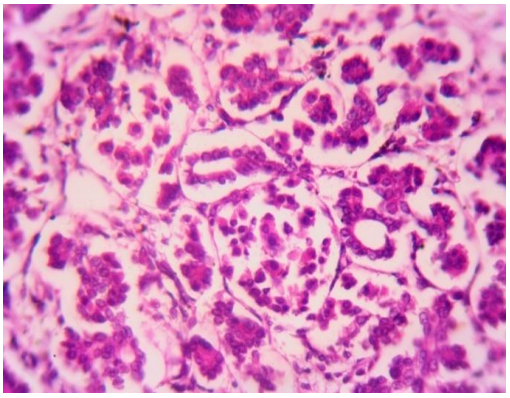


***5. Cassia fistula* 400mg/kg**

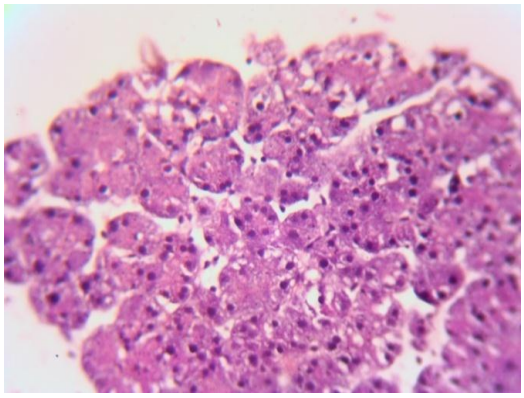
HISTOPATHOLOGICAL OBSERVATION

1. In normal control group animal the histopathology of liver showed portal triad surrounded by cords of hepatocytes.
2. Glibenclamide treated animal the histology of liver showed moderate periportal inflammation when compared with normal control.
3. STZ induced diabetic rat showed no periportal inflammation.
4. *Cassia fistula* L. ethanolic root extract (200mg/kg and 400mg/kg) treated animals showed very mild periportal inflammation compared with diabetic rat.
5. *Cassia fistula* L. ethanolic root extract (200mg/kg and 400mg/kg) treated animals showed mild periportal inflammation when compared with normal control as well as standard drug.

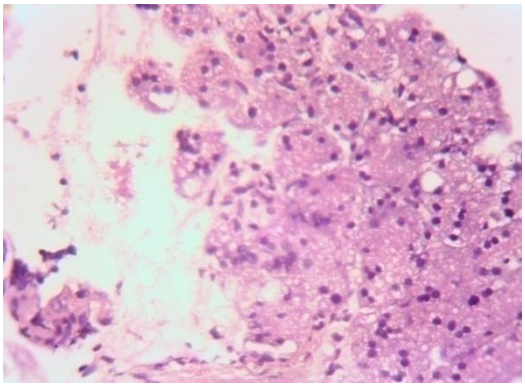
HISTOPATHOLOGICAL STUDY OF PANCREAS



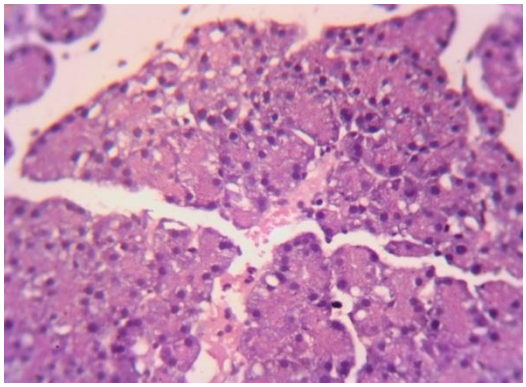
1. NORMAL CONTROL



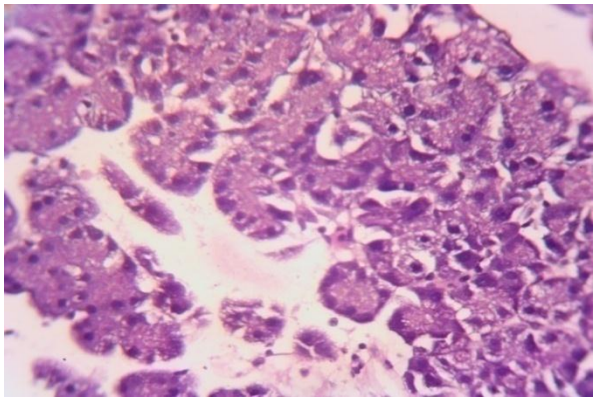
2. DISEASE CONTROL



3. GLIBENCLAMIDE



4. *Cassia fistula* 200mg/kg



5. *Cassia fistula* 400mg/kg

HISTOPATHOLOGICAL OBSERVATION

1. The pancreatic islets of langerhans of normal rat showing alpha cells and beta cells.
2. STZ induced diabetic damaged pancreatic islets showing reduced size and increased damaged beta cells.
3. Glibenclamide (5mg/kg) treated pancreatic islet showed better restoration, when compared to the STZ induced diabetic control rats.
4. *Cassia fistula* extract (200mg/kg) treated pancreatic islet showed partial better restoration, when compared to the STZ induced diabetic rats.
5. *Cassia fistula* extract (400mg/kg) treated pancreatic islets partial restoration of beta cells. The animals revealed better restoration from the STZ induced damage when compared to control and disease control as well as 200mg/kg of extract treated animal.

7. DISCUSSION

Diabetes is a chronic metabolic disorder affecting a major proportion of the population worldwide. A sustained reduction in hyperglycemic will decrease the risk of developing micro vascular disease and reduce their complications [(51)]. The conventional therapies for diabetes have many shortcomings like side effects and high rate of secondary failure. On the other hand herbal extracts are expected to have similar efficacy without side effects as that of conventional drugs. The present investigation reports that antidiabetic effect of *Cassia fistula* on streptozotocin (STZ) induced diabetic rats [(52)].

STZ produces oxygen radicals in the body, which cause pancreatic injury and could be responsible for increased blood sugar seen in animals [(53)]. Pancreas has a relatively weak defense system against oxidative stress, which can be externally strengthened. Antioxidants such as N-acetylcystine and dietary antioxidant such as Vitamin C and E have shown to be beneficial in protecting the β-cells from glucose toxicity in diabetes [(54)].

Medicinal plants could be considered as potential sources for providing a reasonable amount of the required elements other than diet to the patients of diabetes mellitus. Several controlled clinical trials of trace element supplements for glycemic control revealed the beneficial role for supplementation for the control and management of diabetes [(55-57)].

Over 150 plants extracts and some of the active principle including flavonoids are known to be used for the treatment of diabetes. In our study the root of the plant extract of *Cassia fistula L.* indicates the presence of secondary metabolites

like flavonoids, glycosides were used. The presence of these phytochemicals in high concentration account for the significant hypoglycemic effect of *Cassia fistula L*.

Cassia fistula L. is a tropical herb/frob which has been traditionally used in the management DM [39]. This present study has been undertaken to evaluate the preliminary phytochemical analysis, *in vitro*, acute toxicity and *in vivo* studies in diabetic Wistar rats.

The phytochemical analysis of ethanolic extract of *Cassia fistula L*. showed the presence of flavonoids, glycoside **(table 1)**. The medicinal plants with hypoglycemic and anti-diabetic effect usually contain high concentration of flavonoids [58].

Acute toxicity study revealed that the ethanolic extracts of *Cassia fistula L*. was relatively non toxic up to 2000mg/kg/b.w, p.o indirectly pronouncing safety profile of the extract **(table 2)**.

The present finding reveals that *Cassia fistula* L. efficiently inhibit alpha-amylase enzyme *in vitro* in a dose dependent manner. The ethanolic extracts from *Cassia fistula L* root showed a dose dependent inhibitory effect of alpha –amylase activity. There was a dose-dependent increase in percentage inhibitory activity against alpha-amylase enzyme. At a concentration of **200µg/ml** of plant extract showed a percentage inhibition of **31.10%** and for **1200µg/ml** plant extract showed inhibition of **49.14 % (table 4)**.

The *in vitro* antidiabetic evaluation was assessed by glucose diffusion assay. *Cassia fistula L*. ethanolic extract showed maximum decrease in glucose diffusion when compared to control values. Glucose diffusion is useful *in vitro* index to predict the effect of plant fibers on the delay in glucose absorption in GI tract. In addition to

glucose adsorption, the retardation in glucose diffusion might be attributed to the physical obstacle presented by fiber particles towards glucose molecules and entrapment of glucose within the network formed by fibers. The effect of various extracts on glucose diffusion inhibition was depicted **(table 3)**. At the end of 27 hrs, glucose movement of control (without plant extract) in the external solution had reached a plateau with a mean glucose concentration above **300mg/dl (316.66±.76)**. It was evident from the graph **(figure 1)** that the ethanol extract were found to be potent inhibitor of glucose diffusion **(p< 0.001)** compared to control. The ethanolic extract was found to be more potent than other extracts showing the lowest mean glucose concentration of **208±1.15mg/dl** at the end of 27 hrs **(table3)**.

When compared with n-hexane and ethyl acetate extract of *Cassia fistula L*., ethanolic extract showed good activity in alpha-amylase enzyme inhibition and glucose diffusion method. So ethanolic extract of *Cassia fistula L* was selected for *in vivo* study.

In diabetic blood glucose is not utilized by tissue resulting in hyperglycemia. The fatty acids from adipose tissue are mobilized for energy purpose. The reduction in weight is due to loss in muscle adipose tissue, protein and fatty acids. Studies have also reported significant weight reduction in untreated diabetic rats [58, 59].

Control animal were found to be almost stable in their body weight but diabetic induced rats showed significant reduction in body weight. The administration of *Cassia fistula L*. root extracts (200 & 400mg/kg) and Glibenclamide (5mg/kg) to the diabetic rats restored the changes in the body weight **(table 5)**. On comparing 200mg Vs 400mg/kg weight of *Cassia fistula L.* and there was a significant difference in body weight **(p< 0.05)** was observed. The dose dependant antidiabetic property of the ethanolic extract of *Cassia fistula L*. exhibited slight improvement in body weight.

Plants may act on blood glucose through different mechanisms, some of them may have insulin-like substances [60].Some may stimulate β-cells to produce more insulin and other may increase β-cells in the pancreas by activating regeneration of pancreatic cells. The fiber of the plants may also interfere with carbohydrate adsorption; thereby affecting blood glucose [63].

In this present study, after the administration of extract (200 & 400mg/kg), the fall in fasting blood glucose was evident at the end. Fall in fasting blood glucose levels progressively retained till the end of 3rd week.

When the reduction of blood glucose level with 200mg/kg & 400mg/kg were compared, there was a statistically significant reduction in blood glucose levels **(p<0.001)** was exhibited with **400mg/kg** of the ethanolic root extract *Cassia fistula L.* **(table 6)**.

Diabetes induce hyperlipidemia due to excess mobilization of fats from adipose tissue to the under utilization of glucose. High levels of triglyceride, LDL and VLDL also reported and have been associated with heart disease, insulin resistance and diabetes mellitus [61].

The levels of total serum cholesterol and triglycerides were raised in diabetic rats which were lowered significantly with the treatment of Glibenclamide and ethanolic extract of *Cassia fistula Linn.* **(Table7)**. On comparing groups 3,4,5 with group 2 there was a significant reduction in total cholesterol, triglyceride and HDL levels **(p<0.001)** in Streptozotocin induced diabetic rats and its hypolipidemic effect could represent a protective mechanism against the development of atherosclerosis which is usually associated with diabetes.

The rejuvenation of β cells in diabetes has been studied in different animal models. The total β-cells mass reflects the balance between the renewal and loss of these cells. Previous studies suggested that regeneration of islets of β-cells following destruction by streptozotocin, may be the primary cause of the recovery of streptozotocin injected guinea pigs from the effect of the drug [62].

Histological section of endocrine regions of pancreas of STZ induced diabetic rats revealed a significant reduction in the size of the islets when compared to that of normal groups. Further the study revealed the presence of damaged β-cell population. This damage of the β-cells due to STZ induction. The reduction in β-cell number can be as low 50% during diabetes. On the other hand, studies on the supplementation of extracts the diabetic rats revealed restoration of size of the islets along with β-cells repair. This recovery of the β-cells was recorded as dose dependant that is from 200mg to 400mg/kg body weight of the *Cassia fistula L.* extract given animals. The plant extract fed animals revealed better restored β-cells of pancreas from the STZ induced damage. The restoration of β-cells was evident at higher dose level of 400mg/kg body weight extract fed groups.

The data of this present studies may suggested that ethanolic extract of *Cassia fistula L.* has beneficial effects in diabetes mellitus holding the hope for anti-diabetic drugs.

8. CONCLUSION

- From the present study we conclude that the preliminary phytochemical analysis of *Cassia fistula L*. indicated the presence of flavonoids, Glycosides.
- *In vitro* Glucose diffusion properties of *Cassia fistula* L. extract was evaluated using dialysis membrane and GOD-POD kit.
- *In vitro* alpha-amylase enzyme inhibition of *Cassia fistula* L. extract was evaluated.
- Ethanolic extract of *Cassia fistula* L. was safe in dose upto 2000mg/kg by acute toxicity study and there was no toxic effects produced.
- The ethanolic extract of *Cassia fistula* L. (200mg/kg and 400mg/kg) exhibited significant reduction in triglyceride & cholesterol and also reduction in the body weight in streptozotocin induced diabetic rats.
- *In vivo* studies of *Cassia fistula* L. ethanolic extract revealed the glucose lowering effect. Thus it was concluded that the ethanolic extract of *Cassia fistula L.* has anti-diabetic activity.
- In this present study was exhibited significant inhibition activity of ethanolic root extract of *Cassia fistula* L. by using *in vitro* α-amylase and glucose diffusion assay, so further the compound isolation, purification and characterization which is responsible for inhibiting activity, has to be done for the usage of antidiabetic agent.

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