EVALUATION OF ANTIDIABETIC ACTIVITY OF PLANT EXTRACT ON STREPTOZOTOCIN INDUCED DIABETIC RATS

Thesis Submitted for the Partial Fulfillment of the award Degree of Master of Pharmaceutical Technology Faculty of Engineering & Technology Jadavpur University

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CERTIFICATE

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*This is to certify that the thesis entitled "**Evaluation of anti diabetic activity of plant extract on streptozotocin induced diabetic rats**" submitted by **Poulami Majumder,** with registration in the year 2014-2015 for the partial fulfillment of degree of Master of Pharmaceutical Technology, Jadavpur University, is absolutely based upon her own research project work under my supervision. Her thesis has not been submitted before for any degree/diploma or any other academic award elsewhere.*

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Dedicated to my Family

&

Mentor

PREFACE

The present thesis entitled ***"Evaluation of antidiabetic activity of plant extract on streptozotocin induced diabetic rats"*** comprises the work done by the author in Division of Pharmacology, Department of Pharmaceutical Technology, Jadavpur University, Kolkata, for the degree of Master of Pharmaceutical Technology. The immense wealth of the plant kingdom has become a target for the search of new drugs and lead compounds by drug companies. Their easy availability, low toxicity, lesser or almost minimal side effects has prompted us to apply medicinal plants in therapeutic management of different diseases. The traditional uses need scientific background for proper value and so they are currently an important part of research. Thus the thesis covered the above mentioned study in a logical sequence with relation to the other factors related to the study. In conclusion, the detailed study has been linked up in a manner to justify the relation of the work to establish the pharmacological actions, especially anti diabetic activity.

Date: Poulami Majumder

CONTENTS

Introduction

INTRODUCTION

Diabetes is a metabolic disorder of multiple etiologies characterized by chronic hyperglycemias with disturbance of carbohydrate, fat, and protein metabolism.This can be because of the body's inability to produce enough insulin due to destruction of pancreatic beta cells, beta cell deficiency or because cells do not respond to the insulin that is produced. Diabetes is considered as one of the most common public health problems which has already affected a vast population worldwide and will continue to do so in the near future. **"Diabetes**" is a Greek word meaning" to siphon" or pass through and **"mellitus"**, a Latin term which stands for "honeyed or sweet". This name has emerged from the observation that patients with diabetes mellitus produce urine which has been found to contain sugar. This makes it different from **Diabetes Insipidus** which results in huge volumes of dilute urine due to deregulation of response to Antidiuretic Hormone (ADH).Diabetes mellitus is also related to our lifestyle habits and genetic factors. Improper modes of living like, sedentary lifestyle, fast food consumption, lack of exercise leads to obesity which in turn is one of the main cause behind this disease. Pre-diabetes is a condition where a person's blood glucose levels are higher than normal but not high enough to be diagnosed as type 2 diabetes. Sustained hyperglycemia has been shown to affect almost all tissues in the body and is associated with significant complications of multiple organ systems, including the eyes, nerves, kidneys and blood vessels. It has emerged as the major cause of adult morbidity and mortality worldwide

Hyperglycemia occurs because of uncontrolled hepatic glucose output and reduced uptake of glucose by skeletal muscle with reduced glycogen synthesis. When renal threshold of glucose is exceeded it leads to glycosuria (glucose in urine), results in osmotic dieresis (polyuria), which in turn results in dehydration, thirst and increased drinking (polydypsia).(Kumar *et al*., 2012). The pancreas is an elongated organ situated next to the first part of the small intestine. It is both an endocrine gland that produces hormones, such as insulin and glucagon, and an

exocrine gland producing digestive enzymes, such as trypsin and chymotrypsin (Martini, 2004)

The islets of Langerhans have four types of hormone secreting cells. These are the: Alpha cells - secrete glucagon; Beta cells - secrete insulin; Delta cells - secrete gastrin; F cells - produce pancreatic polypeptide. These cells within an islet are not randomly distributed; beta cells occupy the central portion of the islet and are surrounded by alpha, delta and F cells. Insulin is a polypeptide hormone (Porth, 2005). The synthesis of insulin takes place in the beta cells and is stored in granule form in the pancreas (Bonk, 1999).

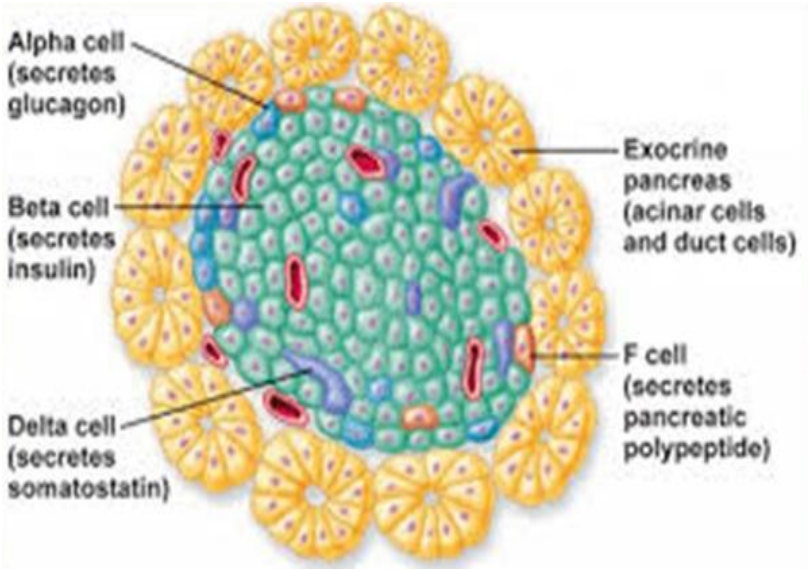


Figure 1.1: Different secretory cells of pancreas

Types of Diabetes Mellitus

TYPE 1 Diabetes

It results from the body's failure to produce insulin and also referred to as insulin-dependent diabetes mellitus, IDDM for short, and juvenile diabetes. There are two possible causes of Type1. One is Immune Mediated which involves cell mediated auto immune destruction of pancreatic beta cells and another may be due to inheritance.

Pathophysiology of Type 1

The autoimmune destruction of pancreatic β-cells, leads to a deficiency of insulin secretion which results in the metabolic derangements associated with IDDM. In addition to that, the function of pancreatic α-cells is also abnormal and promotes excessive secretion of glucagons in IDDM patients. Normally, hyperglycemia leads to reduced glucagon secretion, however, in patients with IDDM, hyperglycemia do not suppress glucagon secretion (Raju and Raju, 2010). The resultant inappropriately elevated glucagon levels aggravates the insulin deficiency mediated metabolic defects. The most pronounced example of this metabolic disruption is that patients with IDDM rapidly develop diabetic ketoacidosis in the absence of insulin administration. Although insulin deficiency is the primary defect in IDDM, there is also a defect in the administration of insulin. Insulin deficiency leads to uncontrolled lipolysis and elevated levels of free fatty acids in the plasma, which suppresses glucose metabolism in peripheral tissues such as skeletal muscle (Raju and Raju, 2010). This impairs glucose utilization and insulin deficiency also decreases GLUT 4 class of glucose transporters in adipose tissue. explained that the major metabolic derangements, which result from insulin deficiency in IDDM namely impaired glucose, lipid and protein metabolism.(Raju and Raju ,2010)

TYPE 2 Diabetes

Type 2 is a condition where the synthesis and secretion of insulin by the islets of Langerhans is diminishing or when there is insulin resistance in spite of too much availability of insulin. The onset tends to be slow and therefore may not be detected in the early stages of the disease. Obesity and lack of exercise are the most common causes of insulin resistance gradually leading to the onset of type 2 diabetes. The age of onset for the disease is becoming more common in those over 50 years (Kumar and Clark, 2005), and in children and young

people. Impaired insulin action affects fat metabolism, resulting in increased free fatty acid flux and triglyceride levels, and reciprocally low high-density lipoprotein (HDL) levels.

Pathophysiology of Type 2

The individuals with impaired glucose tolerance have hyperglycemia inspite of having highest levels of plasma insulin, indicating that they are resistant to the action of insulin. In the progression from impaired glucose tolerance to diabetes mellitus, the level of insulin declines indicating that patients with NIDDM have decreased insulin secretion. Insulin resistance is the primary cause of NIDDM, however some researches contain that insulin deficiency is the primary cause because a moderate degree of insulin resistance is not sufficient to cause NIDDM (Raju and Raju, 2010). Most patients with the common form of NIDDM have both defects. Recent evidence has demonstrated a role for a member of the nuclear hormone receptor super family of proteins in the etiology of type 2 diabetes

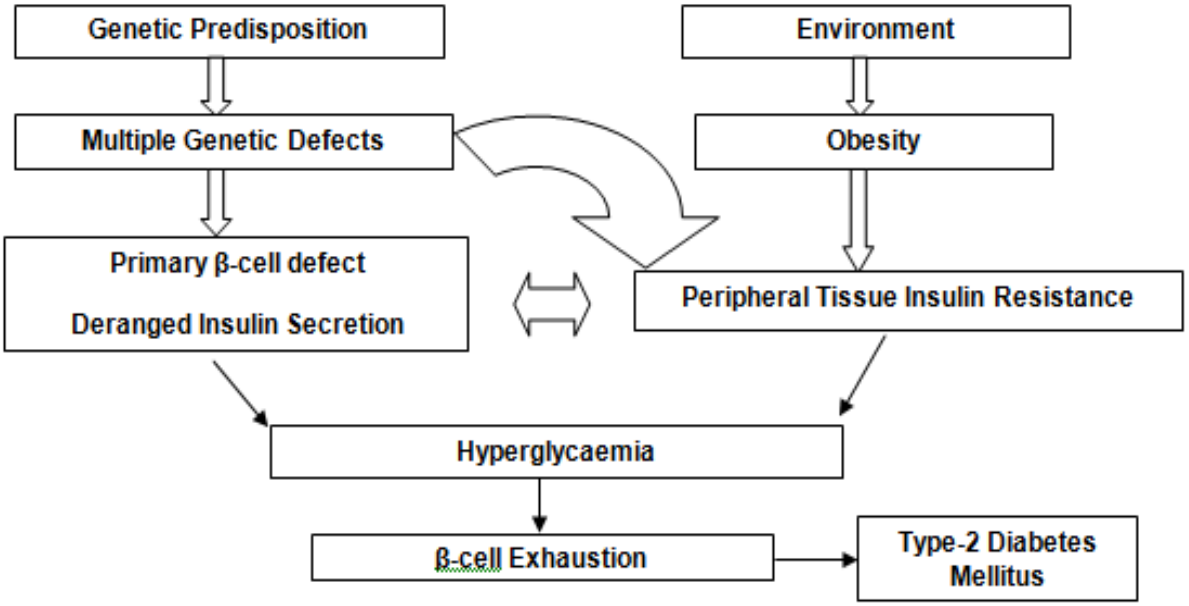


Figure 1.2: Etiology of Type 2 Diabetes Mellitus

Table 1.1: Difference between type 1 and type 2 diabetes mellitus

POINTS	**TYPE 1**	**TYPE 2**
Etiology	Autoimmune destruction of pancreatic beta cells	Insulin resistance with inadequate beta cell function
Insulin levels	Absent or negligible	Typically higher than normal
Age of onset	Typically <30 years	Typically >40 years
Acute complications	Ketoacidosis, wasting	Hyperglycaemia
Chronic complications	Neuropathy, retinopathy, Nephropathy, Peripheral vascular diseases, coronary artery disease	Neuropathy, retinopathy, nephropathy peripheral vascular diseases, coronary artery disease
Pharmacologic interventions	Insulin	Oral hypoglycaemic, insulin preparations

Type 3 Diabetes

The type 3 designation refers to multiple other specific causes of elevated blood glucose: non pancreatic diseases, drug therapy, etc.

Type 4 Diabetes

This type of diabetes occurs in pregnant women, i.e. **Gestational Diabetes**. During pregnancy, the placenta and placental hormones create an insulin resistance that is most pronounced in the last trimester. Risk assessment for diabetes is suggested starting at the first prenatal visit.

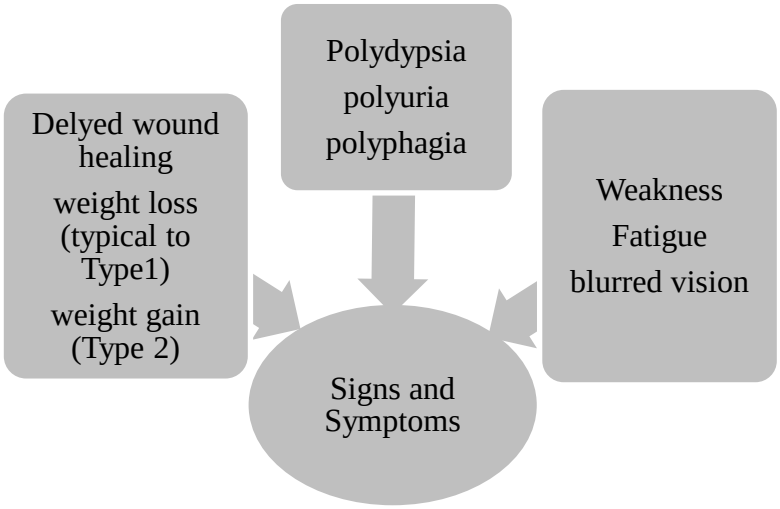


Figure 1.3: Signs and symptoms of Diabetes Mellitus

Table 1.2: Complications

Macro vascular	**Micro vascular**
cerebrovascular	retinopathy
Cardiovascular	nephropathy
Peripheral vascular	neuropathy

Diagnosis of diabetes

(A) Measurement of blood glucose

The fasting plasma glucose greater than 7.0 mmol/l (126 mg/dl) or plasma glucose greater than 11.1 mmol/l (200 mg/dl) indicates that the person may have diabetes. Glucose should be measured immediately after collection by near patient testing, or if a blood sample is collected, plasma should be immediately separated, or the sample should be collected into a container with glycolytic inhibitors and placed on ice-water until separated prior to analysis.

(B) Oral glucose tolerance test (OGTT)

OGTT is used as a diagnostic test as fasting plasma glucose alone fails to diagnose approximately 30% of cases.

(C) GlycatedHaemoglobin (HbA1c)

HbA1c can be used as a diagnostic test for diabetes providing that stringent quality assurance tests are in place and assays are standardized to criteria aligned to the international reference values, and there are no conditions present which preclude its accurate measurement. An HbA1c of 6.5% is recommended as the cut point for diagnosing diabetes. A value of less than 6.5% does not exclude diabetes diagnosed using glucose tests. Currently HbA1c is not considered a suitable diagnostic test for diabetes or intermediate hyperglycemia.

(D) Fasting blood glucose test

Blood glucose levels are checked after fasting for between 12 and 14 hours. Water can be taken during this time, but should strictly avoid any other beverage. Patients with diabetes may be asked to delay their diabetes medication or insulin dose until the test is completed.

(E) Random blood glucose test

Blood glucose levels are checked at various times during the day, and it doesn't matter the time of eating. Blood glucose levels tend to stay constant in a person who doesn't have diabetes.

Current Scenario of Diabetes

Its increasing prevalence is a major issue because diabetes is a complex and multi factorial origin disease due to population growth, aging, urbanization and an increase of obesity and physical inactivity. Unlike in the West, where older persons are most affected, diabetes in Asian countries is disproportionately high in young to middle-aged adults. This could have long-lasting adverse effects on a nation's health and economy, especially for developing countries. IDF Diabetes Atlas estimates that there are about 382 million people living with diabetes all over the world. By 2035,the number will increase to 592 million or one person in ten. More than that currently 316 million people are at high risk of developing type 2 diabetes, with the number expected to rise up to 500 million .Its undiagnosed nature is gradually making this pandemic much more menacing. Nearly 80% of the affected people belong to middle- and low-income communities. Type 2 diabetes mellitus, which constitutes more than 95% of all the diabetic population, has a creeping onset with a long, asymptomatic phase. Of the top 10 countries/territories holding the largest number of diabetic adults, five of them are in Asia. China tops the list with 90.0 million and India follows with 61.3 million people affected by diabetes. India covers quite a large proportion of rural population and the recent reports indicate a rising prevalence of this disease in these areas too .This is primarily due to the rapid socioeconomic changes occurring there. These diseases make major contribution to early morbidity and mortality

Management of Diabetes

Management concentrates on keeping blood sugar levels as close to normal, without causing low blood sugar. This can usually be accomplished with a healthy diet, exercise, weight loss, and use of appropriate medications (insulin in the case of type 1 diabetes; oral medications, as well as possibly insulin, in type 2 diabetes).

Learning about the disease and actively participating in the treatment are important, since complications are far less common and less severe in people who have well-managed blood sugar levels The goal of treatment is an $HbA1_C$ level of 6.5%, but should not be lower than that, and may be set higher. Attention is also paid to other health problems those may accelerate the negative effects of diabetes. These include smoking, elevated cholesterol levels, obesity, high blood pressure, and lack of regular exercise. Specialized footwear is widely used to reduce the risk of ulceration, or re-ulceration, in at-risk diabetic feet. Evidence for the efficacy of this remains equivocal, however.

Lifestyle

People with diabetes can benefit from education about the disease and treatment, good nutrition to achieve a normal body weight, and exercise, with the goal of keeping both short-term and long-term blood glucose levels within acceptable bounds. In addition, given the associated higher risks of cardiovascular disease, lifestyle modifications are recommended to control blood pressure.

Medications

Medications are used to treat diabetes to lower blood sugar levels. There are a number of different classes of anti-diabetic medications. Some are available by mouth, such as metformin, while others are only available by injection like insulin. Type 1diabetes can only be treated with insulin, typically with a combination of regular and NPH insulin, or synthetic insulin analogs.

Metformin is generally recommended as a first line treatment for type 2diabetes, as there is good evidence that it decreases mortality. It works by decreasing production of glucose by the liver. Several other groups of drugs, mostly given by mouth, may also decrease blood sugar in type II DM. These include agents that increase insulin release, agents that decrease absorption of sugar from the intestines, and agents that make the body more sensitive to insulin. When insulin is used in type 2 diabetes, a long-acting formulation is usually added initially, while continuing oral medications.

Regular insulin has a relatively slow onset of action when given subcutaneously, requiring injection 30 minutes prior to meals to prevent delayed postmeal hypoglycemia. **Lispro, aspart,** and **glulisineinsulins** are analogs that are more rapidly absorbed, reach peak faster, and have shorter durations of action than regular insulin. This permits more convenient dosing within 10 minutes of meal (rather than 30 minutes prior), produces better efficacy in lowering postprandial blood glucose than regular insulin in type 1 DM, and minimizes delayed post meal hypoglycemia.

Table 1.3: Oral hypoglycemic and their side effects

Drug	Side Effects
Sulfonylureas, including first generation (e.g., tolbutamide) and second generation (e.g., glyburide) sulfonylureas.	Sulfonylurea therapy is usually associated with weight gain due to hyperinsulinemia.
Biguanides – metformin (e.g. Glucovance®)	Metformin reduces plasma glucose via inhibition of hepatic glucose production and increase of muscle glucose uptake. It also reduces plasma triglyceride and LDL-cholesterol levels.
Alpha-glucosidase inhibitors – acarbose (e.g. Precose®)	The major side effects are gas, bloating, and diarrhea.
Thiazolidinediones troglitazone (e.g. Resulin®), rosiglitazone (e.g. Avandia®) and pioglitazone®.	The major side effects are weight gain and an increase in LDL-cholesterol levels.
Meglitinides – Repaglinide (e.g. Prandin®)	Weight gain, gastrointestinal disturbances, and hypoglycemia are common side effects.

Table 1.4: Insulin analogues

Type of Insulin	Onset	Peak (hr)	Duration (hr)	Maximum Duration (hr)	Appearance
Rapid-acting					
Aspart	15–30 min.	1–2	3–5	5–6	Clear
Lispro	15–30 min.	1–2	3–4	4–6	Clear
Glulisine	15–30 min.	1–2	3–4	5–6	Clear
Short-acting					
Regular	30–60 min.	30–60 min.	3-6	6-8	Clear
Intermediate-acting					
NPH	2–4 hr	4–6	8–12	14–18	Cloudy
Long-acting					
Glargine	4–5 hr	-	22–24	24	Clear
Detemir	2 hr	6–9	14–24	24	Clear

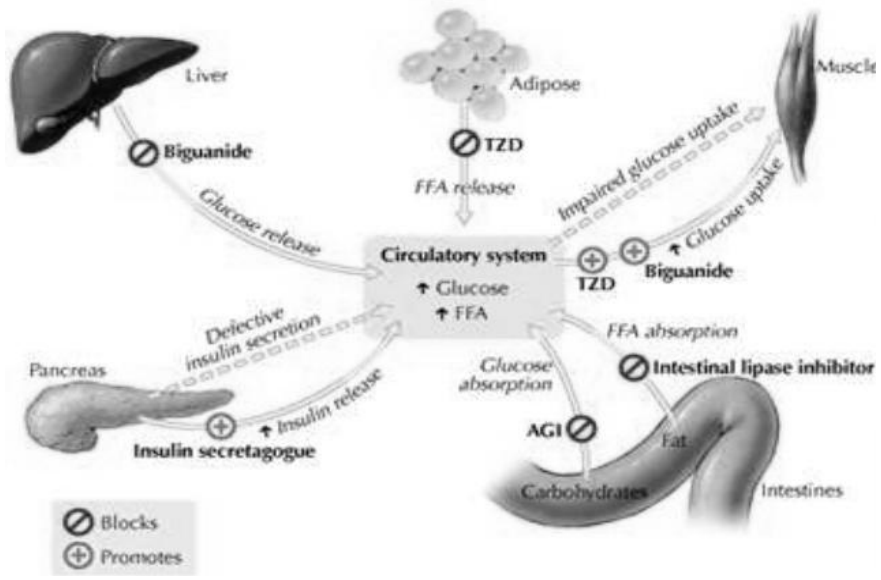


Figure 1.4: Mechanism of action of oral hypoglycemics

Newer Approaches in Antidiabetic Treatment

A new perspective, even a paradigm change, has recently been brought forward by two new classes called incretin hormones and incretin enhancers. Incretins are defined as being responsible for a higher insulin response to oral intake of glucose compared with an equal intravenous glucose load (i.e., reaching equivalent plasma glucose levels) .One such mode of

treatment is GLP-1 based Incretintherapy which includes GLP-1 Analogues and DPP- 4 Inhibitors.

GLP-1 Analogues

Exenatide: Long acting analogue of GLP-1 that was originally isolated from a reptile. It acts as an agonist at human GLP- 1 receptors.The drug must be injectd twice daily and is used in combination with metformin, thiazolidinediones to improve glucose control.It increases secretion of insulin in a glucose dependent manner, suppresses secretion of glucagon,slows gastric emptying .It has some adverse effects like nausea and even pancreatitis.

DPP-4 Inhibitors: These classes of drugs stabilize endogenous GLP-1 and induce insulin secretion in a glucose dependent manner.This increases the circulating levels of endogenous GLP -1 leading to increased secretion of insulin, insulin biosynthesis and inhibits glucagon release. Examples: sitagliptin, saxagliptin, dulogliptin, etc.

Other approaches include

Amylin analogues

Pramlintide: A stable analogue of human amylin,the beta cell hormone that is co-secreted with insulin and helps regulate postprandial glucose levels. It slows gastric emptying, reduces post prandial glucagon and promotes satiety.It can be used in both type 1 and type diabetes mellitus. It is not associated with hypoglycemia until and unless it is administered with other agents that can cause hypoglycemia.

These drugs, inspite of their therapeutic efficacies have certain shortfalls which provokes us to switch to natural ways of treatment.

Nature has always been the mastermind in helping human civilization to grow and flourish to what it is today since ancient times. Besides providing us with our daily needs it has also been the source of medicines, men have been utilizing different plant parts like leaves, roots, stem barks etc. to cure themselves from ailments. These have replaced by synthetic

compounds with the advancement of technology, research and modernization. These traditional approaches prove to be quite effective, easily approachable and popular in today's world but its drawbacks, side effects, contraindications, gradual resistance to diseases have tempted us to look back to the days of natural ways of treatment. Nature has huge collection of products which can be used in varied categories of diseases. It has been beneficial as analgesic, hepatoprotective, anti-inflammtory, antiulcer and in cuts and wounds, fever, cough and cold, diarrhoea, diabetes, cancer, anaemia, cardiac problems, etc. Herbal products or plant products are rich in phenolic compounds, flavonoids, terpenoids, coumarins and other constituents which show reduction in blood glucose level(C.N He *et a*l., M. Jung *et al.,* H.F Ji *et al*.,)

Table 1.5: Plants with antidiabetic activity

Plant Name	**Common Name**	**Parts Used**
Cassia auriculata	Tanner's cassia	flowers
Glycine max	Soya beans	seed
Aeglemarmelos	Golden apple	leaf, Seed, Fruit
Azadirachtaindica	Neem	leaf, Seed
Beta vulgaris	Beet root	whole plant
Allium sativum	Garlic	root
Withaniasomnifera	Winter cherry	leaf

In the following work, the pharmacological screening of *Curcuma caesia* has been studied with special emphasis on its antioxidant, antidiabetic, cytotoxic and anticonvulsant properties.

REFERENCES

Ramachandran A, Snehalatha C, Shetty AS. Trends in prevalence of diabetes in Asian countries. World Journal of Diabetes. 2012; 3(6): 110-7.

Bailey CJ. Antidiabetic drugs other than insulin. Encyclopaedia of Molecular Pharmacology. 2008: 116-25.

Carulli L, Rondinella S, Lombardini S, Canedi I, Loria P, Carulli N. Review article: diabetes, genetics and ethnicity: Aliment Pharmacology Therapy. 2005; (2):16-9.

Diagnosis and Classification of Diabetes Mellitus American Diabetes Association. Diabetes Care. 2010; 33 (1): 62–69.

Drucker DJ. The biology of incretin hormones. Cell Metabolism. 2006; 3: 153-65.

Handelsman Yehuda MD: A Doctor's Diagnosis: Prediabetes. Power of Prevention. 2009; 1(2): 1.

Joshi SR, Das AK, Vijay VJ. Challenges in diabetes care in India: sheer numbers, lack of awareness and inadequate control. Journal of the Associations of Physicians India. 2008; 56: 443-50.

Krentz AJ, Patel MB, Bailey CJ. New Drugs for type 2 diabetes mellitus. Drugs. 2008; 68: 2131-62.

Luna B, Feinglos MN. Oral Agents in the Management of Type 2 Diabetes Mellitus. American Family Physician. 2001; 63: 1747-56.

Nair M. Diabetes mellitus, part 1: physiology and complications. British Journal of Nursing. 2007; 16(3): 185-7.

Nathan DM, Turgeon H, Regans S.Relationship between Glycated haemoglobin levels and mean glucose levels over time. Diabetologia.2007; 50: 239-44.

Offermans S, Rosenthal W. Encyclopedia of Molecular Pharmacology, 2nd Edition. Berlin: Springer, 2009; 116 – 125.

Ozougwu JC, Obimba KC, Belonwu CD, Unakalamba CB. The pathogenesis and pathophysiology of type 1 and type 2 diabetes mellitus. Journal of Physiology and Pathophysiology. 2013; 4(4): 46-57

Parveen Kumar,Michael Clark.Kumar and Clark Clinical Medicine ,Saunders Ltd.; 6 edition, 2005; ISBN-10: 0702027634.

Raju, SM. and Raju, B.(2010) Illustrated Medical Biochemistry. 2nd Edition,Jaypee Brothers Medical Publishers Ltd., New Delhi.

RM Cohn and KS Roth. p 587. Williams and Wilkins, Baltimore. 1996 ISBN 0-683-02049-8. Biochemical Education, 25: 177–178. doi: 10.1016/S0307-4412(97)84445-7

Rother KI: Diabetes treatment–bridging the divide. The New England Journal of Medicine. 2007, 356(15): 1499-501.

Sacks DB, Bruns DE, Goldstein DE, Maclaren NK, McDonald JM, Parrott M. Guidelines and recommendations for laboratory analysis in thediagnosis and management of diabetes mellitus. Clinical Chemistry 2002, 48: 436-72.

Sandozi Tasneem TS. Newer insulins. International journal of pharmaceutical, chemical and biological sciences. 2013; 3(2): 242-6.

Unwin N, Whiting D, Guariguata L, Ghyoot G, Gan D (Eds). The IDF Diabetes Atlas, 5th edition. Brussels, Belgium: International Diabetes Federation. 2011: 7-12.

Vella F. (1997), Biochemistry and disease: Bridging basic science and clinical practice: By RM Cohn and KS Roth. p 587. Williams and Wilkins, Baltimore. 1996 ISBN 0-683-02049-8. Biochemical Education, 25: 177–178. doi: 10.1016/S0307-4412(97)84445-7

PLAN OF WORK


Materials
Procedure
Hypothesis
Conclusion
MARTIN
philipmartin.info

Thesis title: Evaluation of Antidiabetic potential of methanol extract of *Curcuma caesia* rhizome

- Collection and identification of *Curcuma caesia* rhizome
- Extraction of the selected plant
- Preliminary phytochemical study of the extract
- Determination of LD_{50} value (Acute Toxicity)
- Evaluation of *in vitro* anti diabetic potential
    - Alpha amylase inhibition assay
    - Alpha glucosidase inhibition assay
    - Glucose uptake by yeast cell method
- Evaluation of *in vivo* anti diabetic potential
    - Oral glucose tolerance test (OGTT)
    - Blood glucose level
    - Glycosylated haemoglobin (HbA1c)
    - Tissue antioxidant parameters
    - Serum biochemical parameters
    - Histopathology

Literature Review

Plant Description

Name : *Curcuma caesia*

Family : Zingiberaceae

Common Name : Hindi- Kali haldi Krishna kedar

Bengali- kala haldi

Assamese- kala haladhi

Nepali - Kaalo haledo

Mizo - Aihang, Ailaihang

Part used : Rhizomes; leaves

Use : The rhizomes are used in smooth muscle relaxant activity (Arulmozhi *et al*., 2006), hemorrhoids, leprosy, asthma, cancer, epilepsy, fever, wound, vomiting, menstrualdisorder, anthelmentic, aphrodisiac, inflammation, gonorrhoeal discharges, etc. (Sasikuma 2005), antioxidant scavenging activity (Karmakar *et al*., 2011), anxiolytic and CNS depressant activity (Karmakar *et al*., 2011), analgesic activity (Karmakar *et al.*, 2011), anticancer potential by regulating Bax/bcl2 protein expression. (Karmakar *et al*., 2011).

Geographical Distribution

Curcuma caesia, (**black turmeric)** is a perennial herb with bluish-black rhizome, native to North-East and Central India. Black turmeric is also sparsely found in the Papi Hills of East Godavari, West Godavari of Andhra Pradesh. The rhizome of black turmeric has a high economic importance owing to its putative medicinal properties. This species has been regarded as endangered by the central forest department of India due to biopiracy deforestation and unfavourable climate.

Morphology

These are tropical crops used as landscape plants and specially potted plants. A herbaceous and rhizomatous perennial plant composed of an upright pseudostem a corm (an ovate rhizome) underground cylindrical branches or rhizomes and fleshy roots. From March to April axillary buds of the crop and apical buds of the third order rhizomes emerge above the ground as inflorescence.

Phytochemical Distribution

The research on the volatile oil obtained from the rhizomes of *C.caesia* resulted in the identification of 30 component representing 97.48% of oil with camphor (28.3%), ar-turmerone (12.3%), (Z)-ocimene (8.2%), ar-curcumene (6.8%), 1, 8-cineole (5.3%), elemene (4.8%), borneol (4.4%), bornyl acetate (3.3%) and curcumene (2.82 %) as the major constituents.

Cultivation and Harvest

The cultivation and harvest practices are similar to that of common turmeric which is used in recipes. In the fields rhizomes are washed thoroughly and are placed in a wide mouthed cauldron. The water is poured in cauldron to sink the rhizomes. Rhizomes are boiled in covered cauldron until it starts foaming with strong odour. They are then taken out while water is reduced to one third of its content and when their inner portion gets decolourized from blue to dark or pale brown. Drying continues until they are hardened and are then packed for marketing.

a. *Curcuma caesia* whole plant

b. *Curcuma caesia* rhizome

REFERENCES

Nadkarni KM. Indian Material Medica. Vol. 1.; Popular Prakashan; Bombay ,1976. p 414.

Karmakar I. Pharmacological Screening of Curcuma caesia(Zingiberaceae).2011:10-1.

Karmakar I, Dolai N, Saha P, Sarkar N, Bala A, Haldar PK. Scavenging activity of *Curcuma caesia* rhizome against reactive oxygen and nitrogen species. Oriental Pharmacy and Experimental Medicine. 2011; 11:221–8

Karmakar I,Dolai N, Bala A,Haldar PK. Anxiolytic and cns depressant activities of methanol extract of *Curcuma caesia* rhizome. Pharmacologyonline 2. 2011:738-47.

Karmakar I, Saha P,Sarkar N,Bhattacharya S,Haldar PK.Neuropharmacological assessment of *Curcuma caesia* rhizome in experimental animal models. Oriental Pharmacy and Experimental Medicine. 2011; 11:251–5

Karmakar I, Haldar S, Chakraborty M, Chaudhury K, Dewanjee S, Haldar PK. Regulation of apoptosis through bcl-2/bax proteins expression and DNA damage by *Zanthoxylum alatum* . Pharmaceutical Biology.2016; 54(3):503-8.

COLLECTION, EXTRACTION AND CHEMICAL TEST

Collection Identification and Extraction

The rhizome of *Curcuma caesia* plant was collected from the upper hill region of Sikkim, India in the month of August, 2015. The plant species was identified and authenticated (SHRC-5/02/2012-TECH: 367) by Botanical Survey of India, Howrah, India. Air dried whole rhizomes (180 g) were powdered in a mechanical grinder and the plant materials were extracted by methanol using Soxhlet extraction apparatus. The solvent was completely removed under reduced pressure in a rotary evaporator. The concentrated extract was obtained by lyophilization and stored in vacuum desiccators (20°C) for further use. The yield of the methanol fraction was about 18.22%.

Qualitatiive Analysis

Preliminary qualitative analysis has been performed to know the type of compound present in the extract. Chemical group tests were performed for Alkaloids, Flavonoids Saponins, Tannins, Steroids .

Chemical Tests

Tests for Steroids

Liebermann- Burchard Test (Zhou *et al.*, 2004)

- 10 mg of extract was dissolved in 1ml of chloroform. 1 ml of Acetic Anhydride was added following the addition of 2 ml of concentrated sulphuric acid. Formation of reddish violet colour indicated the presence of steroids.

Salkowski Test (Bosila *et al., 2005*)

- 1 ml of concentrated sulphuric acid was added to 10 mg of extract dissolved in 1 ml of chloroform. A reddish blue colour exhibited by chloroform layer and green fluorescence by acid layer indicated the presence of steroid.

Test for Flavonoids (Saija *et al*., 1995)

- 5ml of extract solution was hydrolysed with 10% sulphuric acid and cooled. Thn it was extracted with diethyl ether and divided with three portions in three separate test tubes. 1ml of dilute ammonia 1ml of dilute sodium bicarbonate and 1ml of 0.1(N) sodium hydroxide were added to the first second and third test tube respectively. In each test tube development of yellow colour indicated the presence of flavonoids.
- The extract was dissolved in alcohol. One piece of magnesium followed by concentrated hydrochloric acid was added dropwise to that and heated. Appearance of magenta colour demonstrated the presence of flavonoids.

Test for Saponins (Sinha *et al* ., 1985)

- 1 ml solution of the extract was diluted with distilled water to 20 ml and shake in a graduated cylinder for 15 mins. Development of stable foam suggested the presence of saponins.
- 1 ml extract was treated with 1% lead acetate solution. Formation of white precipitate indicated the presence of saponins.

Test for Tannins (Segelman *et al* .,1969)

- 5 ml of extract solution was allowed to react with 1ml 5% ferric chloride solution. Greenish black colouration indicated the presence of tannins.
- 5 ml of extract was treated with 1 ml of 10% aqueous potassium dichromate solution. Formation of yellowish brown precipitate suggested the presence of tannins.
- 5 ml extract was treated with 1 ml of 10% lead acetate solution in water. Yellow coloured precipitation indicated the presence of tannins.

Test for Alkaloids (Raffauf *et al* ., 1962)

- 1.2 ml of extract was taken in a test tube. 0.2 ml of dilute hydrochloric acid and 0.1 ml of Mayer's reagent were added. Formation of yellowish buff coloured precipitate gives positive test for alkaloids.
- 0.1 ml of dilute hydrochloric acid and 0.1 ml of Dragendroff's reagent were added in 2 ml solution of extract in a test tube. Development of orange brown coloured precipitate suggested the presence of alkaloids.
- 2 ml of extract solution was treated with dilute hydrochloric acid and 0.1 ml Wagner's Reagent. Formation of reddish brown indicated the positive response for alkaloids.
- 2 ml of extract was allowed to react with 0.2 ml of dilute hydrochloric acid and 0.1 ml of Hager's reagent. A yellowish precipitate suggested the presence of alkaloids.

RESULTS

Extract	Steroid	Tannin	Flavonoid	Alkaloid
Methanol	+	+	+	+

(+) indicates the presence of Phytoconstituents

REFERENCES

Bosila HA, Mohamed SM, EI SF, IbrahimSIA. Phytochemical screening of some *in vivo* and *in vitro* date palm tissues: 2005:23-5.

Evans WC. Trease and Evan Pharmacognosy. 14th ed. London, Bailiere Tindall W.B. Saunders Company Ltd; 1996: 293 - 575.

Karmakar I, Dolai N, Bala A, Haldar PK. Anxiolytic and CNS depressant activities of methanol extract of *Curcuma caesia* rhizome. Pharmacologyonline 2.2011; 738-47

Parekh J, Chanda SV. *In vitro* antimicrobial activity and phytochemical analysis of some Indian medicinal plants. Turkish Journal of Biology. 2007; 31: 53-8.

Raffauf RF. A simple field test for alkaloid-containing plants. Economic Botany. 1962; 16: 171-2

Saija A, Scalese M, Lanza M, Marzulla D,Bonina F, Castelli F. Flavonoids as antioxidant agents:importance of their interaction with biomembranes. Free radical Biology and Medicine. 1995; 19:481-6.

Segelman AB, Farnsworth NR, Quimby MD. False-Negative saponins test results induced by the presence of tannins. The Journal of Biological Chemistry. 1969; 32(2): 52 – 8.

Sinha SKP, Dogra JVV. A Survey of The Plants of Bhagalpur and Santhal Pargana for saponin; Flavonoids and Alkaloids. Pharmaceutical Biology.1985; 23:77-86.

Zhou H, Li YS, Tong XT, Liu HQ, Jiang SH, Zhu DY. Serratane –type Triterpenoids from *Huperzia Serrata*. Natural Product Research. 2004; 18:453-9.

TOXICITY STUDY

The term toxicology derived from the word "toxion" means poison and"logos"means science. Toxicology is the science which deals with the harmful effects of chemicals and drugs on living organism. It helps us to determine the quality and quantity of poison.

The potential uses of toxicity testing data include:

- Establishing the therapeutic dose.
- Acquiring information about the harmful effects on specific organs.
- Gathering information about the mode of toxic action .
- Use as a reference standard.

Acute Toxicity Studies (single dose)

These are conducted to determine the median lethal dose (LD_{50}) i.e. the dose which will kill 50% of the animals of a particular species. In addition such studies may also indicate the probable target organ of the chemical and its specific toxic effect. It provides guidance on the doses to be used in prolonged studies. Acute toxicity tests form part of a programme of toxicity testing that provide the basis on which further testing programmes are designed.

Sub-Acute Toxicity Studies (Daily Dose)

This study is conducted to determine the organs affected by different dose levels. It access the nature of toxic effects under more realistic solutions than the acute toxicity studies. The purpose of this test is to determine the maximum tolerated dose and to indicate the nature of toxic reactions, so that suitable chronic toxicity studies can be designed to evaluate fully the toxic potentiality of compound.

Long-term Toxicity Studies (Chronic Toxicity Studies)

The animals are exposed over a long period of time to the toxic effects of the drug in order to mimic more realistic solutions. On the basis of information obtained in sub-acute toxicity studies, the main aim of these long-term studies is to determine the organs affected and

determine whether the drug is potentially carcinogenic or not. These tests may be conducted concurrently with the initial studies in human (phase1clinical trials).

Various Methods of LD_{50} Determination

Litchfield and Wilcoxson Graphic Method (Litchfield et al., 1949)

Here different doses are given to different groups of animals and mortality is to be recorded. Then graph is prepared with percentage of mortality vs. log dose.

Miller Unitary Method (Pulgarin M et al., 2003)

Here any dose is taken say 1mg or 1ml or 1gm or 1mg/ml. The unit dose is given to two mice and observed for 24 hrs. If the dose is tolerated by the animals the dose is modified by multiplication with 3/2. This has to be carried out until a dose causes a death of one animal i.e. 50% death as two animals were used. However this result is not reliable until and unless it is verified with more number of animals. But the method is ease less time consuming and economical.

Up and Down Method (Shetty JA et al., 2007)

OECD (Organisation for economic co-operation and development) guidelines for the testing of chemicals are periodically reviewed in the light of scientific progress or changing assessment practices. The concept of the Up and Down testing approach was first described by Dixon and Mood. In 1985 Bruce proposed to use an up- and- down (UDP) procedure for the determination of acute toxicity of chemicals. There exist several variations of the UDP experimental design for estimating LD_{50}. The method applies for materials that produce death within one or two days. The method is not practical when considerable delayed death is expected. A limit test can be used to identify chemicals that are likely to have low toxicity. The test uses a maximum of 5 animals. A test dose up to 2000 mg/kg or exceptionally 5000mg/kg can be used. The main test consists of a single ordered dose progression in which animals are dosed one at a time at 48 hours interval. The first animal receives a dose a step

below the level of the best estimate of LD_{50}. If the animal survives the dose for the next animal is increased to a factor of 3/2 of original dose but if it dies the dose for the next animal is decreased by a similar dose progression. Each animal should be observed carefully for 48 hours before making decision on the next dose to be given to the next animal. Dosing is stopped when one of the criteria is satisfied i.e. an estimate of LD_{50} and a confidence interval are calculated for the test based on the status of all animals at termination.

Method Used

Acute Toxicity Study

Animals

10 healthy Swiss Albino mice weighing 20-25g were taken in each group and were kept in poly acrylic cages (38cm x10cm) with not more than six animals in each cage. They were maintained under standard laboratory conditions with sufficient food and water ad libitum.

Procedure

As per reported method (Organization for Economic Co-operation and Development 420) fasted animals of single sex were dosed in a stepwise procedure using the fixed dose of 5, 50, 300 and 2000 mg/kg orally (OECD 2000). All the animals were observed for any signs of toxicity or mortality at least 24 hr. All the animals were subjected for sharp observation for a period of 14 days. MECC showed no mortality or toxic effect up to 2000 mg/kg body weight in mice.

RESULT: No deaths were observed when the animals were given a dose of 2000 mg/kg b.w.

DISCUSSION: The index of acute toxicity is LD_{50}. Oral administration of methanol extract of *Curcuma caesia* (MECC) in mice at doses up to 2000 mg/kg did not produce any significant change in behavior, breathing, cutaneous effects, sensory nervous system responses or gastrointestinal effects. During the experiment period no deaths were observed. So from the results it is concluded that MECC is safe up to dose of 2000 mg/kg.

REFERENCES

Litchfield JT, Wilcoxon, F. A simplified method of evaluating dose-effect experiments . Journal of Pharmacology and Experimental therapeutics. 1949; (96):99-113

OECD (2000). Guidance Document on Acute Oral Toxicity. Environmental Health and Safety Monograph Series on Testing and Assessment No.24.

Pulgarin M, Bermejo G. Determination of napropamide in technical formulations, soil and vegetable samples by sensitized fluorescence: validation of the method. Analytica Chimica Acta. 2003; 491(1):37-45

Saha M, Ghosh D, Ghosh D, Garai D, Jaisankar P, Sarkar KK. Studies on the production and purification of an antimicrobial compound and taxonomy of the producer isolated from the marine-environment of the sudarbans. Applied Microbiology and Biotechnology. 2005; 66:497-505.

Shetty JA.Acute toxicity studies and determination of median lethal dose. Current Science. 2007; 93(7):917-20

IN VITRO STUDY

Current management of diabetes includes observation and estimation of *in vivo* biological parameters in diabetes induced rats. Besides these *in vivo* parameters there are also some *in vitro* assay methods to evaluate the antidiabetic potential of the plant extract. Few such *in vitro* assays include enzyme inhibition and glucose uptake by yeast cells. Carbohydrates are normally converted into simple sugars (monosaccharide), which are absorbed through the intestine. So one of the antidiabetic therapeutic approaches is to reduce gastrointestinal glucose production and absorption. Alpha amylase and glucosidase enzymes digest carbohydrates and increase the post prandial blood glucose after a mixed carbohydrate diet. Therefore blood glucose can be controlled by inhibiting these carbohydrate digesting enzymes such as α- amylase and α-glucosidase. Alpha amylase is an enzyme, found in many tissues but mostly found in pancreatic juice and saliva. Salivary amylase is better known as "ptyalin". Alpha amylase hydrolyses the alpha bonds of large alpha linked polysaccharides such as starch and glycogen yielding glucose and maltose. α (1,4 glycosidic) linkage of starch is the major site of action. Starch is broken down into disaccharide like maltose which is further broken down to simpler monosaccharide like glucose. Alpha-glucosidase enzymes in the brush border of the small intestines also digest carbohydrates. Alpha-glucosidase inhibitors act as competitive inhibitors of this enzyme and hamper digestion of carbohydrates. These membrane-bound intestinal glucosidases hydrolyze oligosaccharides, trisaccharides and disaccharides to glucose and other monosaccharide in the small intestine. α-glucosidase Inhibitors (Acarbose) which act as competitive inhibitors of intestinal α (-)glucosidase can delay the digestion and subsequent absorption of elevated blood glucose levels. The different concentrations of extract were pre-incubated with the enzyme before adding the substrate p-Nitrophenyl-α-d glucopyranoside (PNPG). α-glucosidase activity was measured by determining the color developed by the release of p-nitrophenol arising from the hydrolysis of substrate PNPG by α –glucosidase using spectrophotometric method. Glucose transport

across the yeast cell membrane is based on the principle of Facilitated Diffusion down the concentration gradient. Glucose transport occurs only after intracellular glucose is effectively utilized (reduced). Therefore the aim of our desired plant extract should be enhancement of effective glucose utilization thereby controlling blood glucose level.

Materials and Methods

In vitro α-amylase inhibitory activity (Hossan *et al.*, 2009)

This study was performed by a modified starch iodine protocol. In short, 1 mL of plant extract or standard (Acarbose) of different concentration (100, 300,500 and 1000 µg/mL) was taken in prelabeled test tubes. A volume of 20 µL of α-amylase was added to each test tube and incubated for 10 min at 37 °C. After the incubation 200 µL of 1% starch solution was added to each test tube and the mixture was re-incubated for 1 h at 37 °C. Then 200 µL of 1% iodine solution was added to each test tube and after that, 10 mL distilled water was added. Absorbance of the mixture was taken at 565 nm. Sample, substrate and α-amylase blank were undertaken under the same conditions. Each experiment was done in triplicate. IC_{50} value was calculated by using regression analysis.

% α-amylase inhibition= [1- {(SA-SBB)-SMB}/ AAB] x 100

where SA=Sample absorbance, SMB=Sample blank, SBB=Substrate blank, AAB=α-Amylase blank

α-glucosidase inhibition assay (Kim *et al.*,2011)

α-glucosidase (50 µl, 0.5 U/ml) and 0.2 M K3PO4 buffer (pH 6.8, 50 µl) were mixed with 50 µl of the test sample. After pre-incubation at 37 °C for 15 min, 3 mM PNPG (100 µl) was added. The enzymatic reaction was allowed to proceed at 37 °C for 10 min, and was stopped by the addition of 750 µl of 0.1 M Na_2CO_3 . The 4-nitrophenol absorption was measured at 405 nm using a spectrophotometer. A solution without sample was used as a control and a

solution without substrate was used as a blank. The antidiabetic drug acarbose was also assayed as a standard reference.

% inhibition of α-glucosidase =1 – {(Abs sample – Abs blank)/Abs control} × 100%

where Abs sample represents the absorbance of the sample Abs blank is the absorbance of the blank and Abs control is the absorbance of control.

Glucose uptake by Yeast cells (Abirami *et al.*, 2014)

Yeast suspension was prepared by repeated washing (by centrifugation 3,000×g; 5 min) in distilled water until the supernatant fluids were clear. A 10% (v/v) suspension was prepared with the supernatant fluid. 1mL of glucose solution (5, 10 and 25 mM) was added to various concentrations of methanol extract (100, 300, 500 and 1000 µg) and incubated for 10 min at 37 °C. Reaction was started by adding 100 µl of yeast suspension, vortexed and further incubated at 37 °C for 60 min. After 60 min, the reaction mixture was centrifuged (2,500×g, 5 min) and glucose was estimated in the supernatant. Metformin was taken as reference drug. The percentage increase in glucose uptake by yeast cells was calculated using the following formula.

%inhibition = {(Abs Sample – Abs Control)/Abs Sample} x 100 Where, Abs control is the absorbance of the control reaction (containing all reagents except the test sample) and Abs sample is the absorbance of the test sample.

Statistical Analysis

All the results are shown as mean ±SEM. The results were analyzed for statistically significance by one-sway analysis of variance (ANOVA) followed by post hoc Dunnett's test using Graph Pad Prism 5.0 software (Graph Pad Software, USA). *p* values of < 0.05 were considered as statistically significant.

RESULTS

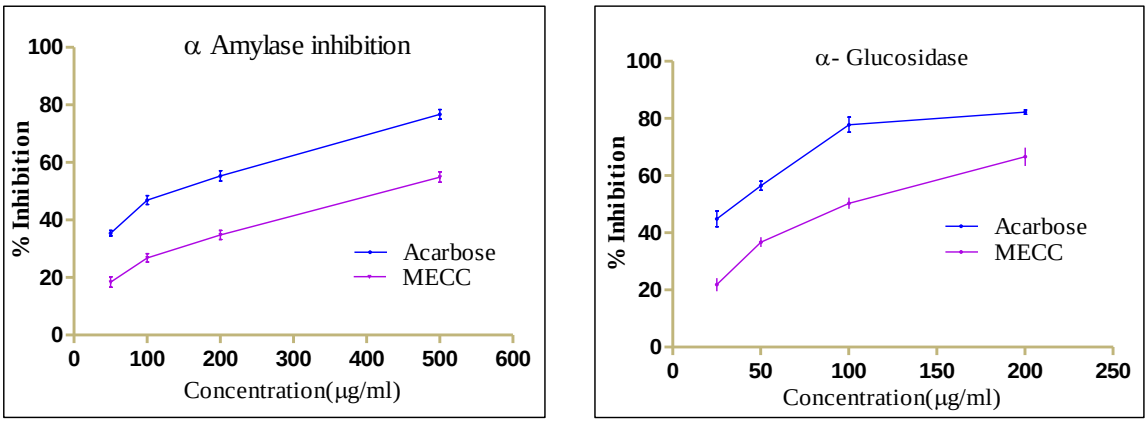


Figure 6.1: % inhibition of α-amylase and α- glucosidase shown by different concentrations of MECC and Acarbose.

Table 6.1: % inhibition of Alpha amylase by MECC and Acarbose

Concentration(µg/ml)	MECC	Acarbose
50	18.41±3.06	35.40 ± 1.62
100	26.82 ±2.55	46.89 ±2.64
200	34.81 ±2.71	55.33± 3.05
500	54.91 ± 3.07	76.69 ±2.93

Each value expressed as Mean ± SEM

Table 6.2: % inhibition of Alpha glucosidase by MECC and Acarbose

Concentration(µg/ml)	MECC	Acarbose
25	21.84±3.97	44.89±4.79
50	36.74±2.98	56.47±2.72
100	50.31±3.40	77.83±4.54
200	66.58±5.53	82.20±1.27

Each value expressed as Mean ± SEM.

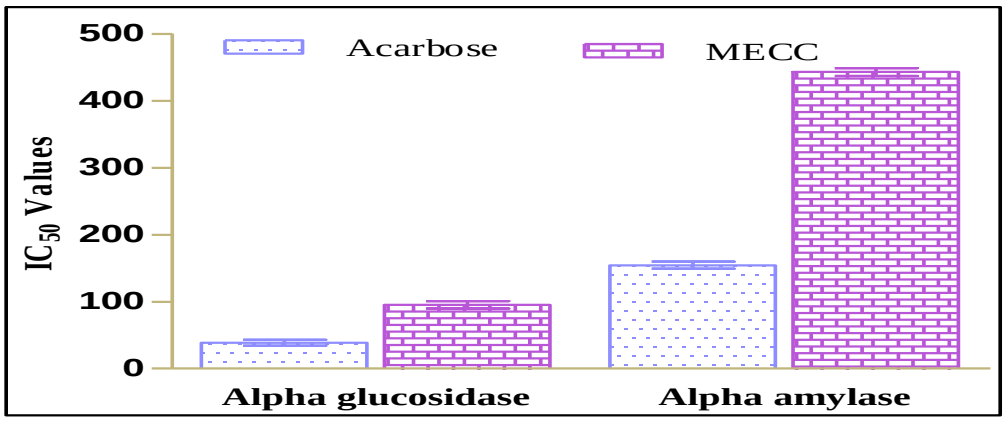


Figure 6.2: IC_{50} values of Acarbose and MECC in alpha glucosidase and alpha amylase.

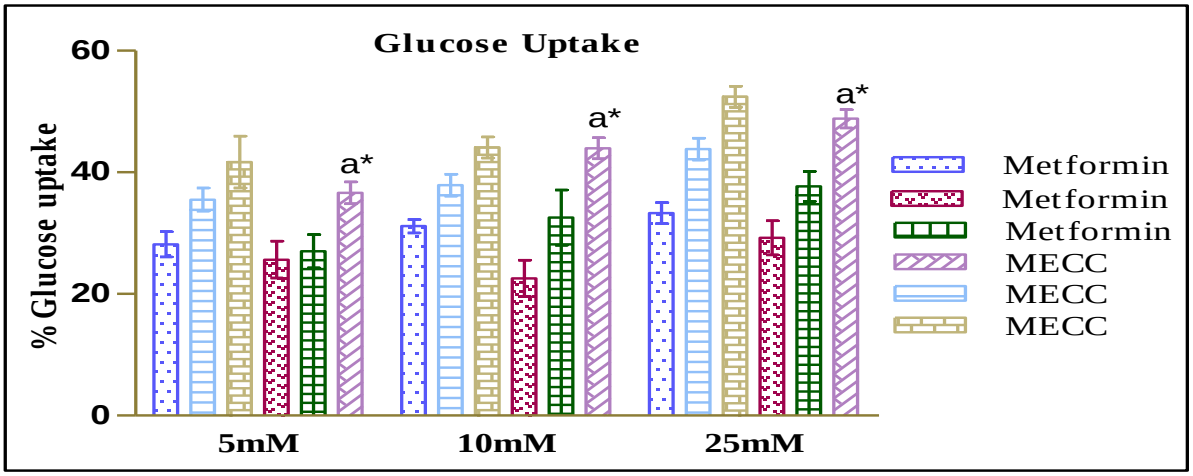


Figure 6.3: % Glucose uptake in Yeast cells as shown by different concentrations of MECC and Metformin.

Table 6.3: % Glucose uptake in yeast cells by MECC and Metformin.

Concentration of glucose (mM)	Concentration (µg/ml)	MECC	Metformin
5	100	25.66 ± 5.21	28.16 ± 3.61
	300	27.02 ± 4.72	35.51 ± 3.26
	500	36.62 ± 3.12	41.69 ± 7.36
10	100	22.55 ± 5.18	31.14 ± 1.91
	300	32.56 ± 7.90	37.91 ± 3.11
	500	43.96 ±3.00	44.07 ± 3.00
25	100	29.23 ± 4.85	33.30 ± 2.95
	300	37.70 ± 4.28	43.82 ± 3.08
	500	48.81 ± 2.62	52.45 ± 3.01

Each value expressed as Mean ± SEM.

The alpha-amylase inhibition study was conducted with different concentrations of MECC and acarbose (50, 100, 200, 500 µg/ml) and the alpha glucosidase inhibition study was conducted with different concentrations of MECC and Acarbose (25, 50,100,200 µg/ml). In alpha amylase, IC_{50} value of MECC was found to be 442.92 ±10.05 µg/ml when compared to that of IC_{50} value of acarbose which was 154.33 ± 9.08 µg/ml. In alpha glucosidase IC_{50} value of MECC was found to be 95.40 ± 9.74 µg/ml when compared to that of acarbose which was 38.63 ± 8.05 µg/ml. The glucose uptake in yeast cell was carried out at different concentrations (5, 10, 25 mM) of glucose by different concentrations of MECC (100, 300,

500 µg/ml) and Metformin (100, 300, 500 µg/ml). The increased uptake of glucose by cells was observed and found to be significant $p<0.05$.

DISCUSSION

The present study aimed to evaluate the *in vitro* anti hyperglycemic activity of methanol extract of *Curcuma caesia* rhizome (MECC). The treatment goal of diabetic patients is to maintain near normal levels of glycemic control, in both fasting and post- prandial conditions. Many natural sources have been investigated with respect to suppression of glucose production from the carbohydrates in the gut or glucose absorption from the intestine (Mastsui *et al*., 2001). The parameters checked were enzyme inhibition capabilities and effect on glucose uptake by yeast cells. Activities of enzymes like alpha-amylase and alpha glucosidase in the body are responsible for postprandial hyperglycemia by break down of dietary carbohydrates to glucose. Hence, the inhibitory effect of extract on these enzymes may lead to reduction in post prandial hyperglycemia in diabetes. Postprandial hyperglycemia has been proposed as an independent risk factor for coronary vascular disease. Therefore, control of postprandial hyperglycemia is considered to be important in the treatment of diabetes and prevention of complications related to diabetes. The results showed that significant inhibition of alpha-amylase and alpha-glucosidase activity and increased glucose uptake by the cells.

REFERENCES

Abirami N, Natarajan B , Sagadevan E. Phytochemical investigation and *in vitro* evaluation of hypoglycemic potential of *Grewia hirsute*. International Journal of Pharma and Bio Sciences. 2014; 5(1):76 – 83.

Adisa RA, Oke J, Olomu SA, Olorunsogo O. Inhibition of human haemoglobin glycosylation by flavonoid containing leaf extract of *Cnestis ferruginea*. Journal of the Cameroon Academy of Sciences.2004; 4: 351-9.

Afifi AF, Kamel EM, Khalil AA, Foaad MA, Fawziand EM, Houseny M. Purification and characterization of alpha-amylase from penicillium olsonii under the effect of some antioxidant vitamins. Global Journal of Biotechnology and Biochemistry. 2008; 3: 14-21.

Halvorson H. Methods in enzymology.Academics Press, New York, 1966; 8: 559-62

Hossan SJ, El-Sayed M , Aoshima H. Antioxidative and anti α-amylase activities of four wild plants consumed by nomads in Egypt. Oriental Pharmacy and Experimental Medicine. 2009; 9(3): 217-224.

Kim JS, Hyun TK, Kim MJ .The inhibitory effects of ethanol extracts from sorghum, foxtail millet and proso millet on α-glucosidase and α-amylase activities. Food Chemistry.2011; 124: 1647–1651.

Kobold U, Jeppsson JO, Dulffer T, Finke A, Hoetzel W, Miedema K.Candidate reference method for hemoglibin A1c based on peptide mapping.Clinical Chemistry 1997; 43: 1944-1951.

Kwon YI, Apostolidis E , Shetty K. Evaluation of pepper (*Capsicum annuum*) for management of diabetes and hypertension. Journal of Food Biochemistry. 2007; 31(3): 370 -85.

Manohar V, Talpur NA, Echard BW, Lieberman S, Preuss HG.Effects of a water-soluble extract of maitake mushroom on circulating glucose/insulin concentrations in KK mice. Diabetes, Obesity and Metabolism.2002; 4(1): 43–8.

Matsui T, Ogunwande IA,. Abesundara KJM, Matsumoto K.Anti-hyperglycemic potential of natural products.Mini-Reviews in Medicinal Chemistry.2006; 6(3): 349–56.

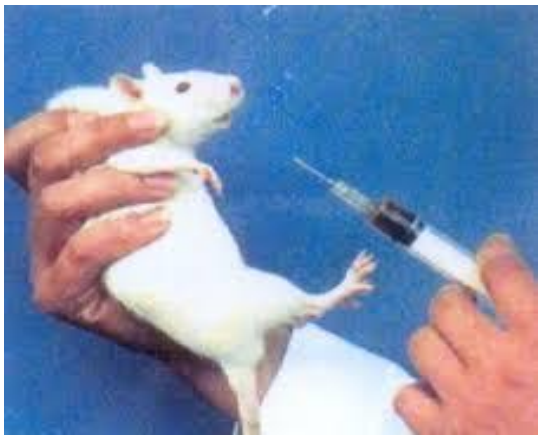
Matsui T, Ueda T, Oki T, Sugita K, Terahara N, Matsumoto K. Alpha-Glucosidase inhibitory action of natural acylated anthocyanins. 1. Survey of natural pigments with potent inhibitory activity. (2001). Journal of Agricultural and Food Chemistry. 49: 1948–51.

Narkhede MB, Ajimire PV, Wagh AE, Mohan M Shivashanmugam AT. *In vitro* antidiabetic activity of Caesalpina digyna (R.) methanol root extract. Asian Journal of Plant Science and Research, 2011, 1 (2): 101-6.

ShimY J, Doo HK, Ahn SY, Kim YS , Seong JK.Inhibitory effect of aqueous extract from the gall of *Rhus chinensis* on alpha-glucosidase activity and postprandial blood glucose. Journal of Ethnopharmacology.2003; 85: 283-7.

Tahara Y, Shima K. Kinetics of HbA1c, glycated albumin, and fructosamine and analysis of their weight functions against preceding plasma glucose level.Diabetes Care, 1995; 18: 440-7.

IN VIVO STUDY



In diabetes increased blood glucose level causes generation of free radicals which belong to ROS (Reactive Oxygen Species) and RNS (Reactive Nitrogen Species) family. The growing level of free radicals demand the necessity of antioxidants, whose inability to scavenge the free radicals leads to oxidative stress. Oxidative stress is the cause of various other degenerative diseases like cancer, rheumatoid arthritis, inflammation, Alzheimer's disease etc. The present chapter deals with the *in vivo* anti diabetic activity of MECC against streptozotocin induced rats.An antibiotic in nature, streptozotocin, destroys the beta cells of the pancreas on prolonged exposure and causes insulin deficiency which is a major factor in commencement of diabetes. The following study deals with various parameters which should be checked as a consequence of diabetes, including oral glucose tolerance test (OGTT), tissue antioxidant, serum parameters, blood glucose level, glcosylated haemoglobin and histopathology of the affected organs.

Materials and Methods

Animals

Healthy Wistar albino rats (weighing 180±10 g) of either sex were used in the study. Rats were kept in standard laboratory conditions *i.e*., 12 hr light/dark cycles at 25-28 °C, relative humidity 55–60 % and were fed with a standard pellet diet and water *ad libitum*. The animals were acclimatized to laboratory condition for one week prior to the experiment. All procedures described were reviewed and approved (ACE / PHARM /1502 /09 /2015) by University Animal Ethics Committee.

Drugs and chemicals

Streptozotocin was purchased from HI media Laboratories Pvt. Ltd. India and glibenclamide (reference drug) was from Sanofi India Ltd. India. Trichloroacetic acid (TCA) from Merck Ltd., Mumbai, India; thiobarbituric acid (TBA), 5,5´-dithio bis-2-nitro benzoic acid(DTNB), phenazonium methosulfate (PMS), nicotinamide adenine dinucleotide (NADH) and reduced

glutathione (GSH) from SISCO Research Laboratory, Mumbai, India; potassium dichromate, glacial acetic acid from Ranbaxy, Mumbai; and all the other reagents kits used were from Span Diagnostics Ltd. India.

Procedure:

OGTT:

- Animals treated with desired doses of plant extract
- Animals were fed glucose solution (2g/kg b.w)
- Blood glucose level was checked at intervals of 0, 30, 60,120,240 mins using one touch glucometer

***In vivo* study protocol outline:**

1. Streptozotocin, 55mg/kg b.w was induced i.p in rats
2. Body weight and blood glucose were checked at 0, 5, 10, 15 days.
3. Animals were sacrificed and tissue antioxidant, serum parameters, HbA1c were measured and histopathology of organs was studied.

Oral Glucose Tolerance Test (OGTT)

The OGTT was performed in overnight fasted Wistar albino rats. Rats were divided into three groups (n=6). Group I served as normal control (received distilled water 5 ml/kg b.w. p.o) groups II and III received MECC at the doses of 200 and 400 mg/kg b.w. p.o. respectively. All the animals received glucose solution (2 g/kg b.w., orally) 30 min after drugs administration. Blood sample was withdrawn from the tail vein at 0, 30, 60, 120 and 240 min. after glucose administration (Haldar *et al*., 2010) and blood glucose level was measured using single touch glucometer (Contour TS, Bayer Health Care USA).

Induction of diabetes in rats

The rats were rendered diabetic by a single intraperitoneal administration of streptozotocin [55mg/kg b.w dissolved in ice cold 0.1 M citrate buffer (pH 4.5)]. After 72 h, fasting blood glucose (FBG) levels were measured and only those animals showing blood glucose level ≥ 250 mg/dl were used for the present investigation (Ewart *et al*., 1975). The day on which hyperglycemia had been confirmed was designated as day 0 (Li *et al*., 2007; Schmatz *et al*., 2009).

Treatment schedule and Estimation of fasting blood glucose (FBG) level

After induction of experimental diabetes, the rats were divided into five groups (n=6). Except group I, which served as normal (non-diabetic) control, all other groups were comprised of diabetic rats. Group II served as diabetic (STZ) control. Groups III and IV received MECC (100 and 200 mg/kg b.w., p.o. respectively) and group V received reference drug glibenclamide (0.5 mg/kg b.w., p.o) daily for 14 days (Naskar *et al*., 2011).FBG level was measured on day 0th, 5th, 10th, and 15th by using a one touch glucometer. After 24th hour of last dose and 18 hours of fasting, blood was collected from all rats in each group by cardiac puncture for estimation of glycosylated hemoglobin (HbA1C), serum lipid profile and

serum biochemical parameters and then the animals were sacrificed for collection of liver tissue to check the different endogenous antioxidant parameters.

Body weight

Body weight of rats from each group was measured on day 0, 5, 10 and 15. Weight was measured using standard digital weight balance to get accuracy.

Estimation of glycosylated hemoglobin (HbA1c)

Glycosylated hemoglobin was analyzed by using commercially available kit (Beacon Diagnostic Pvt. Ltd. India) which is based on the principle of ion exchange resin method.

Estimation of liver biochemical parameters and antioxidant status

Livers and kidneys collected from the sacrificed animals were homogenized separately in 10 ml of phosphate buffer (20mM, pH-7.4) and centrifuged at 12000 rpm for 30 min at 4° C. The supernatants were collected and used for the following experiments as described below:

Lipid peroxidation level (TBARS)

Degree of lipid peroxidation in tissue homogenate was determined in terms of thiobarbituric acid reactive substances (TBARS) formation. (Ohkawa *et al.*, 1979). About 500 µl of each tissue homogenate was mixed with PBS (0.02 M, pH-7.4) and TCA (10% w/v) and kept at room temperature for 30 minutes. Then the mixture was centrifuge at 3000 g for 10 minutes. 1 ml supernatant was mixed with TBA (1% w/v) and heated for 1 hour at 95° C or water bath until a stable pink color formed. The absorbance of the sample was measured at 535 nm using a blank containing all the reagents except the tissue homogenate. As 99% of the TBARS is malondialdehyde (MDA), TBARS concentrations of the samples were calculated using the extinction coefficient of MDA (1.56×10^5 M^{-1} cm^{-1}).

Superoxide dismutase (SOD) activity assay

The SOD activity was measured by following the method of Kakkar *et al*, 1984. About 100 µl tissue homogenate (liver) were mixed with PMS (186 mM), NADH (780 mM), phosphate

buffer saline (100 mM, pH-7.4) and NBT (300 mM). It was then incubated at 30° C for 90 minute. The reaction was then stopped by adding 1 ml glacial acetic acid and absorbance of chromogen formed was measured at 560 nm. One unit of SOD activity is defined as the enzyme concentration required for the inhibition of chromogen production by 50% in one minute under the assay condition.

Estimation of reduced glutathione (GSH) level

GSH level was measured by the method of Ellman *et al*, 1959. About 100 µl of tissue homogenate and EDTA (0.02 M) were mixed and kept on ice bath for 10 minutes. Then 1 ml distilled water and TCA (50%) were added and again kept on ice bath for 10 minutes. After that mixture was centrifuged at 3000 g for 15 minutes. To 1 ml of supernatant, 0.4 M triss buffer (pH-8.9) followed by DTNB (0.01 M) were added and the absorbance was measured at 412 nm. A standard graph was drawn using different concentration of a standard GSH solution (1 mg/ml). With the help of the standard graph, GSH contents in the liver homogenates of the experimental animals were calculated

Serum biochemical parameters

Collected blood was analyzed for various serum biochemical parameters like Serum glutamic oxaloacetic transaminase (SGOT), Serum glutamic pyruvic transaminase (SGPT), Serum alkaline phosphatase (SALP) and Total protein. All the analyses were performed by using commercially available kits from Span Diagnostics Ltd. India.

Serum lipid profiles

Serum lipid profiles like total cholesterol, HDL Cholesterol and triglyceride in STZ-induced diabetic rats were determined by using commercially available kits from Span Diagnostics Ltd. India.

Statistical Analysis

All the results are shown as mean ±SEM. The results were analyzed for statistically significance by one-way analysis of variance (ANOVA) followed by post hoc Dunnett's test using Graph Pad Prism 5.0 software (Graph Pad Software, USA). *p* values of < 0.05 were considered as statistically significant.

RESULTS

Oral glucose tolerance test

Glucose administration to the normal rats increased blood glucose levels from 111.3 ± 3.5 to 119 ± 3.60 in first 30 mins and gradually decreased in 60 min, 120 mins and returned near to normal i.e. 77.33 ± 2.66 at 240 min. Administration of MECC at the doses of 100 and 200 mg/kg significantly ($p < 0.05$) improved glucose tolerance in a dose dependent manner at 120 min with time (Table 7.1).

Table 7.1: Effect of MECC on OGTT

GROUPS	0 mins	30 mins	60 mins	120 mins
Normal Control	81.3 ± 3.5	129± 3.60	121 ±3.78	111.33 ±1.85
MECC 100mg/kg	90 ± 6.6	134.7 ±4.05*	126 ±3.05 *	114 ±4.61 *
MECC 200mg/kg	85.67 ± 4.09	130± 5.19 *	124 ±4.4 *	120 ±4.61 *
STZ+Glibenclamide	80.21±3.21	105.02±2.98 *	117.12±5.02 *	113.56±3.89 *

Values are represented as mean ± SEM, where n = 6. *p<0.05 when compared to normal control.

Body weight

The final body weights were significantly ($p < 0.05$) decreased in the diabetic control group as compared to normal control group. Administration of MECC at the doses of 100 and 200 mg/kg significantly ($p < 0.05$) improved of the body weight when compared to the diabetic control group (Table 7.2).

Table 7.2: Effect of MECC on Body weight

GROUPS	DAY 0	DAY 5	DAY 10	DAY 15
Normal Control(5ml/kg)	131.7 ±0.88	135 ±1.15	133.3 ±0.88	141.7± 0.88
STZ Control(55mg/kg)	151.3±0.88[a*]	136± 1.52 [a*]	135 ±1.55 [a*]	129.7 ±0.88 [a*]
STZ+100mg/kg MECC	131.3±0.88[b*]	123 ±1.55 [b*]	124.7 ±1.2 [b*]	128.7± 1.20 [b*]
STZ+200mg/kg MECC	141.3±0.89[c*]	132.7±1.47[c*]	134.3±1.45 [c*]	138 ±0.89 [c*]
STZ+0.5mg/kg Glibenclamide	163 ±1.73[d*]	145 ±1.55 [d*]	154.3±2.33 [d*]	159.3 ±2.33 [d*]

Each volume expressed as MEAN±SEM, where n=6, a* normal control group vs. diabetic control group (b*, c*, d* all treated group vs. diabetic control group a*) on corresponding day, $p<0.05$.

Fasting blood glucose level

There was significantly ($p < 0.05$) elevated FBG level in STZ-induced diabetic rats as compared to normal control group. Administration of MECC in diabetic rats at the doses of 100 and 200 mg/kg significant ($p < 0.05$) reduced the FBG level towards normal as compared to the diabetic control group (Table 7.3).

Table7.3: Effect of MECC on Fasting Blood Glucose(mg/dl).

GROUPS	DAY 0	DAY 5	DAY 10	DAY 15
Normal Control(5ml/kg)	77.67±4.25	88±2.51	89.67±1.20	86.67±2.72
STZ Control(55mg/kg)	475±1.73[a]*	546±2.33 [a]*	505.7±2.02 [a]*	521.30±2.72 [a]*
STZ+100 mg/kg MECC	459.30 ±1.49	317± 2.30 [b*]	306.7± 1.46 [b*]	195.3 ±1.76 [b*]
STZ+200 mg/kg MECC	422 ±1.45	323.7 ±1.76 [b*]	115 ±1.56 [b*]	96.33± 1.45 [b*]
STZ+0.5mg/kg Glibenclamide	375 ±1.73	205± 1.55 [b*]	103± 1.55 [b*]	94.67 ±0.88 [b*]

Each volume expressed as MEAN±SEM, where n=6, a* normal control group vs. diabetic control group (b* all treated group vs. diabetic control group a*) on corresponding day, $p<0.05$.

Estimation of serum lipid profiles

Serum lipid profiles like total cholesterol and triglyceride in STZ-induced diabetic rats were significantly ($p < 0.05$) elevated and the HDL level significantly ($p < 0.05$) decreased compared to normal control group. Treatment with MECC at the doses of 100 and 200 mg/kg significantly ($p < 0.05$) reduced the total cholesterol, triglyceride level and significantly ($p < 0.05$) increased the HDL level when compared to the diabetic control group (Table 7.4) .

Table 7.4: Effect of MECC on Lipid Profiles

GROUPS	Total Cholesterol	HDL	Triglycerides
Normal Control	38.11 ±1.35	74.11 ± 3.08	55.25 ±3.42
STZ Control(Diabetic)	105.9 ± 3.74^{a*}	30.64 ± 0.93^{a*}	134.4± 3.78^{a*}
STZ+ MECC (100mg/kg)	61.20 ± 4.28^{b*}	38.59 ± 2.57	90.62± 4.33^{b*}
STZ +MECC(200mg/kg)	51.67 ± 2.90^{b*}	55.65 ± 2.44^{b*}	68.90± 5.45^{b*}
STZ+Glibenclamide(0.5mg/kg)	45 ± 2.30^{b*}	59.67 ± 4.20^{b*}	57.66± 3.98^{b*}

Each volume expressed as MEAN±SEM, where n=6, a* normal control group vs. diabetic control group (b* all treated group vs. diabetic control group a* p<0.05).

Figures:

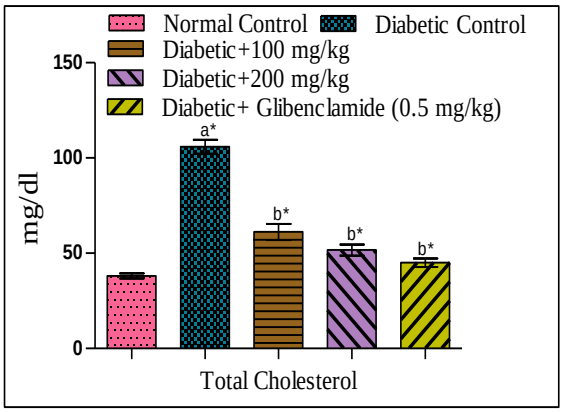


Fig 7.1: Effect of MECC on Total Cholesterol. Each value is expressed as Mean ± SEM where n=6. a* p<0.05 when compared to normal and b* p<0.05 when compared to diabetic control.

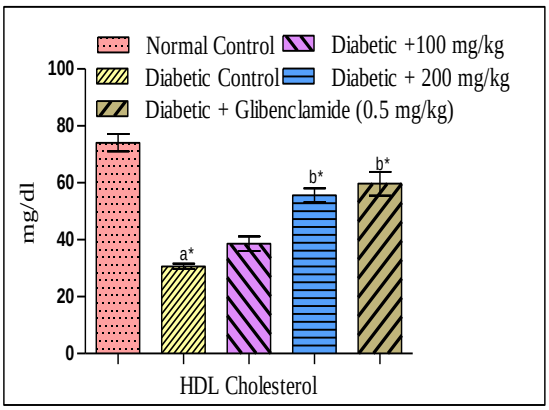


Fig 7.2: Effect of MECC on HDL cholesterol. Each value is expressed as Mean ± SEM where n=6.a* p<0.05when compared to normal , b* p<0.05 when compared to diabetic control.

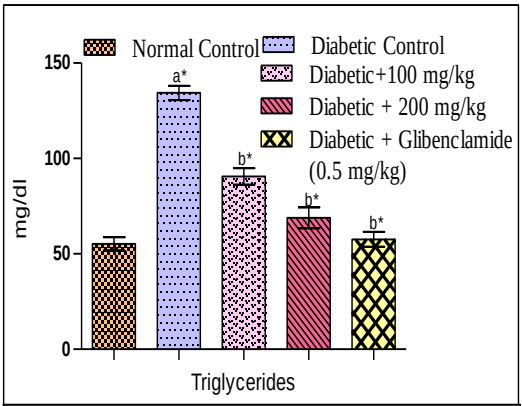


Figure 7.3: Effect of MECC on Triglycerides.Each value is expressed as Mean ± SEM where n=6. a* p<0.05 when compared to normal and b* p<0.05 when compared to diabetic control.

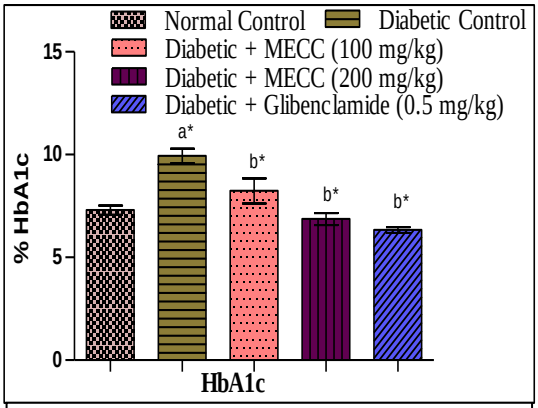


Figure 7.4: Effect of MECC on HbA1c.Each value is expressed as Mean ± SEM where n=6. a* p<0.05 when compared to normal and b* p<0.05 when compared to diabetic control**.**

Estimation of glycosylated hemoglobin (HbA1c)

Glycosylated hemoglobin level in STZ-induced diabetic rats was significantly ($p < 0.001$) increased compared to normal control group. Treatment with MECC at the doses of 100 and 200 mg/kg significantly ($p < 0.05$) reduced the HbA1c level when compared to the diabetic control group (Table 7.5).

Table 7.5: Effect of MECC on HbA1c

Groups	HbA1c (%)
Normal Control	7.3 ± 0.23
STZ Control (Diabetic)	9.93 ± 0.35a*
STZ + MECC (100 mg/kg)	8.23 ± 0.060
STZ+ MECC (200 mg/kg)	6.86 ± 0.29
STZ + Glibenclamide (0.5mg/kg)	6.33 ± 0.14

Values are represented as mean ± SEM, where n = 6. a*p<0.05 when compared to normal control.

Estimation of serum biochemical parameters

Biochemical parameters like SGOT, SGPT and SALP in STZ-induced diabetic rats were significantly ($p < 0.05$) elevated and the total protein content was significantly ($p < 0.05$) decreased compared to the normal control group. Treatment with MECC at the doses of 100 and 200 mg/kg significantly ($p < 0.05$) reduced the SGOT, SGPT and SALP levels and significantly ($p < 0.05$) increased the total protein level as compared to the diabetic control group (Table 7.6).

Table 7.6: Effect of MECC on Serum Biochemical Parameters.

Groups	SGOT (IU/L)	SGPT (IU/L)	ALP (IU/L)	Total Protein (g/dl)
Normal Control	76.95 ±3.62	22.33±1.85	5.05±1.04	9.28± 0.248
STZ Control(Diabetic)	136.6±3.39^{a*}	101.3±2.46^{a*}	18.91±1.09^{a*}	3.35± 0.59^{a*}
STZ+MECC (100mg/kg)	108.1±6.14^{b*}	43.58±2.44^{b*}	14.57±1.24^{b*}	6.40± 0.29^{b*}
STZ+MECC(200mg/kg)	94.17±5.35^{b*}	33.48±2.18^{b*}	9.43± 0.34^{b*}	7.67± 0.16^{b*}
STZ+Glibenclamide(0.5mg/kg)	88.15±4.78^{b*}	29.74±2.09^{b*}	8.28 ±0.38^{b*}	8.43 ±0.25^{b*}

Values are expressed as mean ± S.E.M. (n = 6). a* p < 0.05 when compared to normal control; b***** p < 0.05 when compared to diabetic control.

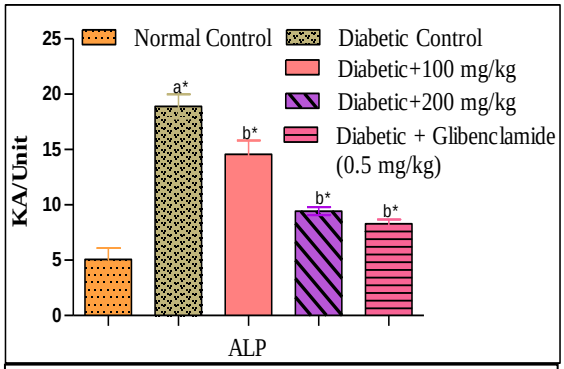


Figure 7.5: Effect of MECC on ALP . Each value is expressed as Mean ± SEM where n=6. a* p<0.05 when compared to normal and b* p<0.05 when compared to diabetic control.

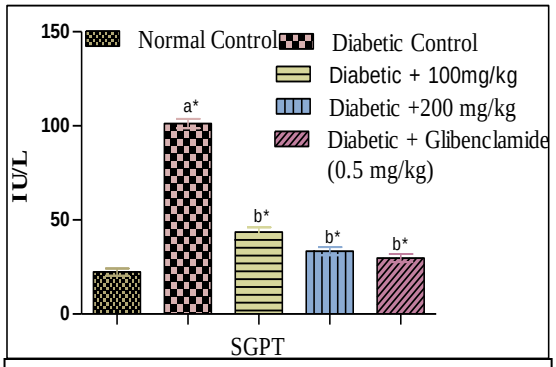


Figure 7.6: Effect of MECC on SGPT. Each value is expressed as Mean ± SEM where n=6. a* p<0.05 when compared to normal and b* p<0.05 when compared to diabetic control

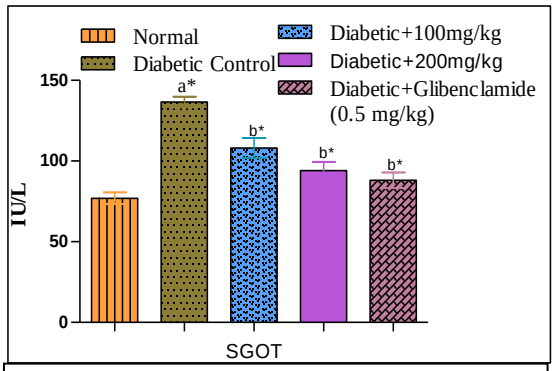


Figure 7.7: Effect of MECC on SGOT. Each value is expressed as Mean ± SEM where n=6. a* p<0.05 when compared to normal and b* p<0.05 when compared to diabetic control

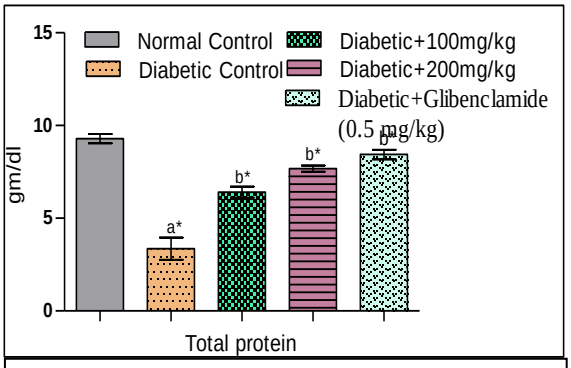


Figure 7.8: Effect of MECC on Total Protein. Each value is expressed as Mean ± SEM where n=6. a* p<0.05 when compared to normal and b* p<0.05when compared to diabetic control.

Estimation of tissue antioxidant parameter

Lipid peroxidation results in the formation of ROS species and subsequently elevates the level of malondialdehyde (MDA) in liver tissue of STZ-induced diabetic rats. In the present study the MDA level was significantly (p < 0.05) increased in STZ-induced diabetic rats

compared to normal control group. Interestingly, treatment with MECC at the doses of 100 and 200 mg/kg significantly ($p < 0.05$) reduced the MDA levels compared to diabetic control group. The levels of reduced glutathione, and SOD were significantly ($p < 0.05$) decreased in STZ-induced diabetic rats compared to normal control group. Administration of MECC at the doses of 100 and 200 mg/kg significantly ($p < 0.05$) increased GSH, SOD levels in the liver of STZ-induced diabetic rats compared to the diabetic control group (Table 7.7 , 7.8, 7.9).

Table 7.7: Effect of MECC on MDA.

Groups	MDA(µM/100gm Tissue Homogenate) Liver	MDA(µM/100gmTissue Homogenate) Kidney
Normal Control	1.6 ±0.0173	1.09 ±0.020
STZControl(Diabetic)	2.48 ±0.0176^{a*}	1.99± 0.008^{a*}
STZ +MECC (100mg/kg)	1.91 ±0.026^{b*}	0.816± 0.098^{b*}
STZ +MECC(200mg/kg)	1.73 ±0.034^{b*}	1.18 ±0.04^{b*}
Diabetic+Glibenclamide(0.5 mg/kg)	1.68 ±0.29^{b*}	1.11± 0.056^{b*}

Values are represented as Mean ± SEM, where n = 6. a*p<0.05 when compared to normal control , b* when compared to diabetic control p<0.05.

Table 7.8: Effect of MECC on SOD

Groups	SOD LIVER(U/mg)	SOD KIDNEY(U/mg)
Normal Control	2.43± 0.23	3.63 ±0.31
STZ Control(Diabetic)	0.83± 0.088^{a*}	1.03± 0.088^{a*}
MECC (100mg/kg)	0.88 ±0.060	1.30 ±0.034
MECC(200mg/kg)	1.55± 0.06^{b*}	1.95± 0.032^{b*}
Diabetic+Glibenclamide(0.5mg/kg)	1.83 ±0.088^{b*}	2.20± 0.05^{b*}

Values are represented as Mean ± SEM, where n = 6. a*p<0.05 when compared to normal control; b*p<0.05, when compared to diabetic control.

Table 7.9: Effect of MECC on GSH

Groups	GSH(µg/mg tissue) Liver	GSH(µg/mg tissue)Kidney
Normal Control	3.36± 0.089	3.78± 0.10
STZ Control(Diabetic)	1.58± 0.080^{a*}	1.77± 0.087^{a*}
MECC (100mg/kg)	2.013 ±0.069^{b*}	2.22± 0.08^{b*}
MECC(200mg/kg)	3.007± 0.072^{b*}	3.56 ±0.11^{b*}
Diabetic+Glibenclamide(0.5mg/kg)	2.63 ±0.078^{b*}	3.2 ±0.07^{b*}

Values are represented as Mean ± SEM, where n = 6. a*p<0.05 when compared to normal control; b*p<0.05, when compared to diabetic control.

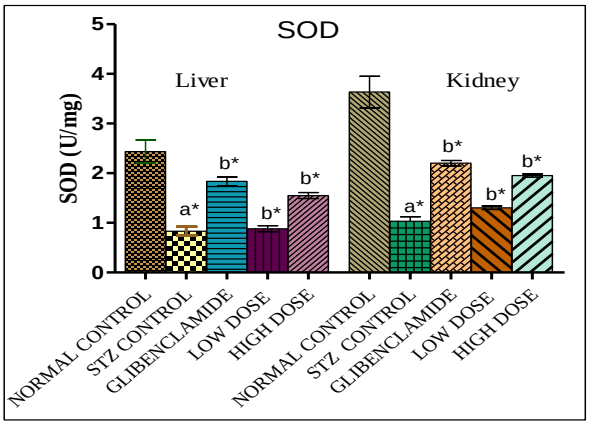


Figure 7.9: Effect of MECC on Superoxide dismutase. Each value is expressed as Mean ± SEM where n=6. a* p<0.05 when compared to normal and b* p<0.05 when compared to diabetic

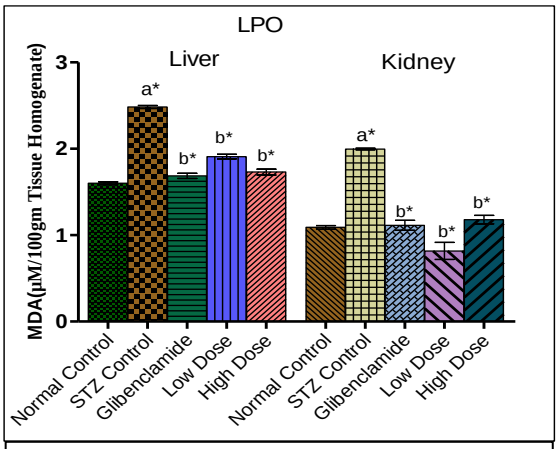


Figure 7.10: Effect of MECC on MDA. Each value is expressed as Mean ± SEM where n=6. a* p<0.05 when compared to normal and b* p<0.05 when compared to diabetic control**.**

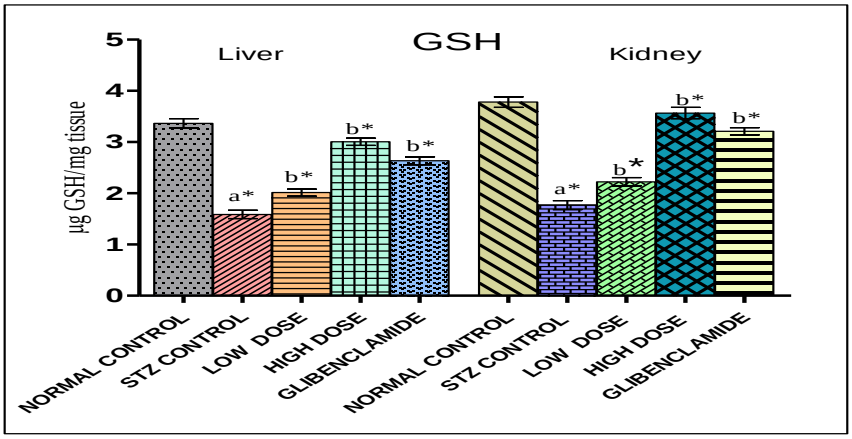


Figure 7.11: Effect of MECC on GSH.Each value is expressed as Mean ± SEM where n=6. a* p<0.05 when compared to normal and b* p<0.05 when compared to diabetic control**.**

Histopathology

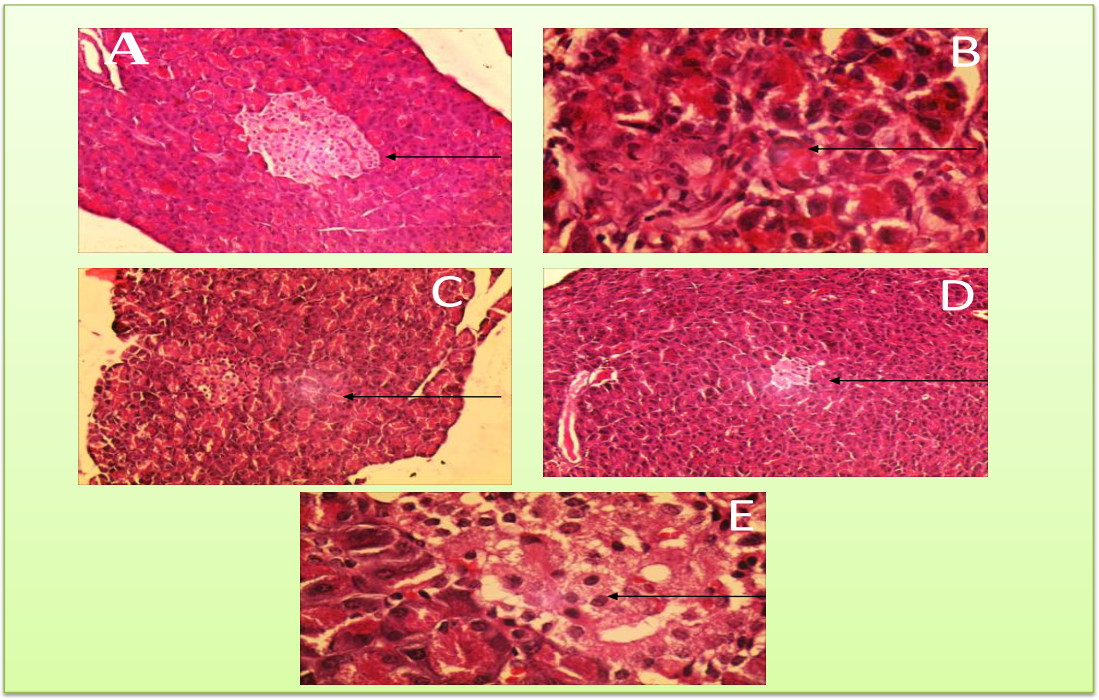


Figure 7.12: A) Beta cells in normal control rats. B) Total destruction of beta cells in STZ control rats. C) Remnants of beta cells in MECC (100 mg/kg) treated rats. D) Gradual regeneration of beta cells in MECC (200 mg/kg) treated rats. E) Functioning secretory granules in the islets of beta cells as seen in Glibenclamide (0.5 mg/kg) treated rats.

DISCUSSION

The present study discusses the antihyperglycemic activity of methanol extract of *Curcuma caesia* rhizome in STZ-induced diabetic rats. It was observed that there was significant increase in the fasting blood glucose (FBG), triglyceride (TG), total cholesterol (TC), serum biochemical parameters (SGOT, SGPT, SALP), blood glycosylated haemoglobin (HbA1c), lipid peroxidation (MDA) and significant decrease in the HDL, total protein, body weight and liver antioxidants (GSH, SOD,) levels in the STZ induced diabetic rats when compared to normal control group. The treatment with MECC at doses of 100 and 200 mg/kg was done significantly and dose dependently normalized elevated blood glucose level, glycosylated hemoglobin, body weight and restored serum and liver biochemical parameters towards normal values in MECC treated group when compared to diabetic control group. India has

the richest plant based traditional medicine system because of its rich biodiversity. As a result of which, traditional methods of treatment are used for treating diabetes in developing countries. These herbal medicines are mainly used for health care due to their cost value, effectiveness and lesser side effects on human body (Sekar *et al*., 2010).WHO estimates that 80% of the world populations currently use herbal drugs for major healthcare. It is believed that plants having antidiabetic activity have been ascertained to be rich in alkaloids, flavonoids and saponin, (Mishra *et al*., 2010), which are known to be bioactive against diabetes. The MECC rhizome has shown the presence of alkaloid, flavonoid, steroid and triterpenoid tannin when phytochemical screening was done. An increase in blood glucose level was observed in normal glucose administered rats thanMECC treated rats while studying oral glucose tolerance test (OGTT). Oral administration of MECC significantly improved the impaired glucose tolerance in the glucose loaded rats in a dose dependent manner. From the above result, the OGTT showed an increased glucoseutilization triggered by insulin production from the beta cells. It was also evident from the result that MECC significantly lowered FBG level in STZ-induced diabetic rats. This gives an idea that the antihyperglycemic effect of MECC may have insulin-like action *i.e*., action at peripheral level to increase cellular glucose uptake or secretion of insulin (Mbaka *et al*., 2012). Induction of diabetes with STZ is associated with a characteristic loss of body weight,

which is due to increased muscle wasting and loss of tissue proteins. Treated with MECC showed significant improvement in body weight. Hence MECC exhibited a marked effect in controlling the loss of body weight of diabetic rats. Streptozotocin results in elevation of triglycerides, total cholesterol and decrease in HDL cholesterol. Hypercholesteremia and hypertriglyceridemia are primary factors involved in the development of atherosclerosis and coronary heart disease which are the secondary complications of diabetes (Ananthan *et al*., 2003). Dyslipidaemia is characterized by high plasma levels of total cholesterol, LDL-cholesterol and triglycerides, with low plasma levels of HDL cholesterol. MECC

significantly reduced serum triglycerides, total cholesterol and increased level of HDL in STZ induced diabetic rats. Streptozotocin mediated persistent hyperglycemia is due to beta cells destruction, mediated and complicated by the enhanced formation of free radicals. (Kaneto *et al*., 2005 & Leung and Chan, 2009). Further the excess accumulation of reactive radicals produces chronic oxidative stress through chain reactions. From the present results hyperglycemia was confirmed in the experimental animals by the significant elevation of glycosylated haemoglobin level as compared with the normal controls. The amount of HbA1c increase is directly proportional to the fasting blood glucose level (Nain *et al*., 2012). Administration of MECC to diabetic rats significantly reduced the glycosylated hemoglobin. Compared to Fasting Blood Glucose (FBG) and Oral Glucose Tolerance Test (OGTT), HbA1c is a better diagnostic tool to determine diabetic conditions. Elevation of serum biomarker enzymes such as SGOT, SGPT, and SALP was observed in diabetic (STZ induced) rats indicating impaired liver function, which was obviously due to hepatocellular necrosis. Therefore, increase in the activities of SGOT, SGPT, and SALP gives an indication on the hepatotoxic effect of STZ. Treatment with MECC restored all the above mentioned biochemical parameters toward the normal values in a dose-dependent manner. Lipid peroxidation (LPO) is usually measured in terms of formation of thiobarbituric acid reactive substance (TBARS) *i.e.*, Malondialdehyde (MDA), as a biomarker of oxidative stress (Venukumar and Latha, 2002). The reduction in liver antioxidant status during diabetes may be the result of counteraction against increased formation of lipid peroxides (Sabu and Kuttan, 2004). A marked increase in the concentration of TBARS in STZ-induced diabetic rats indicated enhanced lipid peroxidation leading to tissue injury and failure of the endogenous antioxidant defence mechanisms to prevent over production of free radicals. Treatment with MECC inhibited hepatic lipid peroxidation in diabetic rats as revealed by the reduction of TBARS levels towards normal, suggesting MECC's ability to improve the pathologic condition of diabetes by inhibiting lipid peroxidation in diabetic rats. It has been

shown that in diabetes, that oxidative stress occurs because of body's inability to scavenge the excess production of free radical. ((Florence *et al*., 2013). The SOD is the enzymatic antioxidant which plays an important role in scavenging and elimination of free radicals in the cells. The decrease in activity of these enzymes can lead to an excess availability of superoxide anion ($O2^-$) and hydrogen peroxide (H_2O_2) in the biological systems, which in turn generate hydroxyl radicals (OH), resulting in initiation and propagation of lipid peroxidation (Latha and Pari, 2003). SOD protects from oxygen free radicals by catalyzing the removal of superoxide radical, which damage the membrane and biological structures. Glutathione plays an important role in the endogenous nonenzymatic antioxidant system. Primarily, it acts as reducing agent and detoxifies hydrogen peroxide in presence of an enzyme, glutathione peroxidase (Biswas *et al*., 2011). The depleted GSH may be due to reduction in GSH synthesis or degradation of GSH by oxidative stress in STZ-induced hyperglycaemic animals (Loven *et al*., 1986).In the present study, extract treated groups showed a significant increase in the hepatic SOD, GSH activities in the diabetic rats. This indicate that the extracts can reduce the potential glycation of enzymes or they may reduce reactive oxygen free radicals and improve the activities of antioxidant enzymes. This result shows evidence that MECC contain a free radical scavenging activity, which could exert a beneficial action against pathological alteration caused by the presence of superoxide radicals and hydrogen peroxide radical. On the basis of this evidence it is possible that these activities of MECC are due to the presence of the above said phytoconstituents . Therefore, it can be concluded that the methanol extract of *Curcua caesia* rhizome is remarkably effective against streptozotocin -induced diabetes in Wistar rats plausibly by virtue of its augmenting the endogenous antioxidant mechanism.

REFERENCES

Ananthan R, Latha M, Ramkumar K Pari , Baskar C, Bai V. Effect of *Gymnema montanum* leaves on serum and tissue lipids in alloxan diabetic rats. Experimental Diabetes and Research . 2003; 4: 183-9.

Biswas M, Kar B, Bhattacharya S, Suresh Kumar RB, Ghosh AK, Haldar PK. Antihyperglycemic activity and antioxidant role of *Terminalia arjuna* leaf in streptozotocin-induced diabetic rats. Pharmaceutical Biology. 2011; 49 (4): 335-40.

Ellman GL. Tissue sulfhydryl groups. Archives of Biochemistry and Biophysics. 1959; 82, 70-7.

Florence NT, Benoit MZ, Jonas K, Alexandra T, Desire DDP, Pierre K,Theophile D. Antidiabetic and antioxidant effects of *Annona muricata (*Annonaceae), aqueous extract on streptozotocin-induced diabetic rats Journal of Ethnopharmacology . 2013; 151: 1-7.

Haldar PK, Kar B, Bhattacharya S, Bala A, Suresh Kumar RB. Antidiabetic activity and modulation of antioxidant status by *Sansevieria roxburghiana* rhizome in streptozotocin-induced diabetic rats. Diabetologia Croatica. . 2010; 39(4): 115-23.

Kaneto H, Kawamori D, Matsuoka TA. Oxidative stress and pancreatic beta cell dysfunction. American Journal of Therapeutics . 2005; 12: 529-33.

Latha M, Pari L. Preventive effects of Cassia auriculata L. flowers on brain lipid peroxidation in rats treated with streptozotocin. Molecular and Cellular Biochemistry 2003; 243: 23-8.

Loven D, Schedl H, Wilson H, Daabees TT, Stegink LD, Diekus M, Oberley L.Effect of insulin and oral glutathione on glutathione levels and superoxide dismutase activities in organs of rats with streptozocin-induced diabetes. Diabetes.1986; 35: 503-7.

Mbaka GO, Ogbonnia SO, Oyenira, KJ, Awopetu PI.. Effect of *Raphia hookeri* Seed Extract on Blood Glucose, Glycosylated Haemoglobin and Lipid Profile of Alloxan Induced Diabetic Rats. British Journal of Medicine & Medical Research .2012; 2 (4): 621-35.

Mishra SB, Rao CHV, Ojha SK, Vijayakumar M, Verma A. An analytical review of plants for antidiabetic activity with their phytoconstituent & mechanism of action.International Journal of pharmaceutical Sciences and Research , 2010;1(1): 29-46.

Nain P, Saini V, Sharma S, Nain J. Antidiabetic and antioxidant potential of *Emblica officinalis* Gaertn. leaves extract in streptozotocin-inducedtype-2 diabetes mellitus (T2DM) rat. Journal of Ethnopharmacology . 2012; 142: 65-71.

Naskar S, Mazumder UK, Pramanik, G, Gupta M, Suresh Kumar RB, Bala A,Islam A. Evaluation of antihyperglycemic activity of *Cocos nucifera* Linn. on streptozotocin induced type 2 diabetic rats. Journal of Ethnopharmacology. 2011;138: 769- 73.

Sabu MC, Kuttan R. Antidiabetic activity of *Aegle marmelos* and its relationship with its antioxidant properties. Indian Journal of Physiology and Pharmacology. 2004; 48:81–8.

Sekar T, Ayyanar M, Gopalakrishnan M. Medicinal plants and herbal drugs.Current Science. 2010; 98(12): 1558-9.

Venukumar MR, Latha MS. Antioxidant activity of *Curculigo orchioides* in carbon tetrachloride induced hepatopathy in rats. Indian Journal of Clinical Biochemistry . 2002; 17: 80–7.


Conclusion

Diabetes mellitus is the seventh leading cause of death and is in third rank when it's fatal complications are considered. Traditional preparations of plant sources are widely used almost everywhere in the world to treat this disease. Therefore, plant materials are considered to be the alternative sources for finding out new leads for hypo-/antihyperglycemic agents. Glibenclamide was used as a standard drug for type 2 model rats. It is well established that glibenclamide, a long acting sulfonylurea, acts mainly by augmenting insulin secretion. On the other hand, insulin activate glucose uptake in various cells including muscles and adipocytes, stimulates hexose uptake, lipogenesis and inhibit lipolysis and stimulate protein synthesis. Administration of glibenclamide to type 2 diabetes almost normalizes serum glucose levels. In the present study plant was selected based on the traditional uses and literature review and authenticated by botanical survey of India, Howrah, West Bengal, India. *Curcuma caesia* popularly known as *kali haldi* in Hindi had been collected from Sikkim in the month August in the year 2015. The rhizome portion of the plant was shade dried, powdered and used for extraction using methanol. After evaporating the solvent, the extract was kept in vacuum dessicator for further use. Phytochemical screening of the extract showed the presence of steroids, flavonoids, tannins and alkaloids. Toxicity study was performed following OECD guidelines and methanol extract of *Curcuma caesia* (MECC) was found to be safe up to 2000 mg/kg body weight. Next, *in vitro* assay methods were carried out to determine the anti diabetic potential of MECC. The principles include enzyme (alpha amylase and alpha glucosidase) inhibition which measures intestinal absorption of monosaccharides and uptake of glucose in yeast cells. MECC showed promising reduction of intestinal glucose absorption and increased glucose uptake in yeast cells. Before proceeding to the *in vivo* study, the glycemic control of MECC was determined by oral glucose tolerance test (OGTT). MECC succeeded in controlling the increasing glucose level with time. The *in vivo* study started with the induction of diabetes in Wistar albino rats by injecting

streptozotocin (55 mg/kg b.w) intraperitoneally. After 72 hours of STZ administration, diabetes in the rats was confirmed by measuring blood glucose level using one-touch glucometer. The animals showing blood glucose level >220 mg/dl were divided into different groups naming normal control, diabetic control, treated groups (low dose and high dose) and treatment with reference group respectively. Dosing was continued for fourteen days and blood glucose was checked every fifth day. On the fifteenth day animals were sacrificed. Antioxidant activity of the liver and kidney homogenates were studies. The results showed good free radical scavenging activity of MECC. The serum parameters involve levels of SGOT, SGPT, ALP, Total protein whereas lipid parameters include levels of Total cholesterol, HDL cholesterol and triglycerides. SGOT, SGPT, ALP, Total cholesterol, triglycerides were found to decrease and total protein, HDL cholesterol increased in treated groups as compare to STZ control. Diabetes being a metabolic disease involving lipid metabolism, the control of lipid parameters is a matter of concern. Thus the blood glucose level, serum and lipid parameters being in check, we can conclude that MECC is quite effective in controlling Type 2 diabetes mellitus.