ANTIHYPERGLYCEMIC ACTIVITY OF *LITSEA CUBEBA* FRUIT AGAINST STREPTOZOTOCIN INDUCED DIABETES

Thesis submitted for the Partial Fulfillment of the award

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By

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CERTIFICATE

This is to certify that ***Waquar Ahmed Goldar*** *has carried out the research on the project entitled* ***"Antihyperglycemic activity of Litsea cubeba fruit against Streptozotocin induced diabetes"*** *under my supervision, in the Division of Pharmacology, Department of Pharmaceutical Technology, Jadavpur University, Kolkata – 700032.*

He has incorporated his findings into this thesis of the same title being submitted by him in partial fulfillment of the requirement for the award of Degree of Master of Pharmaceutical Technology, Jadavpur University. I am satisfied that he has carried out his thesis with proper care and confidence to my entire satisfaction.

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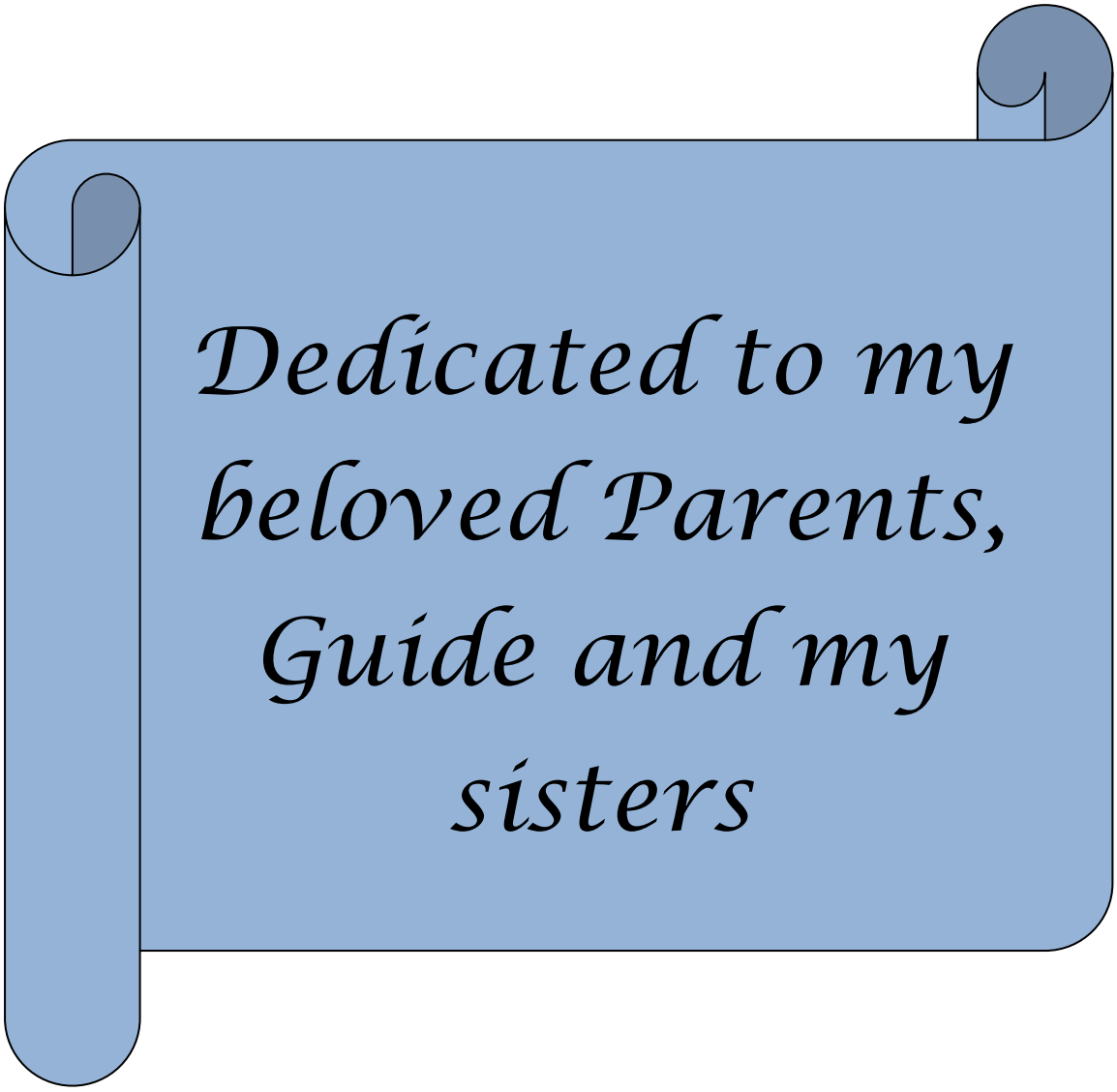
DECLARATION

I declare that **"Antihyperglycemic activity of *Litsea cubeba* fruit against Streptozotocin induced diabetes"** is my own work, that it has not been submitted for any degree or examination in any other university, and that all the sources I have used or quoted have been indicated and acknowledged by complete references.

Signatue of the student: …….....

Full name……

Date……..


Dedicated to my beloved Parents, Guide and my sisters

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Waquar Ahmed Goldar

PREFACE

The present study **"Antihyperglycemic activity of *Litsea cubeba* fruit against Streptozotocin induced diabetes"** covers original research work conducted by the author for the award of Master of Pharmacy in the Department of Pharmaceutical Technology, Jadavpur University, Kolkata.

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.

Waquar Ahmed Goldar

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Introduction

DIABETES : A BRIEF INTRODUCTION

"Laughter is the best medicine –unless you are diabetic, then insulin comes pretty high on the list."-Jasper Carrott.

Diabetes is a growing challenge in India with estimated 8.7% diabetic population in the age group of 20 and 70 years (World Health Organization, Fact Sheet of Diabetes, 2014). In 2000, India (31.7 million) topped the world with the highest number of people with diabetes mellitus followed by China (20.8 million) with the United States (17.7 million) in second and third place respectively. According to Wild et al. the prevalence of diabetes is predicted to double globally from 171 million in 2000 to 366 million in 2030 with a maximum increase in India. (Wild S, Roglic G et al. 2004). It is predicted that by 2030 diabetes mellitus may afflict up to 79.4 million individuals in India, while China (42.3 million) and the United States (30.3 million) will also see significant increases in those affected by the disease.(Whiting Dr, Guariguata L et al. ,2011).India currently faces an uncertain future in relation to the potential burden that diabetes may impose upon the country. Diabetes is a chronic disease, which occurs when the pancreas does not produce enough insulin or when the body cannot effectively use the insulin it produces. This leads to an increased concentration of glucose in the blood. It refers to a group of metabolic disorders that share the phenotype of hyperglycemia, glycosuria, hyperlipaemia, negative nitrogen balance and sometimes ketonaemia. A widespread pathological change is thickening of capillary basement membrane, increase in vessel wall matrix and cellular proliferation resulting in vascular complications like lumen narrowing, early atherosclerosis, sclerosis of glomerular capillaries, retinopathy, neuropathy and peripheral vascular insufficiency (J. Larry Jameson, 2018).

In 2016, diabetes was the direct cause of 1.6 million deaths and in 2012 high blood glucose was the cause of another 2.2 million deaths (World Health Organization, Fact Sheet of Diabetes, 2014).Most patients can be classified clinically as having either Type 1 or Type 2 Diabetic Melitus. The other types of diabetes includes Peridiabetes, Gestational diabetes.

The American Diabetes Association (ADA) criteria for the diagnosis of DM include symptoms polyuria, polydipsia and unexplained weight loss and a random plasma glucose concentration of greater than 200 mg/dL, a fasting plasma glucose concentration of greater than 126 mL/dL, or a plasma glucose concentration of greater than 200 mg/dL, 2 hours after the ingestion of an oral glucose load (Lee Goldman, 2018). HbA1c is the gold standard test

around the world for insulin initiation and intensification but it is not easily available to a large section of Indian population (Seema Abhijeet Kaveeshwar, 2014).

The main function of insulin is to lower blood glucose levels when they rise above normal. When these nutrients, especially glucose, are in excess of immediate needs insulin promotes storage by acting on cell membranes and stimulating uptake and use of glucose by muscle and connective tissue cells, increasing glycogenesis, especially in the liver and skeletal muscles, accelerating uptake of amino acids by cells, and the synthesis of protein, promoting lipogenesis, decreasing glycogenolysis, preventing the breakdown of protein and fat, and gluconeogenesis (Anne Waugh, Allison Grant,2018). Much of the diabetes burden can be prevented or delayed by behavioural changes favouring a healthy diet and regular physical activity.

Virtually all forms of DM result from a decrease in the circulating concentration of insulin i.e. insulin deficiency and a decrease in the response of peripheral tissues to insulin (insulin resistance).

CURRENT SCENARIO OF DIABETES

Global Scenario :

WHO estimates that, globally, 422 million adults aged over 18 years were living with diabetes in 2014. The largest numbers of people with diabetes were estimated for the WHO South-East Asia and Western Pacific Regions accounting for approximately half the diabetes cases in the world. The number of people with diabetes has steadily risen over the past few decades, due to population growth, the increase in the average age of the population, and the rise in prevalence of diabetes at each age. Worldwide, the number of people with diabetes has substantially increased between 1980 and 2014, rising from 108 million to current numbers that are around four times higher. Forty percent of this increase is estimated to result from population growth and ageing, 28% from a rise in age-specific prevalence, and 32% from the interaction of the two (World Health Organization, Fact Sheet of Diabetes,2014).

ESTIMATED PREVALENCE AND NUMBER OF PEOPLE WITH DIABETES (ADULTS 18+ YEARS) (W H O, Fact Sheet of Diabetes,2014)

WHO Region	Prevalence (%)		Number (Millions)	
	1980	**2014**	**1980**	**2014**
African Region	3.1%	7.1%	4	25
Region of the Americas	5%	8.3%	18	62
Eastern Mediterranean Region	5.9%	13.7%	6	43
European Region	5.3%	7.3%	33	64
South-East Asia Region	4.1%	8.6%	17	96
Western Pacific Region	4.4%	8.4%	29	131
Total	4.7%	8.5%	108	422

Indian scenario: Diabetes is fast gaining the status of a potential epidemic in India with more than 62 million diabetic individuals currently diagnosed preliminary results from a large community study conducted by the Indian Council of Medical research (ICMR) revealed that a lower proportion of the population is affected in states of Northern India (Chandigarh 0.12 million, Jharkhand 0.96 million) as compared to Maharashtra (9.2 million) and Tamil Nadu (4.8 million). The National Urban Survey conducted across the metropolitan cities of India reported similar trend: 11.7 percent in Kolkata (Eastern India), 6.1 percent in Kashmir Valley (Northern India), 11.6 percent in New Delhi (Northern India), and 9.3 percent in West India (Mumbai) compared with (13.5 percent in Chennai (South India), 16.6 percent in Hyderabad (south India), and 12.4 percent Bangalore (South India) (U. Singh, 2015). There were over 72.946.400 cases of diabetes in India in 2017.

INDIAN SCENARIO OF PLANT MEDICINE

There an approximate 320,000 medicinal plant species in the world. Herbal medicine is used by more than 50% of the world population. Use of traditional medicine is most common in Africa, Asia and Latin America. In India more than 65% of the population have used traditional medicines. India has rich diversity of medicinal plants. 90% of herbal row materials are used in Ayurvedic, Siddha, Unani and Homeopathy medicine and those mainly collected from wild state.

History of herbal medicine is as old as human civilization. But the problem is that, the information's of these traditional medicines are in the possession of ethnic tribes scattered all over the world. Biodiversity in nature is the huge stone house of traditional medicine. Uncovering of facts about this hidden treasure is a major challenge to present day biologists, where lies the future prospect of our health care system. Ayurveda is the ancient plant product based medicinal system of India practiced since last thousands of years. It gives a lot of valuable information's about diseases and their herbal remedial measures. With the use of sophisticated modern instruments, the plant based ethnopharmaceutical study becomes a common field of innovative research. The annual turnover of Indian herbal medicinal industry is about Rs. 2,300 crores. India has about 25,000 effective plant based formulations used in folk medicine. There are many herbs with strong antidiabetic properties. Herbal treatments for diabetes have been used in patients with insulin dependent and non-insulin dependent diabetes, diabetic retinopathy, diabetic neuropathy *etc*. The families of plants with the most potent hypoglycaemic effects include Leguminoseae, Lamiaceae, Liliaceae, Cucurbitaceae, Asteraceae, Moraceae, Rosaceae, Euphorbiaceae and Araliaceae. The most commonly studied species are: *Opuntia streptacantha*, *Trigonella foenum graecum*, *Momordica charantia*, *Ficus bengalensis*, *Polygala senega*and *Gymnema sylvestre*. Numerous mechanisms of actions have been proposed for plant extracts. Some hypothesis relates to their effects on the activity of pancreatic beta cells, increase in the inhibitory effect against insulinase enzyme, increase of the insulin sensitivity or the insulin-like activity of the plant extracts.

DIABETES MELLITUS

Diabetes Mellitus is a group of heterogeneous disorders in which carbohydrate metabolism is reduced while that of protein and lipid are increased. Hyperglycaemia is a common end point for all type of diabetes mellitus and is an important parameter to evaluate the efficacy of antidiabetic drug. As hyperglycemias increases, there is a loss of glucose through urine (glycosuria) (K.D. Tripathi, 2008). Virtually all forms of Diabetes Mellitus are either a decrease in the circulating level of insulin (insulin deficiency) or a decrease in the response of target tissues to insulin (insulin resistance). Although insulin treatment has largely increased the life expectancy of diabetic patients, diabetes remains the third leading cause of death, the second leading cause of blindness as well as renal failure. The hallmarks of diabetes mellitus are three "polys" : an excessive urine production (Polyuria), an excessive thirst (Polydipsia)

and an excessive eating (Polyphagia). The disease has two major forms: Type-1 and Type-2 DM; but two more diabetes disease state have also been included. These are: Type-1 and Type-2 diabetes mellitus; but two more diabetes disease states have also been induced. These are: Type 3 (other) and Type 4 (gestational Diabetes Mellitus). (Sharma and Sharma, 2017)

TYPES OF DIABETES MELLITUS

It is critical to appreciate that diabetes mellitus is a broad term used for a group of diseases that lead to prolonged hyperglycaemia. The difference in the mechanisms for developing the different types of diabetes forms the basis of their classification.

Type 1 Diabetes Mellitus

T1DM, previously known as insulin dependent diabetes or juvenile onset diabetes is an autoimmune disorder that involves the destruction of the β cells by activated CD4+ and CD8+ T cells and macrophages infiltrating the pancreatic islets. The onset of T1DM usually occurs in childhood and early adulthood (<35 years). Genetic as well as environmental factors are known to contribute to the susceptibility to this diabetes. Genetic studies have shown that the HLA (human leucocyte antigen) gene on chromosome 6 is closely associated with T1DM. The HLA proteins are located on the cell surfaces that help the immune system to distinguish body's normal cells from foreign infectious and non-infectious agents. In T1DM, an abnormality in the HLA proteins leads to an autoimmune reaction against the β cells. DR, another gene in the HLA vicinity plays an important role in T1DM. Evidence exists suggesting that certain viruses may be responsible in triggering T1DM.

Some patients sustain another form of T1DM, called idiopathic diabetes that does not involve autoimmunity. It is less common than the autoimmune T1DM, and is found in African and Asian populations. The aetiology and pathogenesis are not well understood, but the patients lack insulin production and are prone to ketoacidosis in the absence of antibodies to β cells. Fulminant Type 1 Diabetes Mellitus is another subtype of T1DM in which extremely rapid and almost complete destruction of β cells occurs (J. Larry Jameson, 2018) .

Symptoms of Type 1 Diabetes

Some of the symptoms include weight loss, polyurea, polydipsia, polyphagia, constipation fatigue, cramps, blurred vision, and candidiasis dry mouth, pruritis. Long lasting type 1 DM

patients may susceptible to microvascular complications and macrovascular complication (coronary artery, heart and peripheral vascular disease).

Type 2 Diabetes Mellitus

T2DM is a heterogeneous disorder characterized by a progressive decline in insulin action (insulin resistance), followed by the inability of β cells to compensate for insulin resistance (pancreatic beta cell dysfunction). Insulin resistance is a characteristic metabolic defect that precedes overt β cell dysfunction and is primarily associated with resistance to insulin-mediated glucose disposal at the periphery and compensatory hyperinsulinemia. The β cells normally compensate insulin resistance by secreting more amounts of insulin to maintain the glucose homeostasis. In the course of time, however, this β cell function gets impaired leading to deterioration in glucose homeostasis and subsequent development of impaired glucose tolerance and frank diabetes Insulin and Insulin Resistance (Gisela Wilcox,2005). There occurs only a relative insulin deficiency as the day-long circulating insulin concentrations in patients with type 2 diabetes are almost comparable or slightly elevated in absolute terms to the values in normal individuals.

Despite the role of genetic predisposition, aging, obesity and dietetic/sedentary life style are major risk factors involved in the development of type 2 diabetes. Most of the individuals diagnosed with type 2 diabetes are found to be obese (Yanling Wu, 2014).

T2DM is divided into two subgroups, diabetes with obesity and without obesity. The obese T2DM patients usually develop resistance to endogenous insulin due to alterations in cell receptors, and this is associated with distribution of abdominal fat. In non-obese T2DM there is some insulin resistance at the post receptor levels in addition to a deficiency in insulin production and release.

Symptoms of Type 2 Diabetes

Most cases are diagnosed because of complications or incidentally Carries a high risk of large vessel atherosclerosis commonly associated with hypertension, hyperlipidaemia and obesity. Most patients with type 2 diabetes die from cardiovascular complications and end stage renal disease. Geographical variation can contribute in the magnitude of the problems and to overall morbidity and mortality. Some other symptoms are excessive urination and thirst, increased hunger, unexplained weight gain, irritability and fatigue, blurred vision, decelerated

healing, skin yeast infections, sexual dysfunction in men, vaginal infection in female, itchy and flaky skin.

Gestational Diabetes Mellitus

Gestational diabetes mellitus (GDM) refers to the occurrence of diabetes during pregnancy and its consequent resolution at the end of the gestational period. During pregnancy and the gestational period, females go through a great deal of glucose fluctuation and often experience accelerated starvation. This coupled with an increase in insulin secretion by the placenta and a decrease in insulin sensitivity by the end of the first trimester, results in a transient state of insulin resistance. Although this form of diabetes resolves at the end of the gestational period, certain complications may develop which may be irreversible. For example gestational diabetes markedly increases the risk of mortality in both mother and foetus and is a potential teratogen. Furthermore, development of diabetic nephropathy in GDM can lead to pre-eclampsia which in turn is associated with a number of abnormalities in the foetal development, such as intrauterine growth retardation (IUGR), premature delivery and still birth.

Maturity Onset Diabetes of the Young (MODY)

Maturity Onset Diabetes of the Young (MODY) is a monogenic-type of diabetes. It represents a very small percentage of patients with diabetes and is commonly diagnosed by the second decade of the patient's life. The development of the six forms (1-6) of MODY are linked with mutations in a number of genes including hepatocyte nuclear factor 4α (HNF-4α), glucokinase gene (MODY 2) HNF-1α, insulin promoter factor-1 (IPF-1), HNF-1β and NEUROD1. The most common mutation is in the HNF-1 α gene. This mutation accounts for nearly 70% of all MODY patients. MODY 2 and MODY 3 are the most common form of disease, where MODY 3 is characterised by major defect in insulin secretion. Although infrequent, it is important to establish the right diagnosis for MODY and determine the cause of diabetes in order to provide the most appropriate treatment.

Other secondary types of diabetes are induced by pancreatitis, Cushing's syndrome, Klinefelters syndrome and hyperthyroidism. Certain drugs and chemicals such as thiazide diuretic, β-blocker, calcineurin, protease inhibitors and atypical antipsychotic drugs can also cause secondary diabetes (Anne Waugh, Allison Grant,2018).

COMPLICATIONS OF DIABETES MELLITUS

Diabetes is associated with an increased risk of developing vascular complications that contribute to morbidity and mortality of patients. Poor glycaemic and blood pressure control lead to vascular complications that affect large (macrovascular), small (microvascular) vessels, or both.

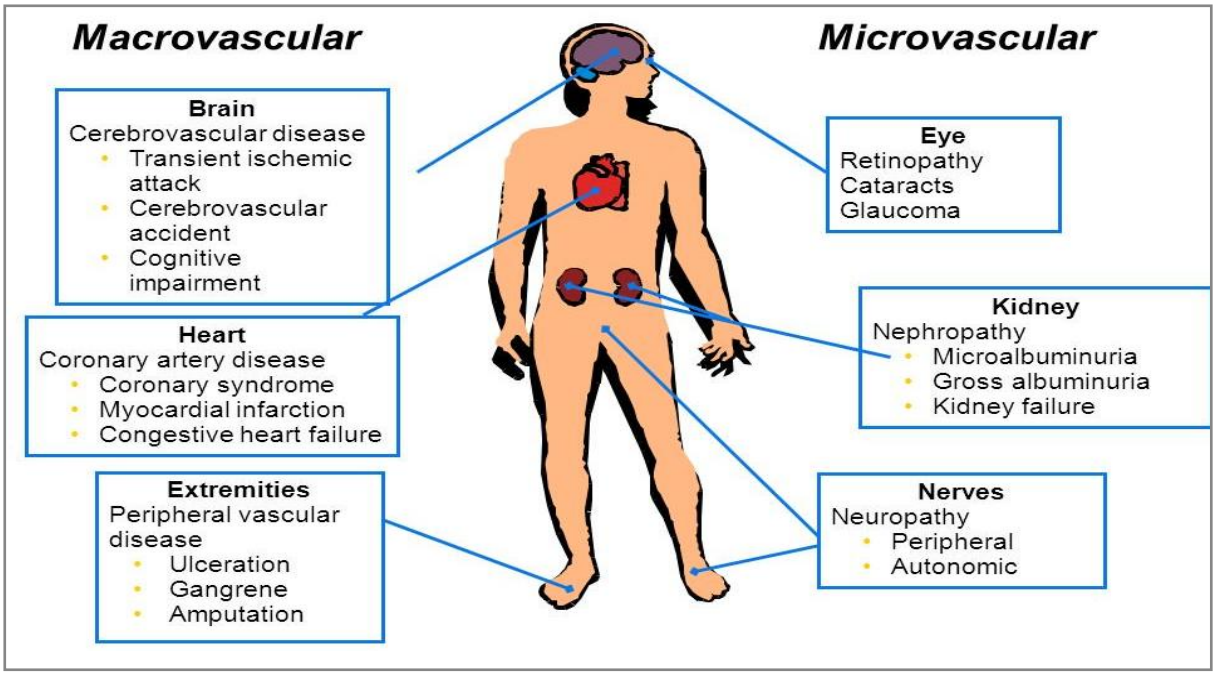

Figure 1. Macrovascular and microvascular complications (secondary to Diabetes Mellitus) Diabetes is a chronic illness closely associated with the development of macrovascular complications (coronary and cerebovascular diseases), in the arteries, and microvascular (retinopathy, neuropathy and nephropathy) complications, in the capillaries. These vasculopathies greatly influence the rate of mortality and the quality of life of the patients.

MACROVASCULAR COMPLICATIONS

The macrovascular complications, which affect the large vessels of the circulatory system may lead in 2 to 4 times higher incidence of stroke (cerebrovascular), coronary heart disease (CHD) and peripheral vascular disease which can lead to ulceration, gangrene and lower extremity amputations. These macrovascular complications are essentially accelerated forms of atherosclerosis involving the migration of leukocytes to site of arterial injury.

MICROVASCULAR COMPLICATIONS

Microvascular complications involve damage to the small blood vessels and contribute to diabetic neuropathy (nerve damage), nephropathy (kidney disease) and retinopathy (eye disease).

DIABETIC NEUROPATHY

Diabetic neuropathy classified as peripheral, proximal, focal and autonomic, is the most common of all the long-term complications of diabetes, with nearly 60% of patients having some form of nerve damage. It is a progressive disease that involves loss of sensation, as well as pain and weakness, and can lead to limb amputations.

DIABETIC NEPHROPATHY

Diabetic nephropathy is the leading cause of end-stage renal disease (ESRD) and the most common cause for kidney transplantation in the developed world. The presence and progressive rise of albumin in urine along with elevated glomerular blood pressure are the biomarkers of nephropathy. In the absence of appropriate intervention, the condition persists and leads to the loss of protein in urine and a decline in renal function in the form of lower glomerular filtration rate. This eventually leads in ESRD and complete renal failure. Clinical evidence suggests that approximately 15-20% of patients with T1DM and 30-40% with Type 2 develop ESRD.

DIABETIC RETINOPATHY

Diabetic retinopathy, caused by damage to the retinal vasculature, is a common cause of blindness and visual impairment in the working age population. The occurrence of diabetic retinopathy can be reduced and/or prevented by adequate and timely treatment.

IMPAIRMENT OF IMMUNE SYSTEM

T1DM is an autoimmune disorder in which the insulin producing β-cells in the islet of langerhans are progressively destroyed and as a result the insulin production stops. The process of autoimmunity is complex and involves both, genetic and environmental factors. B and T cells of immune systems play key roles in autoimmunity, as recent data has highlighted the significant role of T cells in T1DM (J. Larry Jameson ,2018).

PERIODONTAL AND FOOT DISEASES

Since immunity in diabetes can be significantly reduced it can affect the entire body's ability to defend itself from invading foreign pathogens including viral, bacterial, fungal and protozoal infections (P Pozzilli, 2009). Most notable amongst these infections are the periodontal diseases, which can severely affect the teeth if care is not taken seriously (RJ Genco, 2004). However, the interaction between teeth, the antigens and the immune system is fairly complicated.

For the same reasons, fungal infections of feet (especially between the toes) are more frequent in patients with diabetes than in nondiabetics. Lack of cleanliness and humidity in this region are the most common reasons for persistent infection. Another reason for the continual infection is the reduced sensitivity of the skin due to neuropathy. Therefore, it can take a very long time for infection to be diagnosed during which the infection can become deep seated and more difficult to be removed and often turns into gangrene, and finally leads to amputation of the foot (Eckhard M, Lengler A, Liersch J et al.2007) .

DIAGNOSIS OF DIABETES MELLITUS

Random Plasma (Blood) Glucose Test

The criteria for a diagnosis of diabetes with this test is the presence of diabetes symptoms and a blood glucose level of 200 mg/dl or higher. It doesn't require fasting state.

Fasting Plasma Glucose (FPG) Test

A diagnosis of diabetes is made when the fasting blood glucose level is 126 mg/dl or higher on at least two tests conducted on different days confirms a diabetes diagnosis. Values of 100-125 mg/dl indicate prediabetes. A normal fasting blood glucose level is less than 100 mg/dl.

Oral Glucose Tolerance Test (OGTT)

When random plasma glucose test is 148-200 mg/dl and the fasting plasma test is 110-125 mg/dl, then this test is conducted. This blood test evaluates body's response to glucose. This test requires fasting at least 8 but not more than 16 hours. Fasting glucose level is determined,

and then gives 75gm of glucose, 100 gm for pregnant women. The blood is tested every 30 minutes to 1 hour for 2 or 3 hours.

The test is normal if your glucose level at two hours is less than 128 mg/dl, Prediabetes is diagnosed if the two-hour blood glucose level is 128-199 mg/dl. A fasting level of 126 mg/dl or greater and two hour glucose level of 200 mg/dl or Higher confirms a diabetes diagnosis (Gillett MJ, 2009) .

Postprandial Blood Glucose Test

Measures blood glucose levels 2 hours after eating meal. Postprandial blood glucose is usually done in people who have symptoms of hyperglycemia, or when the results of a fasting glucose test suggest possible diabetes, but are inconclusive. Values of 200 mg/dl or more indicate diabetes.

Hemoglobin A1c (HbA1c), also known as the glycosylated hemoglobin or glycohemoglobin test

HbA1c measures the amount of glucose attached to hemoglobin (the oxygen carrying protein in red blood cells), which increases as blood glucose level rise. Since hemoglobin circulates in the blood until the red blood cells die (half the red blood cells are replaced every 12 to 16 weeks), the HbA1c test is a useful tool for measuring average blood glucose values over the previous 2 to 3 months. HbA1c is recommended as an essential indicator for the monitoring of blood glucose control. The blood HbA1c $\geq$ 6.5% is considered as diabetes. (E Selvin et al. 2010).

MANAGEMENT OF DIABETES:

The aim of the treatment is primarily to save life and alleviate symptoms. Secondary aims are to prevent longterm diabetic complications and, by eliminating various risk factors, to increase longevity. Insulin replacement therapy is the mainstay for patients with type 1 DM while diet and lifestyle modifications are considered for the treatment and management of type 2 DM. insulin is also important in type 2 DM when blood glucose levels cannot be controlled by diet, weight loss, exercise and oral medications. Oral hypoglycaemic agents are also useful in the treatment of type 2 DM. The main objective of these drugs is to correct the underlying metabolic disorder, such as insulin resistance and inadequate insulin secretion.

They should be prescribed in combination with an appropriate diet and lifestyle changes. Diabetes is best controlled either by diet alone and exercise (non-pharmacological), or diet with herbal or oral hypoglycaemic agents or insulin (pharmacological).

TREATMENT

Non drug treatment for Diabetes

Lifestyle and diet modification

Dietary and lifestyle modifications are the mainstay of treatment and management for type 2 diabetes. The majority of people with type 2 diabetes are overweight and usually have other metabolic disorders of the insulin resistance syndrome, so the major aims of dietary and lifestyle changes are to reduce weight, improve glycaemia control and reduce the risk of coronary heart disease (CHD), which accounts for 58% to 80% of deaths among those with diabetes. Studies have shown that there was significant reduction in the incidence of type 2 DM with a combination of maintenance of body mass index of 25 kg/m2, eating high fibre and unsaturated fat and diet low in saturated and trans-fat and glycemic index and moderate level of protein (as per WHO recommendation), regular exercise, abstinence from smoking and moderate consumption of alcohol suggesting that majority of type 2 DM can be prevented by lifestyle modification. There is a reduction of CHD among populations who consume diet high in folate and vitamin B6, probably through reducing plasma homocysteine levels (S Bastaki, 2005)

Physical activity and exercise

Regular physical activity is an important component of the primary prevention and management of type 2 Diabetes mellitus. Prospective cohort studies have shown that increased physical activity, independently of other risk factors, has a protective effect against the development of type 2 diabetes. These epidemiological prospective studies demonstrated that various levels of regular physical activity one to several times a week were associated with a decrease incidence of the disease at long-term follow-up (4 and 5 years respectively) in both men and women of different age groups 57,58.

DRUG TREATMENT FOR DIABETES MELLITUS

Insulin

Various preparation of insulin are available. Highly purified recombinant insulin preparaion are available to avoid any hypersensitivities reacions. Various short acting and long acting conventional preparation are also available which modify release of insulin in order to provide better postprandial and between the meals basal control of blood glucose. Various insulin analogues are also prepared to alter the pharmacokinetics to modify the release and increase stability. Insulin preparation can cause hypoglycaemia, weight gain, pharmacological problems complicate insulin therapy. Pharmacokinetic and pharmacodynamic properties of therapeutic insulin preparations which is still under research to control these effect 49. A number of innovation has been made to improve ease and accuracy of insulin administration and tight glycaemic control which include inhaled insulin, insulin pumps, implantable pumps. Insulin is of immense importance in case of type 1 DM, diabetic ketoacidosis associated with type 1 DM, and hyperosmolar non ketotic hyperglycaemic coma associated with type 2 DM . Insulin is used alone or in combination with oral hypoglycemic agents. Augmentation therapy with basal insulin is useful if some β cell function remains. Replacement of basal-bolus insulin is necessary if β cell exhaustion occurs (Mayfield JA, 2004).

ORAL HYPOGLYCAEMIC AGENTS

These drugs are effective to controlling blood glucose and are orally effective, mainly used in type 2 DM

Drug	**Examples**	**Mechanism of Action**	**Beneficiary Effect**	**Side Effect**
Sulphonylureas	First gen: Tolbutamide Chlorpropamide Second gen: Glibenclamide, Glipizide, Glimepiride	Act on sulphonylurea I receptors of B cell membrane thereby ogmentine insulin release by inward ca2+ ion influx in beta cell and by sensitizing target tissue to the action of insulin	Long term improvement in carbohydrate metabolism,improved glucose tolerance	Hypoglycemic episodes, weight gain, hypersensitivity reaction, Cholestatic jaundice (chlorpropamide)

Drug	**Examples**	**Mechanism of Action**	**Beneficiary Effect**	**Side Effect**
Biguanides	Phenformin, Metformin	Metformin reduces plasma glucose via inhibition of hepatic glucose production,increase muscle glucose uptake,retard glucose absorption,promote peripheral glucose utilization by enhancing anerobic glycolysis thereby overcoming insulin resistance	Long acting,improve lipid profile by reducing plasma	Lactic acidosis, Vit B12 deficiency
Meglitinides	Rapeglinide Nateglinide	In similar way as sulphonylurea by closure of ATP dependent K channel leading to depolarization and insulin release.	Rapid onset of action,used as adjuvant to metformin	Short lasting action, Risk of hypoglycemia, weight gain, must be avoided in liver disease
Thiazolidinediones	Rosiglitazone, Troglitazone, Pioglitazone	Selective agonist of paroxysome proliferator activated receptor gama (PPAR, gama) which enhances transcription insulin responsive genes eg. GLUT-4, entry of glucose into muscle and fat cell is improved, increase lypogenesis	Improve glycemic control, lower in circulation HbA1c, lowering of serum triglyceride and increment in serum HDL level.	Weight gain,liver toxicity, fluid retention leading to heart failure
αGlucosidase inhibitor	Acarbose, Milglitol (more potent in inhibiting sucrase)	Inhibition of alpha glucocydase the final enzyme for the digestion of carbohydrate in the brush border of small intestine mucosa.	Lower post prandial glycemia, lower HbA1c, body weight, serum triglyceride	Gas, bloating and diarrohea.

Treatment strategies for initiation of oral therapy

In patients with newly diagnosed type 2 diabetes in whom insulin therapy is not recommended. Pharmocologic therapy is initiated with an oral agent preferably an insulin sensitizer. It is recommended to start with Metformin, a Thiazolidinedione or a sulfonylurea as monotherapy as long as no contraindication is present. Thuis view is based on proven efficacy, safety and long –term clinical experience and is consistent with the guidelines of the ADA. If the blood glucose level is especially high (>280-300mg/dl) and the patient is sympyomatic, insulin should be considered as first line therapy.

FUTURE OF DRUGS AND THERAPIES FOR DIABETES MELLITUS

Growing knowledge in the understanding of diabetes and its pathophysiology has encouraged drug companies into the development of more effective drugs. Some of the new antidiabetic drugs work on the incretin system and include injectable glucagon-like peptide-1 (GLP-1) agonists (Exenatide, Liraglutide, Albiglutide) and oral dipeptidyl peptidase-4 (DPP-4) inhibitors (Alogliptin, Linagliptin, Saxagliptin, Sitagliptin, Vildagliptin) . GLP-1 agonists potentially stimulate insulin secretion. The DPP-4 inhibitors inhibit the DPP-4 enzyme, and increase the circulating incretin hormone GLP-1. In addition, sodium glucose transport protein-2 (SGLT-2) inhibitors (Dapagliflozin, Remogliflozin, Sergliflozin) aiming to block renal glucose re-absorption via the SGLT-2 transporter are also being developed (WN Washburn, 2009).

Immunotherapies which target β-cell depletion and play immunosuppressive roles, such as β-cell depletion using an anti-C20 antibody (Rituximab), T-cell depletion with a CD3-specific antibody (Otelixizumab) and inhibitors of T-cell costimulation (Abatacept), are being developed for the treatment of T1DM.

Transplantation of whole pancreas or purified pancreatic islets continues to be an attractive therapy for T1DM (E Kroon, 2008). The major constraint to both pancreas and islet cell transplant is the availability of suitable donor tissue and rejection even in the presence of immunosuppressant, and lifelong use of these drugs minimizing the ability of immune system to combat the antigens. Ongoing research also aims at developing stem cells to generate insulin-producing β cells using either embryonic stem cells or adult pancreas precursor cells.

These putative treatments are particularly important to the 10% of T1DM patients who display erratic glycaemic control or have hypoglycaemic unawareness.

NATURAL PRODUCTS AND DIABETES

To the existence of human civilization History of medicine dates back practically. Historically and scientifically, the majority of new drugs have been generated from natural products (secondary metabolites) and from compounds derived from natural products (M Lahlou,2013) .Humans have long history to used naturally occurring substances for medical purposes. Most cultures plants plays an important role in medicinal field. Modernization of the science of chemistry at the beginning of the 19th century, plants began to be examined more closely to understand why they were medically useful (Beutler JA, 2009). Many of the currently available drugs have been derived directly or indirectly from plants sources. If we look into the relationship plants use as medicine, it can be traced back to the ancient days and they are as old as mankind itself. Natural product are historically been the major source of pharmaceutical agents which also have an important role in the development of modern medicinal system (YA Kulkarni, 2014).

Traditional medicines (TMs) make use of natural products and are of getting importance day by day. Some common forms of medicine are traditional Chinese medicine (TCM), Ayurveda, Kampo, traditional Korean medicine (TKM), and Unani employ natural products and have been practiced all over the world for hundreds or even thousands of years, and they have blossomed into orderly-regulated systems of medicine. In their various forms, they may have certain defects, but they are still a valuable repository of human knowledge (H Yuan, 2016).

In the area of drug discovery Natural products, have played an invaluable role process. Recently, there has been a renewed interest in natural products research due to the failure of alternative drug discovery methods to deliver many lead compounds in key therapeutic areas such as immunosuppression, anti-infective, and metabolic diseases. However, continuing improvements in natural products research are needed to continue to be competitive with other drug discovery methods, and also to keep pace with the ongoing changes in the drug discovery process.

Natural products have provided a important role to the pharmaceutical industry over the past half century, in the areas of infectious diseases and oncology have benefited much from

numerous drug classes derived from the natural form and as templates for synthetic modification. About 40 new drugs launched on the market between 2000 and 2010, originating from terrestrial plants, terrestrial microorganisms, marine organisms, and terrestrial vertebrates and invertebrates(Y A Kulkarni, 2014). There is an urge to find out an efficient and economic way to manage diabetes and its complications. The scientists are coming up with new synthetic molecules, but they are have a range of limitations including their side effects and the total treatment cost. On this backdrop, the use of natural products is gaining popularity among the people.

LIST OF IMPORTANT PHYTOCONSTITUENTS USED IN THE TREATMENT OF DIABETES MELLITUS

Phytoconstituents	**Plant Name**	**Part used**
Alkaloids		
Berberine	*Berberis* spp. *Tinospora cordifolia*	Roots, stem-bark
Casuarine 6-o-α-Glucoside	*Syzygium malaccense*	Bark
Catharanthine, vindoline and vindolinine	*Catharanthus roseus*	Leaves, stems
Calystegine B2	*Nicandra physalodes*	Fruits
Cryptolepine	*Cryptolepis Sanguinolenta*	Root
Harmane , norharmane,	*Tribulus terrestris*	Leaves, flowers
Jambosine	*Syzygium cumini*	Seeds, fruits, bark
Jatrorrhizine, magnoflorine, palmatine	*Tinospora cordifolia*	Stem
Javaberine A, javaberine Ahexaacetate, javaberine B hexaacetate	*Talinum paniculatum*	Roots
Lepidine and Semilepidine	*Lepidium sativum*	Seeds
Mahanimbine	*Murraya koenigii*	Leaves
Piperumbellactam A	*Piper umbellatum*	Branches
Trigonelline	*Trigonella foenum-graecum*	Seeds
1-deoxynojirimycin	*Morus alba*	Leaves, bark
Glycosides		
Kalopanax	*Kalopanax pictus*	Stem bark
Jamboline or antimellin	*Syzygium cumini*	Seeds

Myrciacitrins I and II A and myrciaphenones and B	*Myrcia multiflora*	Leaves
Neomyrtillin	*Vaccinium myrtillus*	Leaves
Perlargonidin 3-o- α - l Rhamnoside	*Ficus bengalensis*	Bark
Pseudoprototinosaponin AIII & prototinosaponin AIII	*Anemarrhena asphodeloides*	Rhizome
Vitexin, isovitexin and isorhamnetin 3-O- β-D- Rutinoside	*Microcos paniculata*	Leaves
Flavonoids		
Bengalenoside	*Ficus benghalensis*	Stem bark
Epigallocatechin gallate	*Camellia sinensis*	Leaves
(-)-3-O- galloylepicatechin, (-)-3- O-galloylcatechin	*Bergenia ciliate*	
Genistein	*Glycine* spp.	Soya beans
Hesperidin, naringin	*Citrus* spp.	
Prunin	*Amygdalus davidiana* var. *davidiana*	Stems
Kaempferitrin	*Bauhinia forficate*	Leaves
Kaempferol	*Jindai soybean*	Leaves
Kolaviron	*Garcinia kola*	
Leucodelphinidin	*Ficus bengalensis*	Bark
Mangiferin	*Anemarrhena Asphodeloides*	Rhizomes
Marsupsin, pterostilbene	*Pterocarpus marsupium*	Heartwood
Shamimin	*Bombax ceiba*	Leaves
Terpenoids and Steroids		
α -amyrin acetate	*Ficus racemosa*	Fruits
Andrographolide	*Andrographis paniculata*	Leaves
3 β -acetoxy-16 β - hydroxybetulinic acid	*Zanthoxylum gilletii*	Stem bark
Bassic acid	*Bumelia sartorum*	Root bark

Charantin	*Momordica charantia*	Seeds, fruits
Christinin A	*Zizyphus spina-christi*	Leaves
Colosolic acid, maslinic acid	*Lagerstroemia speciosa*	Leaves
Corosolic acid	*Vitex* spp.	Leaves
Elatosides E	*Aralia elata*	Root cortex
Escins-IIA and IIB	*Aesculus hippocastanum*	Seeds
Ginsenosides	*Panax species*	Rhizomes
Gymnemic acid IV	*Gymnema sylvestre*	Leaves
Momordin ic	*Kochia scoparia*	Fruit
Polysaccharides		
Aconitans A-D	*Aconitum carmichaeli*	Roots
Atractans A	*Atractylodes japonica*	Rhizomes
Ganoderans A and B.	*Ganoderma lucidum*	Fruit bodies
Galactomannan gum	*Cyamopsis tetragonolobus* *Amorphophallus konjac*	Seeds Tubers
Miscellaneous		
Allicin	*Allium sativum* *Allium cepa*	Bulbs
Curcuminoids	*Curcuma longa*	Rhizomes
Ellagitannins	*Terminalia chebula*	Fruits
Ferulic acid	*Curcuma longa*	Leaves seeds
Ginseng polypeptides	*Panax ginseng*	Roots
4-hydroxyisoleucine	*Trigonella foenum-Graecum*	Seeds

The world market for safe and effective phytomedicine is 60-100 billion US $ where India's share is 0.1 billion $. Estimated global imports of raw medicinal and aromatic plants and plant parts in the year 2001 exceeded a billion US dollars. India being the second largest exporter, next to China, of raw medicinal plant materials, accounted for about 13 % of global imports. The domestic market for medicinal plants or related products is about Rs. 4000 crores. This along with as export level of Rs. 1200 crores makes the commercialization of Indian medicinal plant sector at Rs. 5200 crores (A Subramoniam,2014) .Quality control of traditional medicines is a critical and essential issue to be considered in assuring the therapeutic efficacy, safety and to rationalize their use in the health care. For the quality control of traditional medicine, the traditional methods are procured, studied, documented

and then the traditional information about identification and quality assessment is interpreted properly in terms of modern assessment. Quality assurance is an integral part of traditional medicine, which ensures that it delivers the required quantity of quality medicament. Today, quality assurance is the thrust area for traditional formulations like churnas, bhasmas, liquid orals, Lehas, etc. (PK Mukherjee,2002)

Safety Issue of Herbal Medicines

Traditional herbal products are heterogeneous in nature. They impose a number of challenges to qualify control, quality assurance and the regulatory process. Most herbal products on the market today have not been subjected to drug approval process to demonstrate their safety and effectiveness. Some of them contain mercury, lead, arsenic and corticosteroids and poisonous organic substances in harmful amount. Hepatic failure and even death following ingestion of herbal medicine have been reported. A prospective study shows that 25% of the corneal ulcer in Tanzania and 26% of the childhood blindness in Nigeria and Malawi were associated with the use of traditional eye medicine. Side effect of some medicinal plant is currently reviewed.

Need for Clinical Trials To gain public trust

To bring herbal product into mainstream of today health care system, the researchers, the manufacturers and the regulatory agencies must apply rigorous scientific methodologies and clinical trails to ensure the quality and lot-to-lot consistency of the traditional herbal products. Since the identities of the final products are not well defined and there are essentially no purification steps involved in the productions of herbal products, the quality and lot-to-lot consistency of the products rely mostly on the quality control of source materials and their manufacturing into the final products. Using modern technologies the quality and consistency of the heterogeneous herbal products can be monitored. A well-designed clinical trial is the method of choice to prove the safety and effectiveness of a therapeutical product. Manufacturers of the herbal products must adhere to the requirements of good manufacturing practices (GMPs) and preclinical testing before these products can be tested on human.

Future Prospects of Herbal Medicine

According to WHO about 25% of modern medicines are descended from plants first used traditionally. Many others are synthetic analogues built on prototype compounds isolated

from plants. Almost, 70% modern medicines in India are derived from natural products. Proper utilization of these resources and tools in bioprospecting will certainly help in discovering novel lead molecules from plants by employing modern drug discovery techniques and the coordinated efforts of various disciplines. Tribal healers in most of the countries, where ethnomedical treatment is frequently used to treat cut wounds, skin infection, swelling, aging, mental illness, cancer, asthma, diabetes, jaundice, scabies, eczema, venereal diseases, snakebite and gastric ulcer, provide instructions to local people as how to prepare medicine from plants. They keep no records and the information is mainly passed on verbally from generation to generation. World Health Organization (WHO) has shown great interest in documenting the use of medicinal plants used by tribal from different parts of the world. Many developing countries have intensified their efforts in documenting the ethnomedicinal data on medicinal plants. Research to find out scientific evidence for claims by tribal healers on Indian herb has been intensified. Once these local ethno medical preparations are scientifically evaluated and disseminated properly, people will be better informed regarding efficacious drug treatment and improved health status (Pathak K.2013).

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Plan of Work

Title of Project: "Antihyperglycemic activity of *Litsea cubeba* fruit against Streptozotocin induced diabetes"

- Collection and identification of *Litsea Cubeba* fruit.
- Extraction of the selected fruit.
- 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:-

***Litsea Cubeba* Flower**

***Litsea Cubeba* Fruit**

SCIENTIFIC NAME:- *Litsea Cubeba Lour. Pers.*

SYNONYMS*:-* *Laurus cubeba Lour. (1790),*

Litsea citrata Blume (1826),

*Tetranthera polyantha Wallich ex Nees var. citrata Meissner (1864).(*Bhuinya Trina *et al.,* 2010*)*

LOCAL NAME:-

Assamese : Mejankeri, Mejankori.

Garo : Zeng jir, Zeng-jil, Zengjir, Zong-jil.

Khasi : Dieng sying.

Lepcha : Siltimbur.

Mizoram : Sernam.

Naga : Angetchang, Atazi, Chona, Cie, Khamthi, Kiorotond, Mekhi, Voting

Nepali : Siltemur, Siltimur, Timur.

Sanskrit : Kankolam (Chaudhury, S. N. *et al.,* 1981)

Taxonomy:

Kingdom: Plantae – Plants

Subkingdom: Tracheobionta - Vascular plants

Superdivision: Spermatophyta – Seed plants

Division: Magnoliophyta – Flowering plants

Class: Magnoliopsida – Dicotyledons

Subclass: Magnoliidae

Order: Laurales

Family: Lauraceae – Laurel family

Genus: Litsea Lam. – litsea P

Species: Litsea cubeba (Lour.) Pers. – litsea P (Chopra *et al*., 1956*)*

GEOGRAPHICAL DISTRIBUTION:-

Southeast Asian countries: India, China, Bhutan, Nepal, Myanmar, Vietnam, Korea, Taiwan and Indonesia.

India: Arunachal Pradesh, Assam, Manipur, Meghalaya, Mizoram, Nagaland, Sikkim, Uttaranchal, Uttar Pradesh and West Bengal (Chopra *et al.*, 1956).

MORPHOLOGY:-

Details of whole plant:- Small evergreen tree, unisexual, dioecious, with aromatic leaves, branches and flowers. Young shoots silky and leaf buds naked. Leaves 5.2 - 13.5 cm x 1.4 - 3.9 cm, simple, alternate, exstipulate, lanceolate, caudate-acuminate at apex, entire along margin, membraneous, bright green, glabrous; secondary veins obscure above, 10-12 pairs, inter secondary veins very common. Inflorescence in umbels, 1 0 - 1 2 mm x 6 - 7 mm, axillary, solitary, arranged in short corymbs, 4 flowered, pedunculate, bracteates; peduncles 4 – 7 mm long, glabrous; bracts 6, in 2 rows, outer two 4.5 - 5.5 mm x 4 - 6 mm, orbicular, coriaceous, glabrous, inner four 3 - 4 mm x 5 - 6 mm, concave, membranous, gland-dotted; flowers 3.7-5 mm x 4 – 5 mm, yellowish white; pedicel 1-2 mm long, green, puberulous; perianth lobes 6,2 - 3 mm x 1.5 - 2 mm, elliptic, membranous, gland-dotted, hairy; perianth

tube 0.5 - 1 mm long, puberulous. Male flowers: stamens 12, in 4 rows, outer 2 rows 3.5-4 mm long, exerted, inner 2 tows 2 - 3 . 5 mm long, glandular, filaments slender, hairy, anthers 1 - 2 mm long, 4 celled, upper 2 cells introrse, lower 2 cells partly latrorse; glands 0.5 - 0.8 mm long, 2 each at base of inner rows of stamens, 2 lobed, stalked; pistillode c. 0.5 mm long, rudimentary. Female flowers with 12 staminodes, in 4 rows, outer 2 rows 1.5-2 mm long, exerted, inner 2 rows 1-1.8 mm long, glandular, filaments tomentose; glands 0.5 - 0.8 mm long, 2 each at base of inner rows of staminodes, 2 lobed, stalked; pistil 2.5 - 4 mm long, stigma knotched at centre, ovary ellipsoid. Fruits berry, globose, 4 - 6 mm in diameter, glabrous, green (black when ripe), seated on persistent perianth tube, 1.5 - 2.5 mm diameter, plate-like; fruit pedicel 2 - 4 mm long; fruit peduncle 4 - 8 mm long. (Fig. I and II). Phenology - Flowering: November to March. Fruiting: February to July (Bhuinya Trina *et al.,* 2010).

Distribution:-

Fairly abundant in subtropical forestslopes, 300 - 1500 m. Often growing gregarious on abandoned fire lines or jhummed areas on hills, sunny slopes or thicket (Keville *et al.*, 1995).

PHYOCHEMISTRY:

Priliminary pharmacognosic phytochmical screening of methanolic extract of fruit showed that the fruit contain quinones, tannins, saponins, anthocyanins, carotenes, phlobatanins, alkaloids, flavonoids, phenolsa & cardiac glycosides.

GC-MS analyses of the methanolic extracts were performed using JEOL GCMATE II to identify the bioactive constituents. The spectrums obtained through GC-MS were finally compared with the MS data library of National Institute of Standards and Technology (NIST) for the identification of bioactive compounds such as:-

1. Butanamide, N,N,3,3-tetramethyl
2. 1,2- Cyclooctanediol
3. 2-Isopropylidene-5-methylhex-4-enal
4. 7-oxabicyclo(4,1,0)heptan-2-one,3-methyl-6-(1-methylethyl) [carvenone oxide]
5. 4,7,7-Trimethyl-3,9-dioxatricyclo[6,1,0,0(2,4)]nonan-5-one
6. α-methyl- α[4-methyl-3-pentenyl]oxiranemethanol (1,2-oxolinalool)
7. (-)-Spathulenol
8. 4H-1-Benzopyran-4-one,7-Hydroxy-2-(4-hydroxyphenyl)

9. Isoaromadendrene Epoxide
10. Corymbolone
11. 1,3,3-Trimethyl-2-oxabicyclo-(2,2,2)octane,6,7-endo, endo-diol
12. Estra-1,3,5(10)-trien-17a-ol
13. 2-Methoxy-4-[(2-pyridin-4-yl-ethylimino)-methyl]-phenol
14. Sandaracopimar-7,15-dien-6-one
15. Oleic acid
16. 4H-1-Benzopyran-4-one, 5,7-dihydroxy-2-(4-hydroxyphenyl) [Apigenin]
17. 4H-1- Benzopyran-4-one, 5,7-dihydroxy-2-(4-hydroxyphenyl)-3,7-dimethoxy
18. Morin
19. 4H-1- Benzopyran-4-one, 5,7-dihydroxy-2-(4-hydroxy-3-methoxyphenyl)-3-methoxy[10]

Free radical scavenging activity is considered to be one of the desired criteria for selection of an antidiabetic drug. In this context, several radical scavenging assays were performed to evaluate the antioxidant activities of the plant extracts in vitro e.g. DPPH redical scavenging assay, ABTS redical scavenging assay, FRAP assay, Nitric Oxide Scavenging Assay, Hydroxyl redical scavenging assay (Chakraborty Rakhi*, et al.,* 2018).

ETHNOPHARMACOLOGICAL EVIDANCE:

Genus Litsea, mainly distributed in the tropical and subtropical regions has been used in traditional and indigenous Chinese medicines for the treatment of diarrhea, stomachache, dyspepsia, gastroenteritis, diabetes, edema, cold, arthritis, asthma, pain, traumatic injury etc. for a long history. Litsea Cubeba also a traditional tee used in Relieving pain, promoting blood circulation, as well as treating stomach distension, asthma, emesia, diarrhea, turbid urine and traumatic injury (Kong De Gang *et al.,* 2014). Due to its action against psychosomaticdisorders, L. cubeba along with several other plants were regarded as sacred plants in the rituals and religious ceremonies associated with traditional healing among the ancient Chinese (Bhuinya Trina *et al.,* 2010). The small rounded fruits are used as carminative. It is also used in headache, dizziness, hysteria, paralysis Loss of memory and as a substitute for piper cubeba wall. The fruits are used dried or fresh against stomach disorders. Usually one or two fruits are used at a time. It has long been used as a folk remedy in Traditional Chinese Medicine (TCM) and Dai Ethnopharmacy for the treatment of rheumatic diseases, common cold and stomach ache in south western China (Bing Lin *et. al.,* 2012). It is also reported that fruit, bark, leaves and fruit are used as Flavoring, carminative,

diuretic, expectorant, stimulant, stomachic, antiasthmatic, arthritis, sedative, antidysentericand antiseptic, cough, cold, hair tonic, indigestion, good sleep (Wang Yun-Song *et. al.,* 2015). One raw fruit chewed as masticatory two times daily for 4–6 weeks to reduce the diabetes (Chhetri D.R.*et al*., 2005).

SCIENTIFICALLY PROVEN AND REPORED ACTIVITY:

Antibacterial Activity: The Antibacterial activity showed large variations in the composition among the different oils. The major components in the oils from roots and fruits, from stems, leaves, and alabastra, and from flowers were citral B (neral), b-phellandrene, and b-terpinene, respectively. The inhibition zone (DD) and MIC values for the bacterial strains tested, which were all sensitive to the essential oil of *Litsea cubeba* were in the range of 10.1 – 35.0 mm and 100 – 1000 mg/ml, respectively. Hence, the oils of the various parts showed moderate activity against the tested bacteria. The antibacterial activity of *Litsea cubeba* was attributed to the essential oils, thus they can be a potential medicinal resource (Wang Hongwu *et al*., 2010).

Antifungal activity: Antifungal activity of Litsea cubeba Pers. at various dilutions revealed the possibility of its utilization as an antifungal agent. The oil isolated from the fruits of *L. cubeba* was tested in vitro for its activity at different concentrations against Fusarium moniliforme, Fusarium solani, Alternaria alternata and Aspevgillus niger. The oil was found to be effective against these fungal pathogens under laboratory screening. The antifungal activities of the oil increased with an increase in the concentration. Minimum effective concentrations of the oil against fungal pathogen was also different (Hwang Jae-Kwan *et al.,* 2005).

Antimicrobial Activity: The "antimicrobial impact" (AI) is a new term that combines the effects of minimal microbicidal concentration (MMC) and quantity of an antimicrobial substance. The AI can quantitatively reflect the relative importance of individual components of the EO on the entire antimicrobial activity of the EO. The MMCs of the LC-EO were 3000, 6000, and 12,000 μg/g for *L. monocytogenes* in tofu stored at 4 °C, 25 °C, and 37 °C, respectively. The temperature affected the bacterial growth which consequently influenced the MMCs of the LC-EO. The MMCs of the LC-EO were 3000, 6000, and 375 μg/g for Vibrio spp. in oysters, *L. plantarum* in orange–milk beverage, and *H. anomala* in soy sauce, respectively (Liu Tai-Ti *et al.,* 2012).

Antioxidant Activity: The antioxidant activity of Litsea cubeba was studied in terms of three different assay systems: DPPH assay, peroxidise / guaiacol assay, and TBA test. The *L. cubeba* methanol extract and its fractions showed remarkable antioxidant activity in comparison with a-tocopherol and ascorbic acid (Hwang Jae-Kwan *et al.,* 2005).

Antianxiey Activity: Oral administration of 100, 300 and 500 mg/kg of *L. cubeba* fruit oil significantly prolonged pentobarbitone induced mouse sleeping time by 20.0, 110.8, and 159.6 %, respectively. In addition, after administration of *L. cubeba* oil, mice significantly increased the time spent in the open arms and number of entries into the open arms of an elevated plus maze compared to saline-treated mice suggesting that *L. cubeba* oil has anxiolytic activity. A tail-flick test conducted after treatment of mice with 500 mg/kg *Litsea Cubeba* fruit oil also suggested that this oil has potent analgetic activity (Chi-Jung Chenn *et al.,* 2012).

Antiinflamatory Activity: Litsea cubeba bark extract and its fractions significantly decreased the activity of myeloperoxidase catalyzing oxidation of chloride to HOCl and O production was reduced by methanol extract (0.05 y 2 mg/ml), chloroform fraction (0.025–0.05 mg/ml) and butanol fraction (0.005 mg/ml) These findings suggest that L. cubeba is beneficial for inflammatory conditions and may contain compound(s) with anti-inflammatory properties (Eun-Mi Choi *et al.,* 2003).

Anticancer Activity: Anticancer activities of the essential oils of we tested their effect on the viability of three human cancer cell lines: human oral squamous OEC-M1 cells, human hepatocellular carcinoma J5 cells, and human lung adenocarcinoma A549 cells. Cells were incubated with various concentrations of essential oils for 24 h and 48 h, and the cell viability was measured by the alamarBlue® proliferation assay. The results showed that fruit oil treatment for 24 h reduced the viability of OEC-M1 cells, J5 cells, and A549 cells, with IC50 values of around 50, 50, and 100 ppm, respectively. This is the first report on the anticancer activities of Litsea cubeba fruit essential oil against human lung, liver and oral cancer cells (Chen-Lung Ho *et al.,* 2010).

***In vitro* Hypoglycaemic and Antioxidant Activities**: Phytochemical screening of secondary metabolites in different solvent extracts showed the presence of phenols, flavonoids, alkaloids, cardiac glycosides, tannins, saponins, and anthocyanins. Methanolic extract exhibited highest antidiabetic potential with IC50 values of 514.9 µg/ml and 1435.7 µg/ml in

α-amylase and α-glucosidase inhibition assay respectively followed by ethanol extract. Significant free radical scavenging activities were also found in the alcohol extracts. GC-MS analysis revealed the presence of principle compounds like oleic acid, morin, apigenin etc. which might be responsible for hypoglycemic activity (Chakraborty Rakhi, *et al.*, 2018).

Antiasthamatic and Antianaphylactic Activites: Litsea Cubeba oil was shown to inhibit the passive cutaneous anaphylaxis (PCA) reaction in rats, and the inhibition obtained was 47.4±4.5% that of the control. In guineapigs sensitized with egg albumin, significant inhibitory effects both on anaphylactic shock induced by inhalation of antigen aerosol in vivo and on spasmodic contraction of guinea-pig ileum induced by antigen challenge in vitro were observed. Besides, the oil (30 μl/ml) was found to block and counteract the contractive activity of SRS-A on the isolated guinea pig ileum (Bochu Qian *et. al.*, 1980).

RATIONAL FOR SELCTION THIS PLANT:

Treatment of type 2 diabetes mellitus patients with sulfonlureas and biguanides is always associated with side effects. So many herbal medicine have been recommended for the treatment of diabetes. Traditionally plant based medicines are used throughout the world for a range of diabetic presentation. Some pharmacological study suggest that this plant process significant anti-diabetic activity but scientific documents are still remain unexplored. Hence primary object of this study to evaluate and substantiate the potential of the methanol extract of *Litsea cubeba* for the treatment of diabetes.

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Collection, Extraction and Phytochemical Screening

Collection Identification and Extraction

The fruis of *Litsea Cubeba* plant was collected from the Sikkim, India in the month of August. The plant species was identified and authenticated by Botanical Survey of India, Howrah. Air dried fruirts (500 grams) were powdered in a mechanical grinder and the plant materials was extracted by methanol using cold maceration method. 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^oC) for further use. The yield of the methanol fraction was about 10.22% w/w.

Qualitative Analysis

The medicinal plants are useful for healing as well as for curing of human diseases because of the presence of phytochemical constituents (A. Nostro *et al.,* 2000). Phytochemicals are non-nutritive plant chemicals that have protective or disease preventive properties. Plants produce these chemicals to protect themselves but recent research demonstrates that they can also protect against disease (M. G. Ajuru *et al.,* 2017). Therefore, determination of the phytoconstituents in a plant material or its extract is of utmost importance. Preliminary qualitative analysis has been performed to know the type of compound present in the extract. Chemical group test were performed for Alkaloids, Flavonoids, Saponins, Tannins, Steroids, Glycosides, Carbohydrates.

Chemical Tests

Test for Steroids

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

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

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

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

Test for Flavonoids

Alkaline reagent test (Ugochukwu SC *et. al.,* 2013)

2ml of extracts was treated with few drops of 20% sodium hydroxide solution. Formation of intense yellow color which becomes colorless on addition of dilute hydrochloric acid, indicates the presence of flavonoids.

Shinoda test (Palanisamy P *et al.,* 2012)

Small quantity of the extract was dissolved in alcohol. Two to three piece of magnesium followed by concentrated hydrochloric acid was added and heated. Appearance of magenta colour demonstrates presence of flavonoids.

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

1ml solution of the extract was diluted with distilled water to 20 ml and shake in a graduated cylinder for 15 mins. Development of srable 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 1 ml 5% ferric chloride solution. Greenish black coloration indicated the presence of tannins.

5 ml of extract was treated with 1ml of 10% aqueous potassium dichromate solution. Formation of yellowish brown precipitate suggested the presence of tannins.

5ml extract was treated with 1ml of 10% lead acetate solution in water. Yellow coloured precipitate indicated the presence of tannins.

Test for Glycoside (Salwaan *et al.,* 2012)

Legal's test

The extract was dissolved in pyridine and sodium nitroprusside solution added to make it alkaline. The formation of pink red to red colour shows the presence of glycosides.

Brontrager's test

A few ml of dilute sulphuric acid added to 1 ml of the extract solution. Boiled, filtered and extracted the filtrate with chloroform. The chloroform layer was treated with 1ml of ammonia. The formation of red colour shows the presence of anthraquinone glycosides.

Test for Carbohydrate

Benedict's test (Bhandary *et al*., 2012)

Test solution was mixed with few drops of Benedict's reagent (alkaline solution containing cupric citrate complex) boiled in water bath, observed for the formation of reddish brown precipitate to show a positive result for the formation of carbohydrate.

Molish test (Salwaan *et al.*, 2012)

To 2ml of the extract, added 1ml of α-naphthol solution, and concentrated sulphuric acid through the sides of test tubes. Purple or reddish violet colour at the junction of the two liquid reveals the presence of carbohydrates.

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

Mayer's test

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 colored precipitate gives positive test for alkaloids.

Dragendroff's test

0.1 ml of dilute hydrochloric acid and 0.1 ml of Dragendroff's reagent were added in 2ml solution of extract in a test tube. Development of orange brown colored precipitate suggested the presence of alkaloids.

Wagner's test

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.

Hager's test

2ml of the 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.

Test for Phenols

Test solution was mixed with 3-4 drops of $FeCl_3$. Formation of bluish black color indicates the presence of phenol.

Test for Triterpenoid

Salkowski test (Nayak *et al.*, 2011)

The test extract was treated with few drops of concentrated sulphuric acid. Formayion of yellow colour at the lower layer suggested the presence of triterpenoids.

RESULTS

S. NO.	PHYTOCONSTITUENT	PRESENCE/ ABSENCE
1.	FLAVONOIDS	+++
2.	PHENOLS	+++
3.	TANINS	++
4.	ALKALOIDS	++
5.	SAPONINS	++
6.	GLYCOSIDES	+
7.	CARBOHYDRADE	-
8.	PHENOLS	-
9.	TRITERPENOIDS	-

(+) indicates the presence of particular constituents whereas (-) indicates absence

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Toxicity Study

INTRODUCTION

The amount of pharmacological substances and chemicals being used in the human community today, have increased to almost an innumerable amount (Sperling F,1979). These may be presented today in the form or as constituents of food substances, medicines, beverages, other industrial and household products. However, these chemicals or pharmacological substances may result in chronic toxicity in the living system when used over a long period of time or acute toxicity may also occur when large quantities capable of eliciting immediate toxic effect are used. These effects may be mild or severe, depending on the nature of substance.

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 systems. It helps us to determine the quality and quantity of chemical which will turn it into poison.

The potential uses of toxicity testing data include:

Establishing the therapeutic dose.

Acquiring information about the harmful effects on specific organs.

Establishment of the mode of toxic action.

Establishment of the toxic substance as a future reference. (Sperling F, 1979).

Acute Toxicity Studies (single dose)

The aim of this study is to determine the median lethal dose (LD50) 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 is an initial assessment of toxic manifestation of the compound under investigation 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 basic on which further testing programmes are designed. These studies are done at least on two animal species and the drug is given in graded doses to several group of animals by at least two routes, one of which should be proposed route to be used in human beings. Observation period is generally 7-14 days.

It was developed in 1920's and called "classical LD50" involved 100 animals for 5 dose-groups, later in 1981 it was modified by the Organization for Economic Co-operation and Development(OECD) and reduced number upto 30 for 3 dose-groups. Due to excess of animal sacrifice we should go to alternative methods which minimize the number of animals required.

FRAME (Fund for the Replacement of Animals in Medical Experiment) believes that the lethal dose test is unnecessarily cruel and scientifically invalid. Several countries, including UK, have taken steps to ban the oral LD50. The OECD, the international governments' advisory body abolished the requirement for the oral test in 2001. Three alternative methods and these are: Fixed Dose Procedure (FDP)-OECD TG 4203, Acute Toxic Class method (ATC)—OECD TG 4234, Up-and-Down Procedure (UDP)-OECD TG 4255. These methods only consider signs of toxicity in place of death signs recorded during studies like; increased motor activity, anaesthesia, tremors, arching and rolling. Alternative methods save numbers of experimental animals (Deora PS et. al., 2010).

OTHER METHODS OF TOXICITY STUDY INCLUDE:

Sub-Acute Toxicity Studies (Daily Dose)

The aim is to identify the target organs susceptible to drug toxicity. 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. The duration of study may range from one or two years and can extend up to 7 years. 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).

Special toxicity studies

Nowadays, toxicological data on teratogenic (including the effects on reproductive functions) mutagenicity and carcinogenicity, local toxicity (for skin diseases) have become mandatory after the unfortunate episode of thalidomide disaster in 1961 which left more than 10000 newborns congenitally deformed and crippled due to phocomelia.

VARIOUS METHODS OF LD50 DETERMINATION

Litchfield and Wilcoxon 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.

Karber's method (Turner R et. al., 1965)

This method involves the administration of different doses of test substance to various groups, which has five animals each. The first group of animals is administered with the vehicle in which the test substance was dissolved or diluted in (e.g., water or normal saline). However, from the second group onward receives different doses of the test substance. The animals in each group receives specific doses, while increment in dose progresses from group to group (starting from group 2 which receives the lowest dose). The interval mean of a number of mortality recorded in each group and dose difference across the groups are key parameters in this method5. The LD is calculated using the arithmetical method of Karber.

Which is as follow:

$LD50 = LD100 - \sum(a * b/n)$

Where,

LD= Median lethal dose

LD100= Least dose required to kill 100%

a= Dose difference

b= Mean mortality

n= Group population

Lorke's method (Lorke D., 1983)

This method has two phases which are phases 1 and 2 respectively.

Phase 1: This phase requires nine animals. The nine animals are divided into three groups of three animals each. Each group of animals are administered different doses (10, 100 and

1000mg/kg) of test substance. The animals are placed under observation for 24 hours to monitor their behaviour as well as if mortality will occur.

Phase 2: This phase involves the use of three animals, which are distributed into three groups of one animal each. The animals are administered higher doses (1600, 2900 and 5000mg/kg) of test substance and then observed for 24 hours for behaviour as well as mortality4.

Then the LD is calculated by the formula:

LD50= √D0 * D100

Where,

D0= Highest dose that gave no mortality

D100= Lowest dose that produced mortality

Up and Down Method (Shetty JA., 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 LD50. 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 LD50. 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 LD50 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, 500, 1000, 1500 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. Methanolic extract of Litsa Cubeba showed no mortality or toxic effect up to 1500 mg/kg body weight in mice.

Acute Toxicity Studies

RESULT

No deaths were observed when the animals were given a dose of 1500 mg/kg body.

DISCUSSION

The index of acute toxicity is LD50. Oral administration of methanol extract of Methanolic extract of Litsa Cubeba in mice at doses up to 1500 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 Methanolic extract of Litsa Cubeba is safe up to dose of 1500 mg/kg.

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In vitro Antidiabetic Activity

INTRODUCTION

Diabetes is characterized by hyperglycemia, altered lipids, carbohydrates and proteins metabolism which affect the patient quality of life in terms of social, psychological wellbeing as well as physical health. Two forms of diabetes differ in their pathogenesis but both have hyperglycemia as a common hall marked diabetes related cardiovascular complication occur due to altered lipoprotein metabolism mediated atherosclerosis, stroke etc. Although different classes of drugs are available to control type II diabetes, still it is a challenging task to bring a better molecule which is devoid of undesirable adverse effects than existing drugs. Current management of diabetes includes observation and estimation of in vivo biological parameters in diabetes induced mices. 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 alpha 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 alpha amylase and alpha 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. Alpha (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. Alpha glucosidase inhibitors (Acarbose) which act as competitive inhibitors of intestinal alpha 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). Alpha 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 (Nair SS *et al*., 2013).

MATERIALS AND METHODS

In vitro alpha amylase inhibitory activity (Wickramaratne MN *et al*., 2016)

The α-amylase inhibition assay was performed using the 3,5-dinitrosalicylic acid (DNSA) method (GL Miller, 1959) . The MELC was dissolved in minimum amount of 10% DMSO and was further dissolved in buffer ((Na2HPO4/NaH2PO4 (0.02 M), NaCl (0.006 M) at pH 6.9) to give concentrations ranging from 10 to 1000 µg/ml. A volume of 200 µl of α-amylase solution (2 units/ml) was mixed with 200 µl of the extract and was incubated for 10 min at 30 °C. Thereafter 200 µl of the starch solution (1% in water (w/v)) was added to each tube and incubated for 3 min. The reaction was terminated by the addition of 200 µl DNSA reagent (12 g of sodium potassium tartrate tetrahydrate in 8.0 mL of 2 M NaOH and 20 mL of 96 mM of 3,5-dinitrosalicylic acid solution) and was boiled for 10 min in a water bath at 85–90 °C. The mixture was cooled to ambient temperature and was diluted with 5 ml of distilled water, and the absorbance was measured at 540 nm using a UV-Visible spectrophotometer. The blank with 100% enzyme activity was prepared by replacing the plant extract with 200 µl of buffer. A blank reaction was similarly prepared using the plant extract at each concentration in the absence of the enzyme solution. A positive control sample was prepared using Acarbose (100 µg/ml–2 µg/ml) and the reaction was performed similarly to the reaction with plant extract as mentioned above. The α-amylase inhibitory activity was expressed as percent inhibition and was calculated using the equation given below: The % α-amylase inhibition was plotted against the extract concentration and the IC50values were obtained from the graph.

% α amylase inhibition =100*((Abs100% control−AbsSample) / Abs100% Control)

Alpha-glucosidase inhibition assay (Elya Berna *et al*., 2012)

The inhibition of alpha-glucosidase activity was determined using the modified published method (R.T. Dewi *et al*., 2007). One mg of alpha-glucosidase (Saccharomyces cerevisiae, Sigma-Aldrich, USA) was dissolved in 100mL of phosphate buffer (pH 6.8) containing 200

mg of bovine serum albumin (Merck, German). The reaction mixture consisting 10 µL of sample at varying concentrations (0.52 to 33 µg/mL) was premixed with 490 µL phosphate buffer pH 6.8 and 250 µL of 5mM p-nitrophenyl α-D-glucopyranoside (Sigma-Aldrich, Switzerland). After preincubating at 37◦C for 5min, 250 µL α-glucosidase (0.15 unit/mL) was added and incubated at 37◦C for 15 min. The reaction was terminated by the addition of 2000 µL Na2CO3 200 mM. α-glucosidase activity was determined spectrophotometrically at 400nm on spectrophotometer UV-Vis Specroscopy by measuring the quantity of p-nitrophenol released from p-NPG. Acarbose was used as positive control of α-glucosidase inhibitor. The concentration of the extract required to inhibit 50% of α-glucosidase activity under the assay conditions was defined as the IC50 value.

% α glucosidase inhibition =100*((Abs100% control−AbsSample) / Abs100% Control)

Glucose uptake by Yeast cells (Abirami N *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.

% of 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-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.

RESULT:

Table 5.1: % inhibition of Alpha amylase by MELC and Acarbose

Concentration (µg/ml)	**MELC**	**Acarbose**
50	11.26 ± 1.24	35.40 ± 0.93
100	23.08 ± 1.42	46.89 ±1.529
200	33.59 ± 1.62	55.33 ± 1.76
500	53.74 ± 1.45	76.69 ±1.93

Each value expressed as Mean ± SEM

Table5.2: % inhibition of Alpha glucosidase by MESM and Acarbose

Concenration (µg/ml)	**MELC**	**Acarbose**
200	11.91 ± 3.97	82.20 ± 4.79
400	21.24 ±2.98	169.5 ± 2.72
600	35.67 ± 3.40	322.3 ± 4.54
800	47.23 ± 5.53	697.3 ± 1.27
1000	55.57 ± 6.35	820 ± 5.783

Each value expressed as Mean ± SEM.

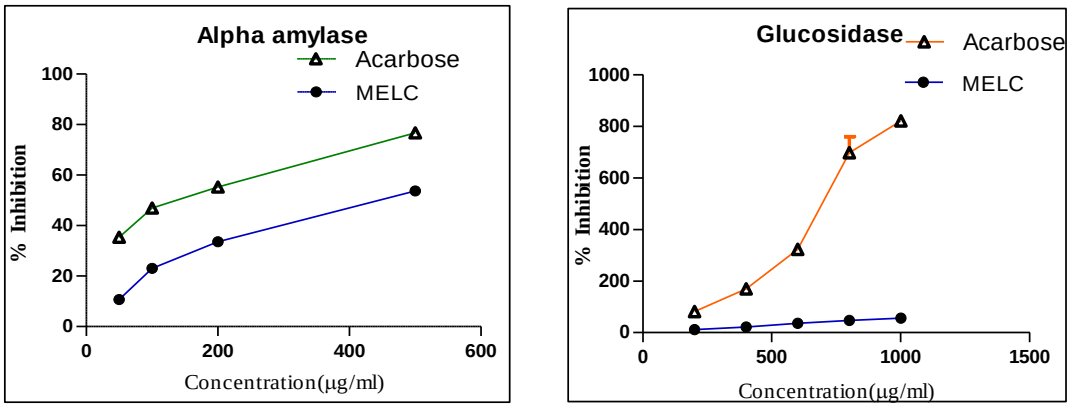

Figure 1: % inhibition of alpha amylase and alpha glucosidase shown by different concentrations of MELC and Acarbose

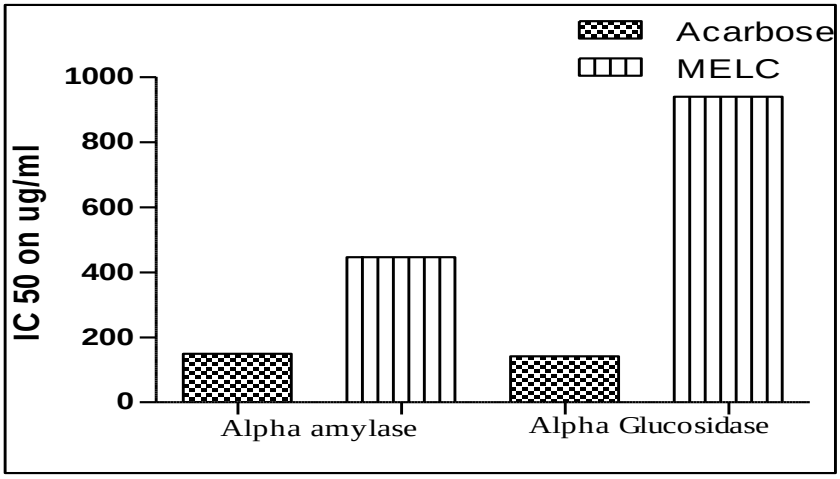

Figure 2: IC_{50} values of Acarbose and MELC in alpha glucosidase and alpha amylase.

Table 3: % Glucose uptake in yeast cells by MELC and Metformin. Each value expressed as Mean ± SEM.

Concentration of glucose (mM)	Concentration (µg/ml)	MELC	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

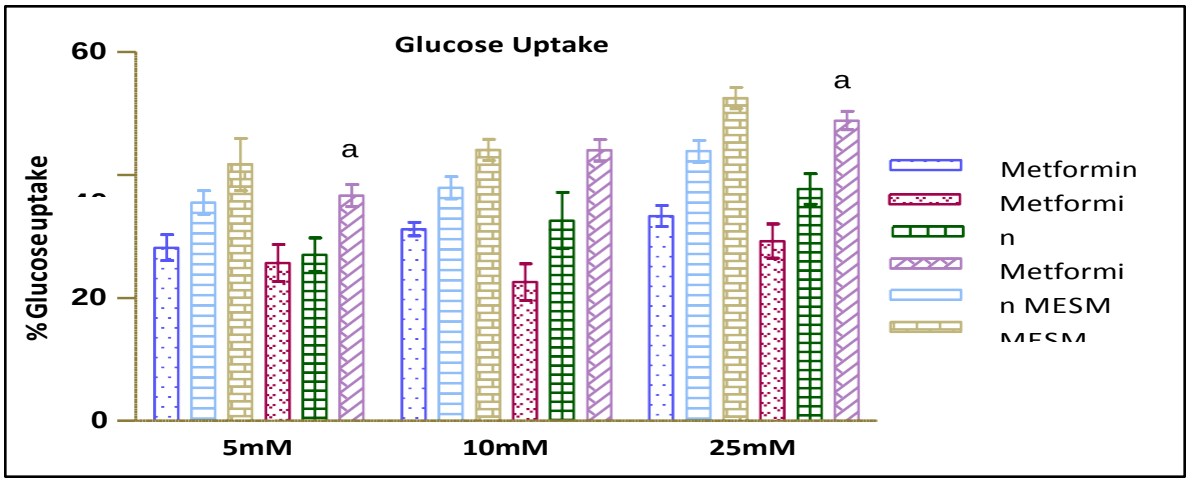

Figure % Glucose uptake in Yeast cells as shown by different concentrations of MELC and Metformin

The alpha-amylase inhibition study was conducted with different concentrations of MELC and acarbose (50, 100, 200, 500 µg/ml) and the alpha glucosidase inhibition study was conducted with different concentrations of MELC and Acarbose (25, 50,100,200, 400, 600, 800 µg/ml). In alpha amylase, IC_{50} value of MELC was found to be 446±10.05 µg/ml when compared to that of IC_{50} value of acarbose which was 149.2 ± 9.08 µg/ml. In alpha glucosidase IC_{50} value of MESM was found to be 939 ± 9.74 µg/ml when compared to that of acarbose which was 142 ± 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 MESM (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 *Litsea Cubeba fruit* (MELC). 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 (Matsui T *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 cell.

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In Vivo Antidiabetic Activity

INTRODUCTION:

Patients developing type 2 diabetes have often gone through a state of obesity associated with reduced insulin sensitivity along with an activated β-cell compensatory mechanism, such as excess basal insulin secretion and hyperproinsulinemia, as a part of their metabolic profile (Kahn SE *et al*., 1997). These pathological conditions occur early in the disease progression of type 2 diabetes (Tabak A G *et al*., 2009), and before the β-cells severely fail in late stage (insulin-dependent) type 2 diabetes (Prentki M *et al*., 2006). Nutritional overload in long term leads to obesity transition from a metabolically healthy state to an obese (Wang J *et al*., 2001) and prediabetic state involves a vicious cycle comprising hyperinsulinemia, insulin resistance, dyslipidemia inflamed and dysfunctional adipose tissue (Ravussin E *et al*., 2002), ectopic fat deposition in liver and muscle (Roden M *et al*., 1996), and failure of β-cells. To combat type 2 diabetes, there is an urgent need for more effective treatments and therapeutic regimens. Thoroughly characterized and clinically relevant type 2 diabetes animal models are required to achieve this aim of testing new and better therapeutics. Both genetic spontaneous diabetes models and experimentally-induced non-spontaneous diabetes models exist. An example of an experimentally-induced animal model of diabetes is the streptozotocin (STZ) mice model. This model involves in some cases sugar, to bring about hyperinsulinemia, insulin resistance and glucose intolerance followed by treatment with the β-cell toxin STZ, which results in a severe reduction in functional β-cell mass (Skovso S *et al*., 2014). Streptozotocin enters the pancreatic-cell via a glucose transporter-GLUT2 and causes alkylation of deoxyribonucleic acid (DNA). Furthermore STZ induces activation of poly adenosine diphosphate ribosylation and nitric oxide release. As a result of HFD/STZ action, pancreatic cells are destroyed by necrosis (Mythili *et al*., 2004). The potential problem with STZ is that its toxic effects are not restricted to pancreatic-cells since it may cause renal injury, oxidative stress inflammation and endothelial dysfunction (Lei Y C *et al*., 2005), so ways are needed to be searched to lessen its dose so that side effects could be curbed. Together, these two stressors are designed to mimic the pathology of type 2 diabetes, though on a shorter timescale than found in the human condition. The present chapter deals with the in vivo anti diabetic activity of MELC against streptozotocin induced mices. The following study deals with various parameters which should be checked as a consequence of diabetes, tissue antioxidant, serum parameters, blood glucose level, glycosylated haemoglobin and histopathology of the affected organs.

Materials and Methods

Animals

Healthy Swis Albino mices (weighing 25±5 g) of either sex were used in the study. Mices were kept in standard laborary 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 labotary condition for one week prior to the experiment.

Drugs and chemicals

Streptozotocin was purchased from HI media Labomiceories Pvt. Ltd. India and Metformin (reference drug) was from USV Private Limited, Himachal Pradesh. 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 Labomiceory, Mumbai, India; potassium dichromate, glacial acetic acid from Ranbaxy, Mumbai; and all the other reagents kits used were from Span Diagnostics Ltd. India.

Procedure:

Normoglycaemic mice: The blood glucose concentration was determined at zero time. Then by means of an oesophageal catheter, the aqueous extract 1g/kg (0.1mL) was administered orally and the blood glucose values were determined 30, 60, 90, 120, 180 and 240 min later. The values obtained were compared with the corresponding control studies from mice receiving water only. Similarly, chlorpropamide 200mg/kg (2%, 0.2mL) was given orally to fasted mice at the same intervals for the same duration (Gupta et al. 1984; Perfumi et al., 1991).

Glucose-induced hyperglycaemic mice. Initial glycaemia was determined in fasting mice. The first experiment was carried out by giving the aqueous extract 1g/kg (0.1mL) and glucose simultaneously. A dose of 1g/kg of glucose solution (50%) was administered orally. Blood samples were collected as for the normoglycaemic mice. Control studies were carried out using glucose alone. Similarly, chlorpropamide 200mg/kg (2%, 0.2mL) was given orally to fasted mice at the same interval for the same duration.

Streptozotocin-induced diabetic mice. Diabetes was induced by giving intraperitoneally (100mg/kg) 0.05 M streptozotocin dissolved in sodium citrate buffer (pH 4.5). The hyperglycaemic mice were allowed to rest for 3 days to stabilize the blood glucose level and then fasted for 18 h. The blood glucose level at zero time was determined prior to the aqueous extract 1g/kg (0.1mL) and at 30, 60, 90, 120, 180 and 240 min after administration. Control studies were carried out using water only. Similarly, chlorpropamide 200mg/kg (2%, 0.2mL) was given to diabetic mice at the same interval for the same duration. Determinations. Blood samples were added to 0.05mL disodium edetate solution. The glucose levels, in blood were analysed using the ortho-toluidine method (Bauer et al., 1974).

Induction of diabetes in mices

Male mices were fed with high-fat diet comprising 22% fat, 48% carbohydmicee, and 20% protein for 4 weeks. After the period of dietary manipulation, mices were injected intraperitoneally with low dose of STZ (40 mg/kg b.w) for consecutive five days dissolved in ice cold 0.1M citrate buffer (pH 4.5). Then animals had free acess to water and standard food (Ghorbanzadeh V *et al*., 2016). One week after streptozotocin injection, the fasting blood glucose levels were appraised and the animals exhibiting fasting blood glucose levels greater than 250 ± 30 mg/dl were considered to be type-2 diabetic (T2D) mices and included for the present investigation (Bhattacharjee N *et al*., 2016) the day on which hyperglycemia has been confirmed was designed as day 0.

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

T2DM was induced by diet ad libitum and low-dose of streptozotocin as per (Ghorbanzadeh *et al*., 2016) with some modification (Bhattacharjee N *et al*., 2016). Briefly, the mices were fed diets ad libitum for 4 weeks and then treated with single dose of streptozotocin (40mg/kg body weight intraperitoneally for consecutive five days). After 7^{th} days streptozotocin injection, the fasting blood glucose levels were appraised and the animals exhibiting fasting blood glucose levels of 250 ± 30 mg/dl were considered to be type 2 diabetic (T2D) mices and included for the further experiments. The mices were continued with high fat diets throughout the course of the study. The animals were divided into five groups (n=6) and received the treatment for 15 days:

Group I: Normal control mices were administered normal saline (0.5ml/kg orally by oral gavage) daily.

Group II: Diabetic control mices were administered normal saline (0.5ml/kg daily).

Group III: Diabetic mices were administered MELC (100mg/kg body weight) orally daily.

Group IV: Diabetic mices were administered MELC (200mg/kg body weight) orally daily.

Group V: Diabetic mices were administered MELC (300mg/kg body weight) orally daily.

Group VI: Diabetic mices were administered Metformin (200mg/kg body weight) orally daily.

FBG level was measured on day 0^{th}, 7^{th}, 14^{th}, 21^{th} and 28^{th} by using a one touch glucometer. After 24^{th} hour of last dose and 18 hours of fasting, blood was collected from all mices 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 mices from each group was measured on day 0^{th}, 7^{th}, 14^{th}, 21^{th} and 28^{th}. 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 sepamiceely 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 H *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 concentmiceions 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 200 µl tissue homogenate (liver) were mixed with PMS (186 mM), NADH (780 mM), phosphate buffer saline (200 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 200 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

Serum Liver Functin Test

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

Serum Kidney Function Test

Collected blood was analyzed for various serum biochemical parameters like Creatinine, Urea, Uric Acid. 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, triglyceride and LDL cholesterol in STZ-induced diabetic mices 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

Normoglycaemic mice glucose-induced hyperglycemic mice and streptozotocin-induced diabetic mice: The Administration of MELC at the doses of 1 g/kg significantly ($p<0.05$) lowering of the blood glucose level at both 120 and 180 min. Similarly chlorpropamide produced significant hypoglycaemia within 2 hours(Table 7.1,7.2 & 7.3).

Table 7.1: Effect of MELC and Chlorpropamid on normoglycemic mice.

GROUS	0 mins	30 mins	60 mins	90 min	120 mins	180 min	240 mins
Normal Control	125 ± 3.149	129 ± 3.60*	121 ± 3.78*	127 ± 3.98*	126 ± 5.2*	110 ± 3.1*	111.33 ±1.85*
MELC 1gm/kg	120 ±1.555	105 ±3.198*	95 ±1.915*	85 ± 2.23*	80 ± 3.312*	75 ± 2.12*	73± 1.915*
Chlorpropamide 200 mg/kg	125 ± 2.963	88 ± 2.646*	76 ± 1.155*	65 ± 1.23*	56 ± 2.23*	51 ± 2.12*	44 ± 1.202*

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

Table 7.2: Effect of MELC and Chlorpropamid on Glucose-induced hyperglycaemic mice

GROUS	0 mins	30 mins	60 mins	90 min	120 mins	180 min	240 mins
Normal Control	83 ± 2.08	129 ± 2.33*	140 ± 1.73*	117 ± 3.76*	103 ± 3.12*	95 ± 3.1*	90 ± 2.85*
MELC 1gm/kg	70 ± 2.38	112 ± 2.5*	167 ± 3.26*	85 ± 2.37*	80 ± 3.12*	75 ± 3.5*	73± 1.915*
Chlorpropamide 200 mg/kg	61 ± 2.5	102 ± 3.26*	103 ± 2.02*	65 ± 3.1*	56 ± 3.38*	51 ± 3.12*	44 ± 2.5*

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

Table 7.3: Effect of MELC and Chlorpropamid on Streptozotocin-induced diabetic mice

GROUS	0 mins	30 mins	60 mins	90 min	120 mins	180 min	240 mins
Normal Control	361 ± 5.45	354 ± 3.2*	376 ± 3.8*	354 ± 3.5*	346 ± 5.2*	330 ± 3.1*	331.33 ±1.85*
MELC 1gm/kg	251 ±1.555	212 ±3.198*	151 ±1.915*	125 ± 2.23*	100 ± 3.312*	86 ± 2.12*	73± 1.915*
Chlorpropamide 200 mg/kg	391 ± 2.963	291 ± 2.646*	250 ± 1.155*	125 ± 1.23*	113 ± 2.23*	106 ± 2.12*	123 ± 1.202

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 MELC at the doses of100, 200 and 300 mg/kg significantly ($p < 0.05$) improved of the body weight when compared to the diabetic control group (Table 7.4).

Table 7.4: Effect of MELC on Body weight

GROUPS	DAY 0	DAY 7	DAY 14	DAY 21	DAY 28
Normal Control	25 ±2.08	28 ± 2.16	32 ± 1.76	33 ± 1.75	35 ± 2.75
STZ Control	25 ±2.61*	23 ±2.35*	21 ±2.03*	21 ±4.49*	20 ±2.1*
STZ + 100 mg/kg MELC	28 ±2.33	26 ±2.19	25 ±1.45	23 ±4.29	20 ±2.9
STZ + 200 mg/kg MELC	27 ± 2.31	23±2.40	24 ±3.18*	21 ±1.45*	22 ±2.45*
STZ + 300 mg/kg MELC	28 ±1.48	24 ±2.62	22 ±3.70	23 ±6.42	22 ± 4.2
STZ+200mg/ kg Metformin	26 ± 2.963	24 ± 2.646*	25 ± 1.155*	28 ± 3.985*	28 ± 2.85*

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

Fasting blood glucose level

There was significantly ($p < 0.05$) elevated FBG level in HFD/STZ-induced diabetic mices as compared to normal control group. Administration of MELC in diabetic mices at the doses of 100, 200 and 400 mg/kg significantly ($p < 0.05$) reduced the FBG level towards normal as compared to the diabetic control group (Table 7.5).

Table7.5: Effect of MELC on Fasting Blood Glucose(mg/dl).

GROUPS	DAY 0	DAY 7	DAY 14	DAY 21	DAY 28
Normal Control	121.0±2.08	134.0±2.16	118.3±1.76	141.3±1.75	145 ±2.75
STZ Control	335.7±2.61*	379.3±2.35*	394.9±2.03*	424.8±4.49*	440 ± 5.9*
STZ + 100 mg/kg MELC	381.7±2.33	389.6±2.19	345.5±1.45	355.6±4.29	350.6±2.9
STZ + 200 mg/kg MELC	374.2±2.31	342.3±2.40	340 ±3.18*	309.5±1.45*	280.5±3.45*
STZ + 300 mg/kg MELC	406.8 ±1.48	393.6 ±2.62	376.5±3.70	354.2±6.42	301.2±3.42
STZ+200mg/ kg Metformin	391 ± 2.963	291 ± 2.646*	250 ± 1.155*	266 ± 3.985*	230 ± 3.985*

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 glycosylated hemoglobin (HbA1c)

Glycosylated hemoglobin level in STZ-induced diabetic mices was significantly ($p < 0.001$) increased compared to normal control group. Treatment with MELC at the doses of 200 and 300 mg/kg significantly ($p < 0.05$) reduced the HbA1c level when compared to the diabetic control group (Table 7.6).

Table 7.6: Effect of MELC on HbA1c

Groups	HbA1c (%)
Normal Control	5.940 ± 0.22
STZ Control (Diabetic)	9.865 ± 1.215[a]*
Diabetic + MELC(100 mg/kg)	7.565 ± 0.5050[b]*
Diabetic + MELC(200 mg/kg)	6.080 ± 0.4580b*
Diabetic + MELC(300 mg/kg)	6.345 ± 0.1250b*
Diabetic + Metformin (150mg/kg)	6.100 ± 0.400b*

Values are represented as mean ± SEM, where n=6. (a* $p<0.05$ when compared to normal control, b* all treated group vs. diabetic control group a* $p<0.05$).

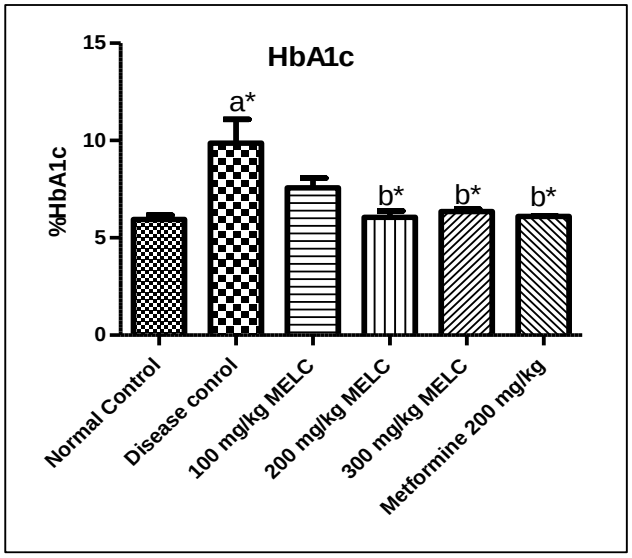


Figure 7.1: Effect of MELC on HbA1c. Each value is expressed as Mean ± SEM where n=6, a* p<0.05 when comared to normal control and b*<0.05 when compared to diabetic control

Estimation of serum lipid profiles

Serum lipid profiles like total cholesterol, triglyceride & LDL cholestrol in STZ-induced diabetic mices were significantly ($p < 0.05$) elevated and the HDL level significantly ($p < 0.05$) decreased compared to normal control group. Treatment with MELC at the doses of 200 and 400 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 7.7).

Table 7.7: Effect of MELC on Lipid Profiles

Groups	Total Cholesterol	HDL Cholesterol	Triglycerides	LDL Cholesterol
Normal Control	101.8 ± 6.195	73.99 ± 4.973	115.6 ± 11.95	29.48 ± 3.183
STZ Control (Diabetic)	201.8 ± 11.57[a]*	27.42 ± 5.817[a]*	183.7± 6.33[a]*	92.03 ± 9.048[a]*
Diabetic + MELC (100mg/kg)	157.5 ± 9.055[b]*	37.33±5.812[b]*	154.7± 4.33[b]*	82 ± 9.074 [b]*
Diabetic + MELC (200mg/kg)	141.8 ± 7.679[b]*	47.33 ± 5.812[b]*	136.7±5.487[b]*	74.67 ± 9.61 [b]*
Diabetic + MELC (300mg/kg)	133.4 ± 6.668[b]*	57.33 ± 5.812[b]*	139.7± 7.86[b]*	60 ± 5.774[b]*
Diabetic + Metformin (200mg/kg)	130.5 ± 5.804 [b]*	74 ± 3.055[b]*	122.7± 13.53[b]*	55 ± 5.77 [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* $p<0.05$).

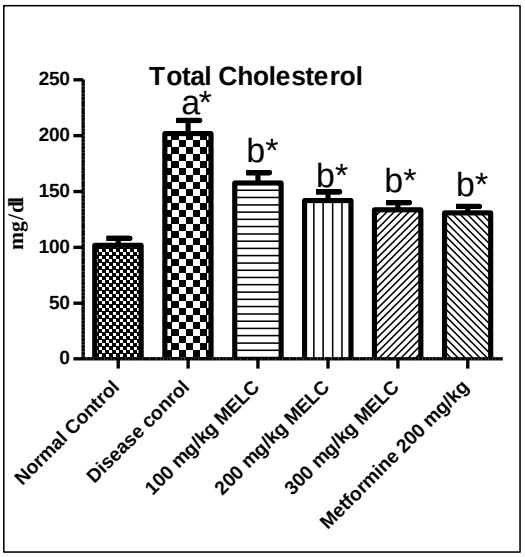


Figure 7.2: Effect of MELC on Total Cholesterol. Each value is expressed as Mean ± SEM where n=6, a* p<0.05 when comared to normal control and b*<0.05 when compared to diabetic control

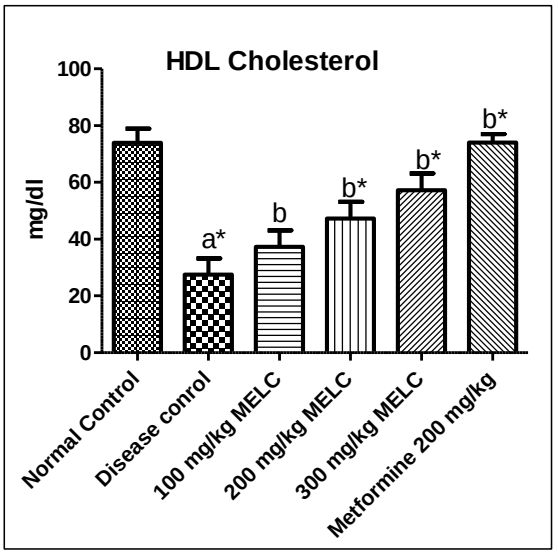


Figure 7.3: Effect of MELC on HDL Cholesterol. Each value is expressed as Mean ± SEM where n=6, a* p<0.05 when comared to normal control and b*<0.05 when compared to diabetic control

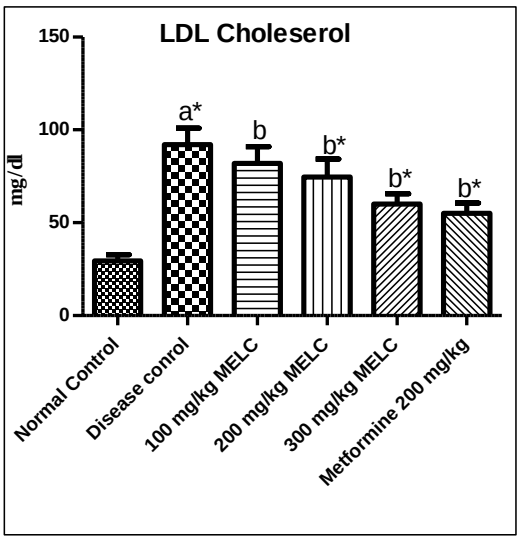


Figure 7.4: Effect of MELC on LDL Cholesterol. Each value is expressed as Mean ± SEM where n=6, a* p<0.05 when comared to normal control and b*<0.05 when compared to diabetic control

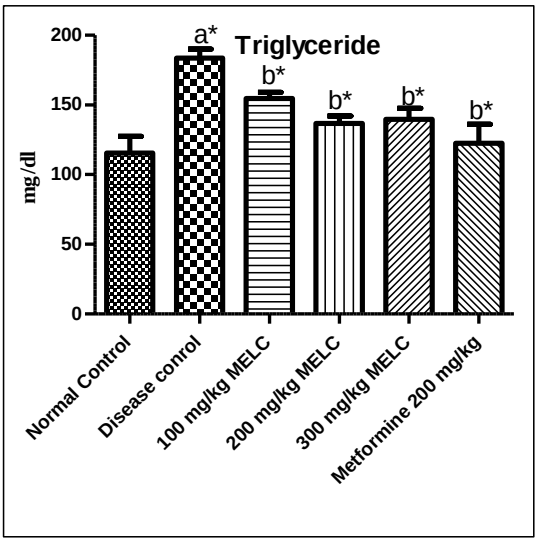


Figure 7.5: Effect of MELC on Triglyceride. Each value is expressed as Mean ± SEM where n=6, a* p<0.05 when comared to normal control and b*<0.05 when compared to diabetic control

Estimation of serum liver function test parameters

Biochemical parameters like SGOT, SGPT, SALP, Total Protein, Total Bilirubin & Albumin in STZ-induced diabetic mices 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 MELC at the doses of 100, 200 and 300 mg/kg significantly ($p < 0.05$) reduced the SGOT, SGPT, SALP, Total Protein, Total Bilirubin & Albumin levels and significantly ($p < 0.05$) increased the total protein level as compared to the diabetic control group (Table 7.8).

Table 7.8: Effect of MELC on Serum Biochemical Parameters.

Groups	SGOT (IU/L)	SGPT (IU/L)	ALP (IU/L)	Total Protein	Total Bilirubin	Albumin
Normal Control	201.7 ± 10.14	35.05 ± 5.573	25.42 ± 2.734	9.607 ± 0.373	0.751±0.152	6.33 ± 0.33
STZ Control (Diabetic)	323.3 ± 8.819	141.5 ± 5.223[a]*	44.86 ± 2.09[a]*	3.43 ± 0.546	3.851 ± 0.319	2.307 ± 0.527
Diabetic + MELC (100mg/kg)	273.3 ± 8.819	88 ± 4.163[b]*	38.02 ± 1.414[b]*	5 ± 0.316	3.072 ± 0.282	2.651 ± 0.339
Diabetic + MELC (200mg/kg)	240 ± 16.07	78.33 ± 6.566[b]*	32.08 ± 2.539[b]*	6.197 ± 0.411	2.005 ± 0.292	2.812 ± 0.676
Diabetic + MELC (300mg/kg)	225 ± 7.68	64.33 ± 7.126[b]*	30.89 ± 2.371[b]*	7.122 ± 1.142	1.355 ± 0.263	3.785 ± 0.437
Diabetic + Metformin (200mg/kg)	216.7 ± 12.02	52 ± 5.859	31.22 ± 2.026	7.371 ± 0.554	0.785 ± 0.42	4.667 ± 0.33

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

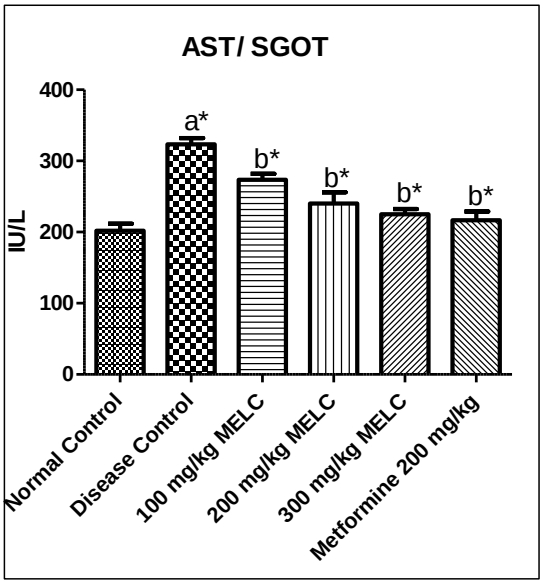


Figure 7.6: Effect of MELC on SGOT. Each value is expressed as Mean ± SEM where n=6, a* p<0.05 when comared to normal control and b*<0.05 when compared to diabetic control

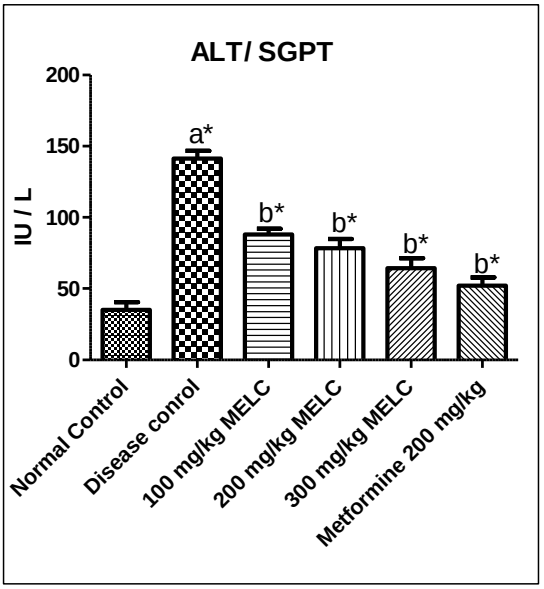


Figure 7.7: Effect of MELC on SGPT. Each value is expressed as Mean ± SEM where n=6, a* p<0.05 when comared to normal control and b*<0.05 when compared to diabetic control

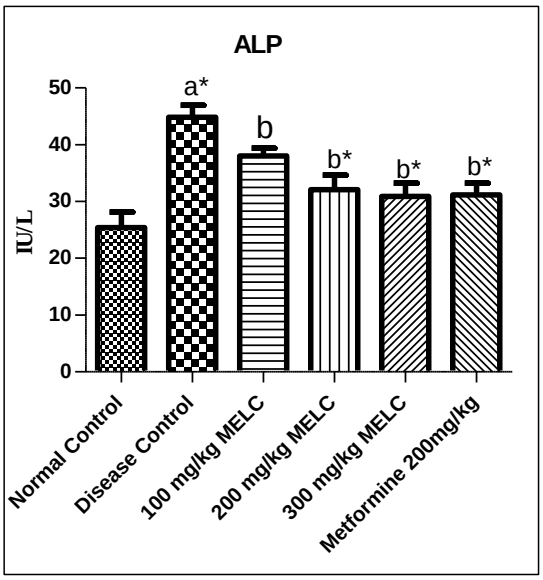


Figure 7.8: Effect of MELC on ALP. Each value is expressed as Mean ± SEM where n=6, a* p<0.05 when comared to normal control and b*<0.05 when compared to diabetic control.

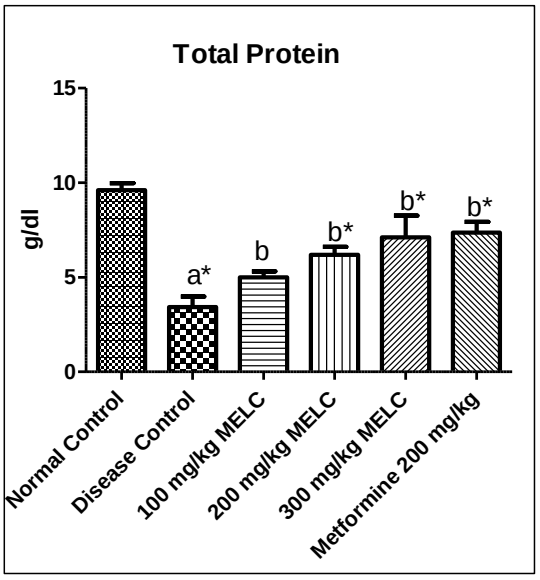


Figure 7.9: Effect of MELC on Total Protein. Each value is expressed as Mean ± SEM where n=6, a* p<0.05 when comared to normal control and b*<0.05 when compared to diabetic control.

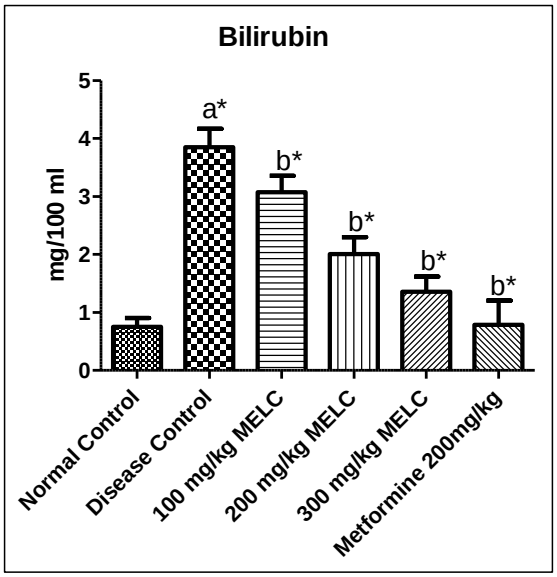


Figure 7.10: Effect of MELC on Bilirubin. Each value is expressed as Mean ± SEM where n=6, a* p<0.05 when comared to normal control and b*<0.05 when compared to diabetic control.

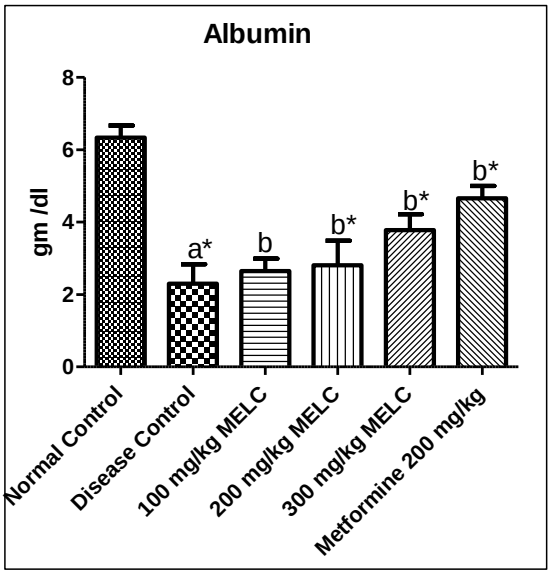


Figure 7.11: Effect of MELC on Albumin. Each value is expressed as Mean ± SEM where n=6, a* p<0.05 when comared to normal control and b*<0.05 when compared to diabetic control.

Estimation of serum kidney function test parameters

Biochemical parameters like Creatinine, Urea, Uric acid in STZ-induced diabetic mices were significantly ($p < 0.05$) elevated as compared to the normal control group. Treatment with MELC at the doses of 100, 200 and 300 mg/kg significantly ($p < 0.05$) reduced the Creatinine, Urea, Uric acid levels (Table 7.9).

Table 7.9: Effect of MELC on Serum Creatinine, Urea, Uric acid Parameters.

Groups	Creatinine	Urea	Uric Acid	Urea Nitrogen
Normal Control	2.026 ± .596	20.94 ± 1.72	3.048 ± 0.569	1.423 ± .265
STZ Control (Diabetic)	6.783 ± .714	53.23 ± 4.109	7.414 ± 0.448	3.462 ± .209
Diabetic + MELC(100mg/kg)	3.317 ± .583	35.98 ± 3.031	5.11 ± 0.506	2.386 ± 0.236
Diabetic + MELC (200mg/kg)	2.703 ± .350	28.18 ± 1.674	4.607 ± 0.510	2.151 ± 0.238
Diabetic + MELC (300mg/kg)	1.931 ± .454	22.74 ± 3.67	3.42 ± 0.392	1.597 ± 0.183
Diabetic + Metformin (200mg/kg)	1.961 ± .414	20.37 ± 3.464	3.017 ± 0.519	1.409 ± 0.242

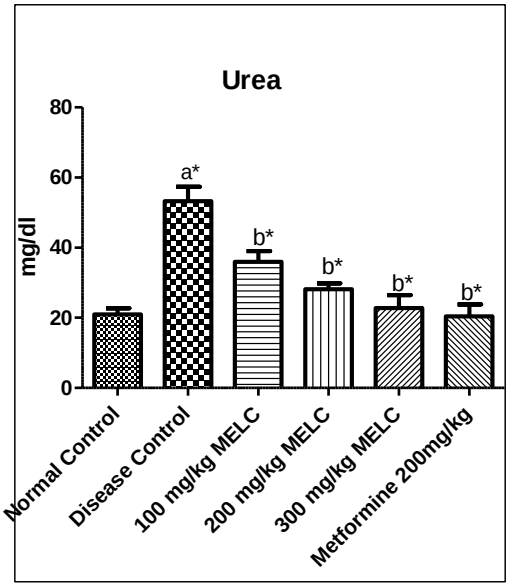


Figure 7.12: Effect of MELC on Urea. Each value is expressed as Mean ± SEM where n=6, a* p<0.05 when comared to normal control and b*<0.05 when compared to diabetic control

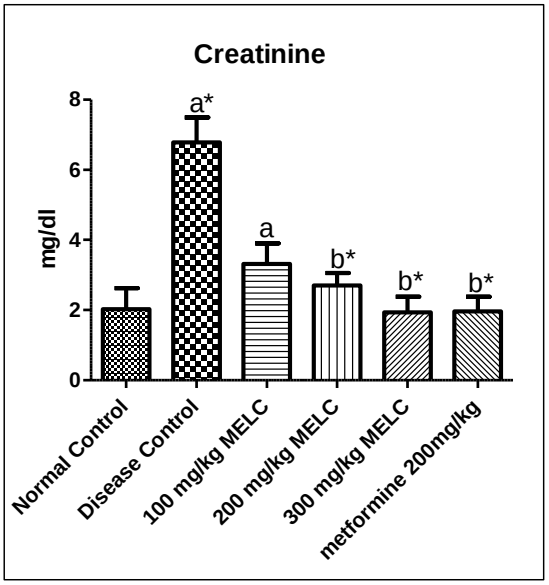


Figure 7.13: Effect of MELC on Creatinine. Each value is expressed as Mean ± SEM where n=6, a* p<0.05 when comared to normal control and b*<0.05 when compared to diabetic control

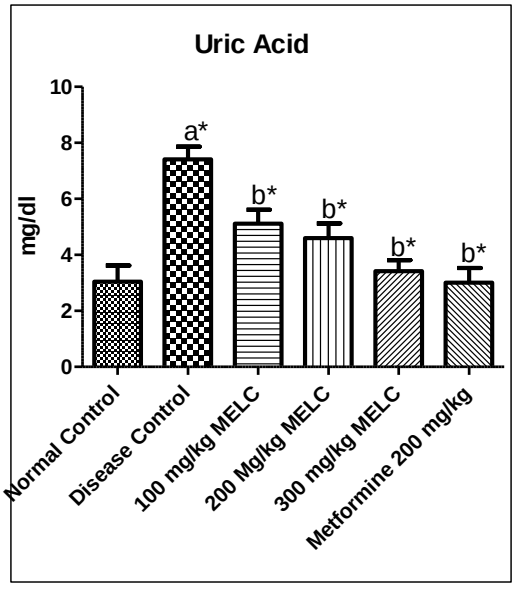


Figure 7.14: Effect of MELC on Uric Acid . Each value is expressed as Mean ± SEM where n=6, a* p<0.05 when comared to normal control and b*<0.05 when compared to diabetic control

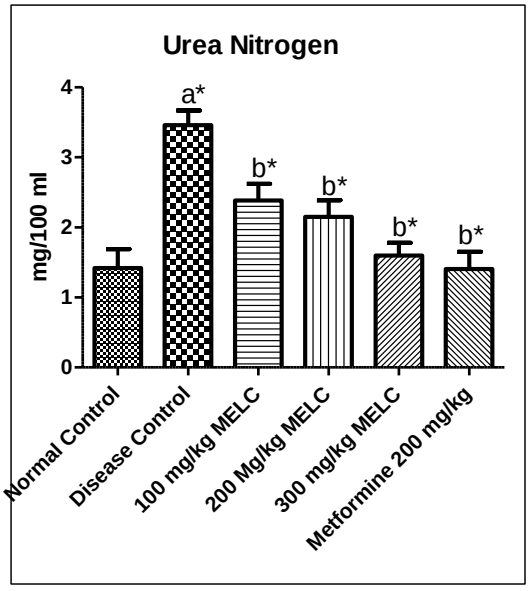


Figure 7.15: Effect of MELC on Urea Nitrogen . Each value is expressed as Mean ± SEM where n=6, a* p<0.05 when comared to normal control and b*<0.05 when compared to diabetic control

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 mices. In the present study the MDA level was significantly (p < increased in HFD/STZ-induced diabetic mices compared to normal control group. Interestingly, treatment with MELC at the doses of 100, 200 and 400 mg/kg significantly (p < 0.05) reduced the MDA levels compared to diabetic control group. The levels of reduced GSH and SOD were significantly (p <0.05) decreased in HFD/STZ-induced diabetic mices compared to normal control group. Administration of MELC at the doses of 100, 200 and 400 mg/kg significantly (p < 0.05) increased GSH, SOD antioxidant enzyme levels in the liver of HFD/STZ- induced diabetic mices compared to the diabetic control group (Table 7.10, 7.11, 7.12).

Table 7.10: Effect of MELC on MDA.

Groups	**MDA (µM/100gm Tissue Homogenate) Liver**	**MDA (µM/100gmTissue Homogenate) Kidney**
Normal Control	1.507±0.1126	1.237±0.7688
STZControl(Diabetic)	3.017±0.1184^{a*}	3.060±0.7550^{a*}
MELC (100mg/kg)	2.3±0.2082^{b*}	2.9±0.1528^{b*}
MELC (200mg/kg)	2.147±0.0933^{b*}	2.320±0.1155^{b*}
MELC (300mg/kg)	1.913±0.7219^{b*}	1.853±0.4978^{b*}
Diabetic+ Metformin (200 mg/kg)	1.973±0.1335^{b*}	1.707±0.8413^{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 7.11: Effect of MELC on SOD

Groups	SOD LIVER (U/mg)	SOD KIDNEY (U/mg)
Normal Control	3.437±0.1683	3.750±0.1790
STZControl(Diabetic)	1.043±0.6960[a*]	1.201±0.06[a*]
MELC (100mg/kg)	1.360±0.2663[b*]	1.33±0.667
MELC (200mg/kg)	1.807±0.0913[b*]	1.587±0.1225[b*]
MELC (300mg/kg)	2.560±0.2417[b*]	2.040±0.1002[b*]
Diabetic+ Metformin (200 mg/kg)	2.883±0.06[b*]	2.643±0.2631[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 7.12: Effect of MELC on GSH

Groups	GSH (µg/mg tissue) Liver	GSH(µg/mg tissue) Kidney
Normal Control	3.663±0.07688	3.570±0.1629
STZControl(Diabetic)	1.710±0.04163[a*]	1.823±0.05812[a*]
MELC (100mg/kg)	1.797±0.03930[b*]	1.89±0.03055[b*]
MELC (200mg/kg)	1.927±0.0589[b*]	1.970±0.01732[b*]
MELC (300mg/kg)	2.450±0.07550[b*]	2.177±0.091[b*]
Diabetic+ Metformin (200 mg/kg)	2.790±0.1136[b*]	2.803±0.1155[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

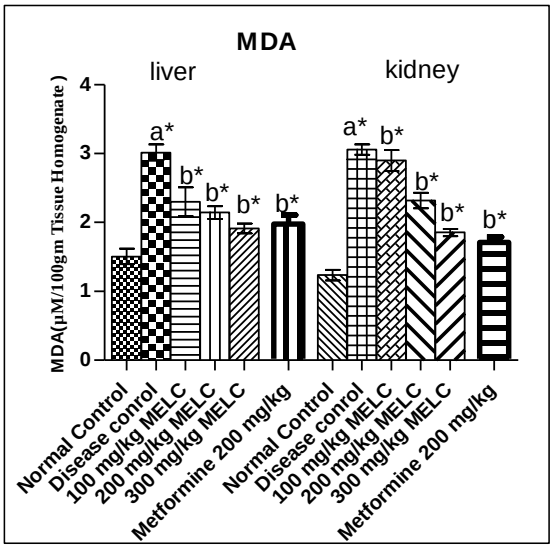


Figure 7.16: Effect of MELC on MDA. Each value is expressed as Mean ± SEM where n=6, a* p<0.05 when comared to normal control and b*<0.05 when compared to diabetic control

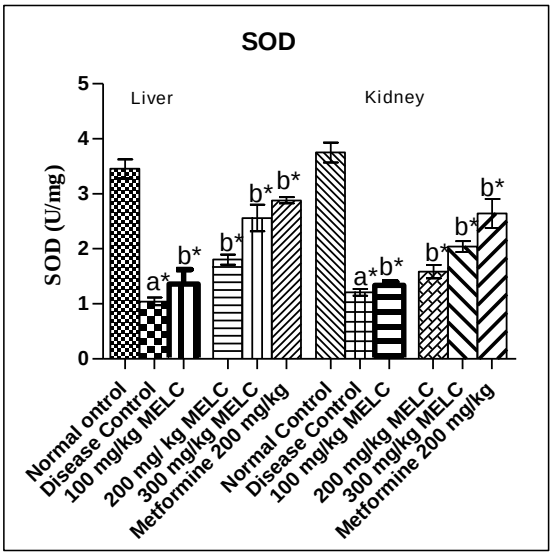


Figure 7.17: Effect of MELC on SOD . Each value is expressed as Mean ± SEM where n=6, a* p<0.05 when comared to normal control and b*<0.05 when compared to diabetic control

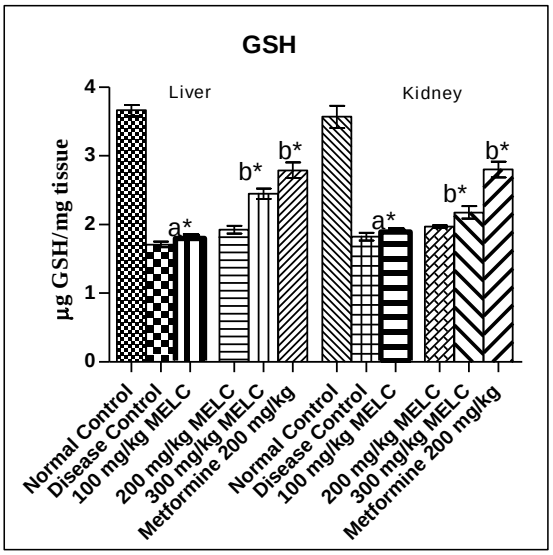
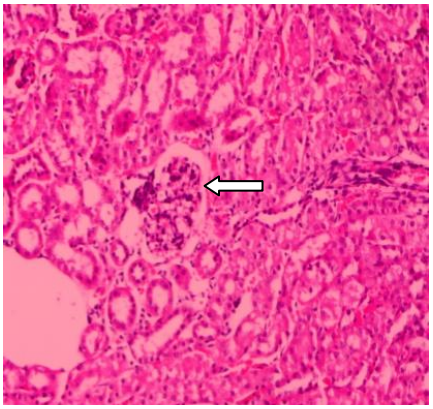
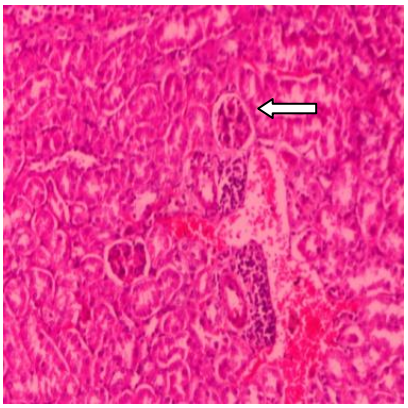


Figure 7.18: Effect of MELC on GSH. Each value is expressed as Mean ± SEM where n=6, a* p<0.05 when comared to normal control and b*<0.05 when compared to diabetic control

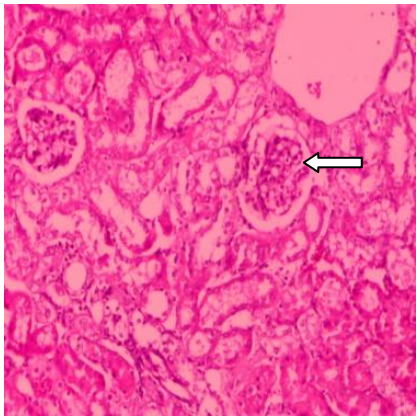
Histopathology: The sccrified animals were quickly dissected. Sample of pancreases were removed and fixed in 10% neutral formalin and Slide prepared by ASHOK LABORTARY. slides examined under olymphus microscope in 10x resolution**.**



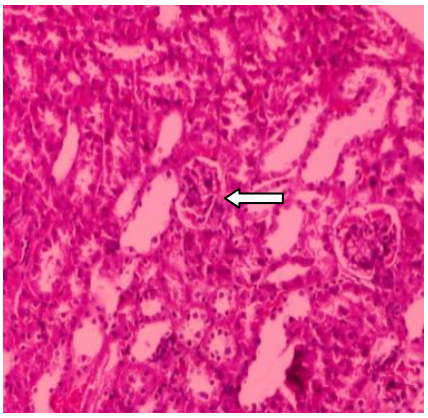
A -Kidney Normal Control



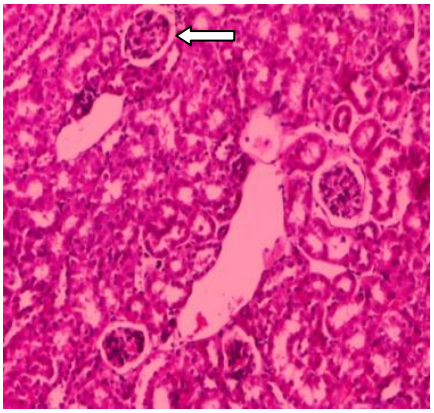
B-KIDNEY Disease Control



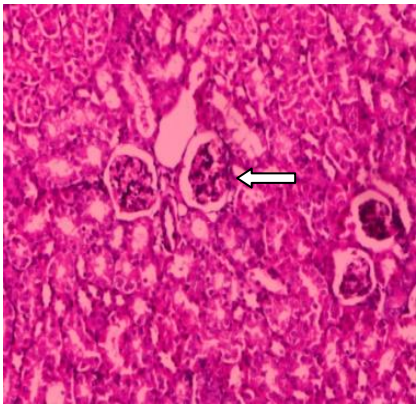
C- KIDNEY 100 mg/kg MELC



D- KIDNEY 200 mg/kg MELC

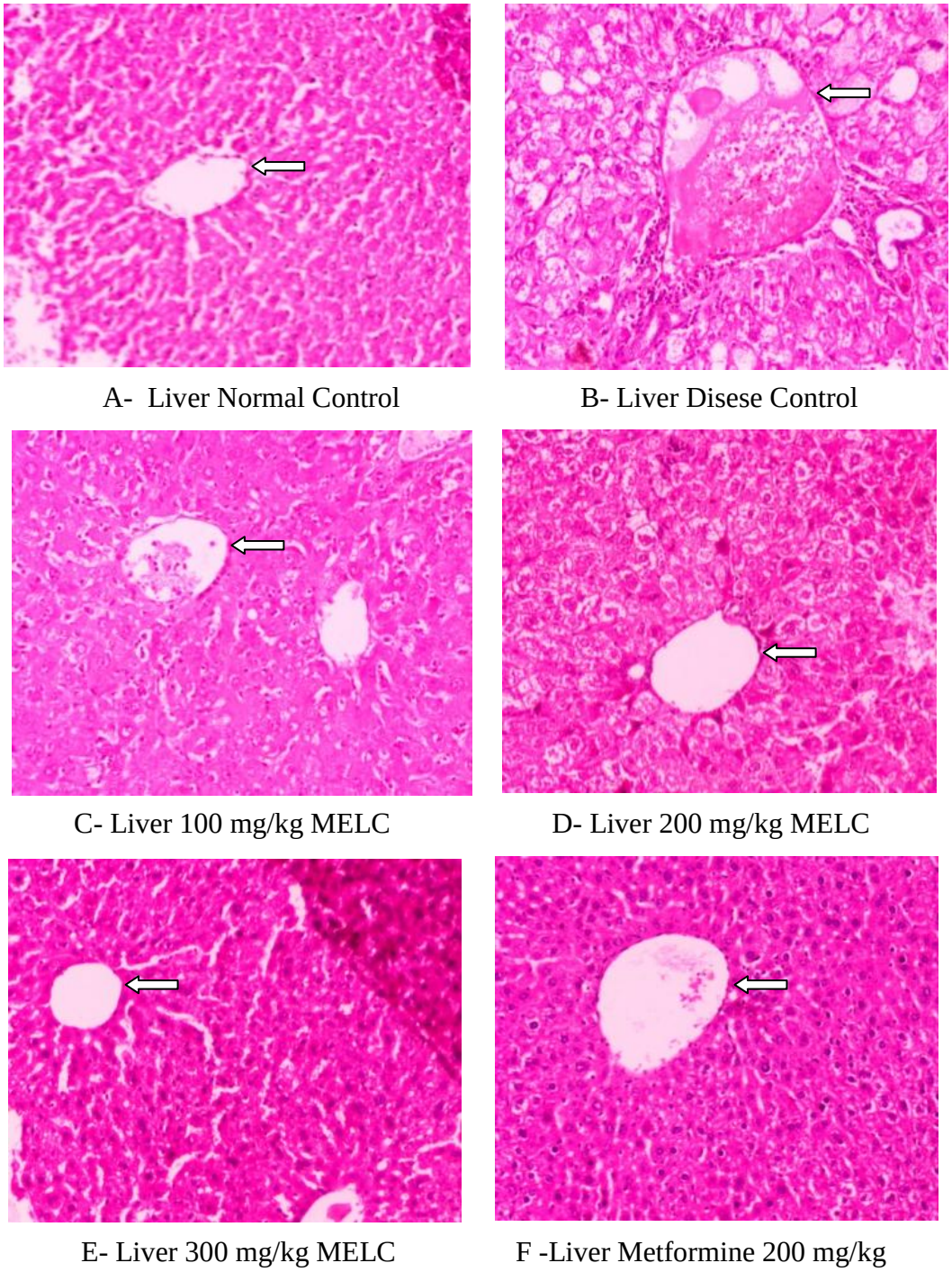


E- KIDNEY 300 mg/kg MELC



F- KIDNEY Metformine 200 mg/kg

Figure 6.12:A) Kidney gromerulus in normal control mices. B) Damage kidney gromerulus of in STZ control mices. C & D) Remnants of kidney gromerulus in MELC (100mg/kg) & (200 mg/kg) treated mices. D) Regenemiceion of kidney gromerulus in MELC (300mg/kg) treated mices. E) kidney gromerulus in good condition as seen in Metformin(200mg/kg) treated mices.



A- Liver Normal Control

B- Liver Disese Control

C- Liver 100 mg/kg MELC

D- Liver 200 mg/kg MELC

E- Liver 300 mg/kg MELC

F -Liver Metformine 200 mg/kg

Figure 6.12: A) Hepatic portal vein in normal control mices. B) Destruction of hepatic portal vein in STZ control mices. C &D) Remnants of hepatic portal vein in MELC (100mg/kg) & (200 mg/kg)c treated mices. E) Gradual regenemiceion of hepatic portal vein in MELC (300mg/kg) treated mices. E) Hepatic portal vein is very good condition as seen in Metformin(200mg/kg) treated mices.

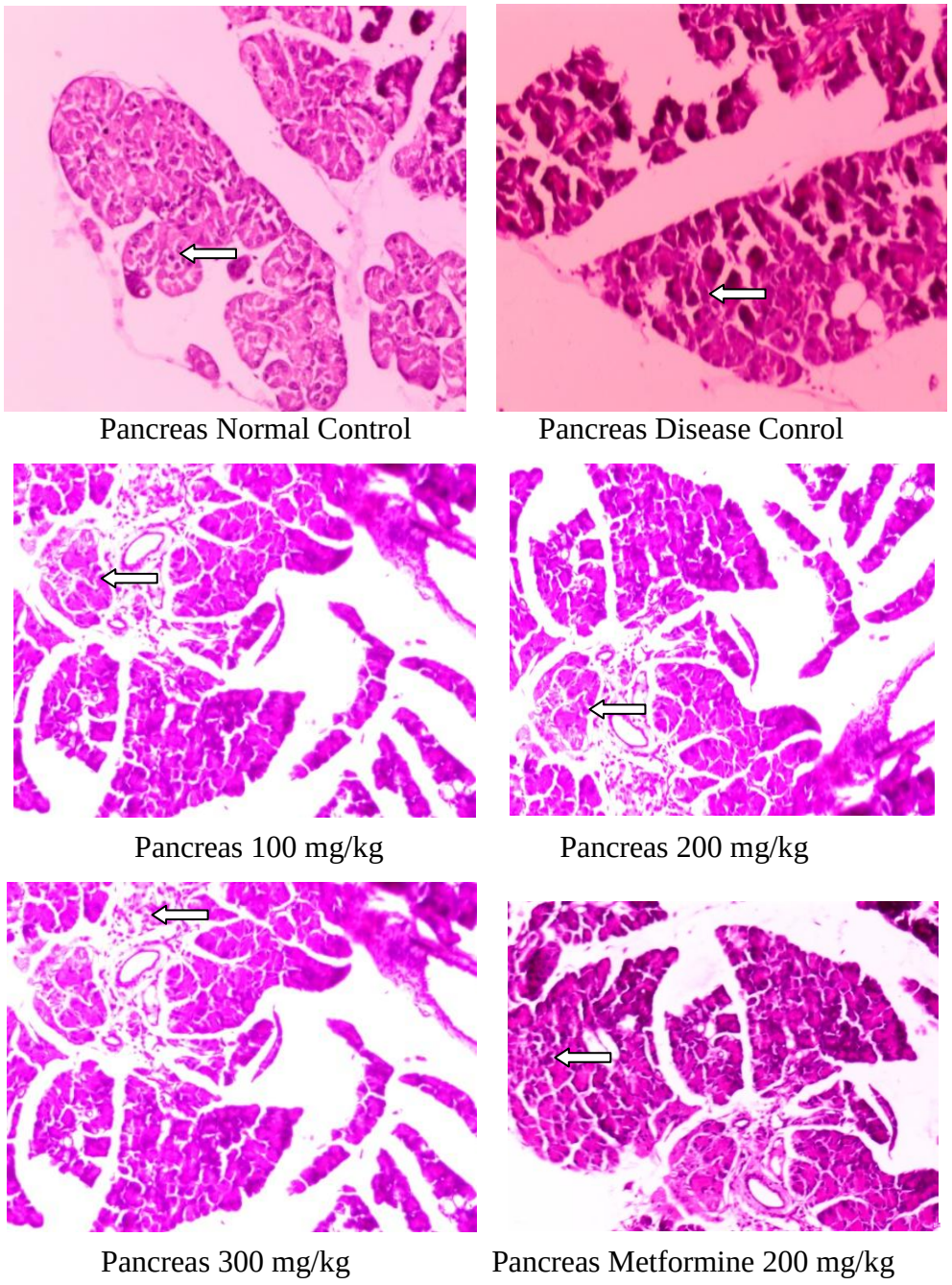


Figure 6.12: A) Pancreas in normal control mices. B) Total destruction of β-cells in STZ control mices. C & D) Remnants of β-cells in MELC (100mg/kg) & (200mg/kg) treated mices. D) Gradual regenemiceion of β-cells in MELC (400mg/kg) treated mices. E) Functioning secretory granules in the islets of β-cells as seen in Metformin(200 mg/kg) treated mices.

DISCUSSION

The present study discusses the antihyperglycemic activity of methanol extract of *Litsea cubeba* leaves in diabetic mice. It was observed that there was significant increase in the fasting blood glucose (FBG), weight, 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 mices when compared to normal control group. The treatment with MELC at doses of 100, 200 and 300 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 MELC 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 MELC leaf extract has shown the presence of alkaloid, flavonoid, steroid, triterpenoid, tannin when phytochemical screening was done. An increase in blood glucose level was observed in normal glucose administered mices than MELC treated mices while studying oral glucose tolerance test (OGTT). Oral administmiceion of MELC significantly improved the impaired glucose tolerance in the glucose loaded mices in a dose dependent manner. From the above result, the OGTT showed an increased glucose utilization triggered by insulin production from the beta cells. It was also evident from the result that MELC significantly lowered FBG level in STZ-induced diabetic mices. This gives an idea that the antihyperglycemic effect of MELC may have insulin-like action *i.e.*, action at peripheral level to increase cellular glucose uptake or secretion of insulin (Mbaka *et al.,* 2012).

The body weight was measured as an indicator of obesity Greater consumption of high energy content foods leads to an increase in the fat mass (adiposity) and fat cell enlargement (hypertrophy), producing the characteristic pathology of obesity. Adipose tissue

plays a major role in regulating whole body insulin resistance. The increase in the prevalence of obesity has been accompanied by a parallel increase in the prevalence of T2DM (Panigrahi *et al.,* 2016). In order to find the similarity in the development of diabetes in humans, induction of HFD with low dose STZ is the preferred. The mices will increase their energy expenditure and then lead towards the progression of insulin resistant in the organs. Moreover, low dose STZ induced hyperglycemia in mices where it defects the secretion of insulin. This progressive development of type 2 DM is similar to humans where several researchers agree (Reuter, 2007) Warwick et al. (2002) has reported that the amount of fat needed in high fat diet must be in the range 30% to 60% out of total diet and that is because this amount allows the changes of body weight composition, endocrine secretion and metabolic (Warwick *et al.,* 2002). The body weight of diabetic control group was decreased but at a smaller micee during the treatment period in our study. The characteristic loss of body weight is caused due to the loss of tissue protein and increased muscle wasting so there was slight decrease in weight of mices taken at various time intervals. But as atherogenic diet was continued so weight decrease was not that evident MELC fruit extract at doses 200mg/kg and 300mg/kg significantly caused weight loss in diabetic mices in Groups 3, 4 compared to diabetic control mices. The mechanism of this extract may be similar to that of metformin, because treatment with metformin is associated with weight loss, it is the first choice of drug in treatment of diabetes presented with obesity (Tripathi, 2010).

Insulin mediated glucose uptake mentioned STZ causes hyperglycemia in mices (Ananthan *et al.,* 2003). Hyperglycemia has an important role in the pathogenesis of long-term complications during diabetes. However, MELC exhibited a hypoglycaemic effect at 200,300mg/kg and significantly ($p<0.05$) decreased the glucose level at dose 400mg/kg comparable to metformin and effects were evident from the very 1st week. From this observation the inference could be drawn that MELC increase the glucose utilization which could be due to its extrapancreatic reaction (that is stimulation of cellular mechanism that consume glucose) and decrease glucose absorption.

Atherogenic diet and 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 (Bansal *et al.,* 2012). Dyslipidaemia is characterized by high plasma levels of total cholesterol, LDL cholesterol and triglycerides, with low plasma

levels of HDL cholesterol. The abnormality in lipid metabolism in type 2 diabetes mellitus has caused hyperlipidaemia in diabetic patient. Thus, diabetic mices thathave been induced low dose STZ also showed similar situations, hyperlipidaemia. This finding further support the idea of Lombardo and Chicco, where it is shown that those mices administered with HFD cause dyslipidemia and other syndromes in diabetics (Lombardo and Chicco, 2006). Besides, defect in insulin secretion due to STZ also causes defect in lipogenic activity. Insulin plays an important role in stimulating lipogenesis in mammals, by low secretions of insulin it implicates of high level of lipid in plasma (Rossi *et al.,* 2010; Gomes *et al.,* 1995) MELC significantly reduced serum triglycerides, total cholesterol and increased level of HDL in STZ induced diabetic mices.

Streptozotocin mediated persistent hyperglycemia is due to beta cells destruction, mediated and complicated by the enhanced formation of free radicals (Kaneto *et al.,*2005) . 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). Administmiceion of MELC to diabetic mices significantly reduced the glycosylated haemoglobin. Compared to Fasting Blood Glucose (FBG) and Oral Glucose Tolerance Test (OGTT), HbA1c is a better diagnostic tool to determine diabetic conditions.

Hepatotoxicity and nephropathy are complications from T2DM. Hepatocytes damage due to hepatotoxicity cause ALT and GGT enzymes leaking out into blood circulation. Elevation of serum biomarker enzymes such as SGOT, SGPT, and ALP was observed in diabetic STZ induced) mices indicating impaired liver function. Therefore, increased in the activities of SGOT, SGPT, and ALP gives an indication on the hepatotoxic effect of STZ. These findings are consistent with those of Bolkent et al. who found that high cholesterol level could cause damage to the liver (Bolkent *et al.,* 2004). So here the increase in enzymatic level may be related to hepatocellular necrosis and high cholesterol level since in our study diabetic mices also showed high cholesterol level, so it supports the idea that high cholesterol level leads to liver damage. Treatment with MELC restored all the above mentioned biochemical parameters toward the normal values in a dose-dependent manner. These observation indicates the MELC prevents further defects in liver functions.

Oxidative stress generally causes damage to the membrane polyunsatumiceed fatty acid (PUFA) leading to genemiceion of malondialdehyde (MDA) (Venukumar and Latha, 2002), a thiobarbituric acid reacting substance (TBARS). Several studies have indicated an increase in serum TBARS and a decrease in plasma SOD, GSH activity signifying an imbalance between the prooxidant and antioxidant states in the body, leading to an imbalance in systemic redox status. In the present study, we found significant decrease in serum SOD, GSH and elevated MDA content activity in diabetic control animals as compared to normal control animals, signifying an imbalance between the prooxidant and antioxidant states. The reduction in liver antioxidant status during diabetes may be result of concentmiceion against increased formation of lipid peroxides (Sabu and Kuttan, 2004). A marked increase in the concentmiceion of TBARS in STZ-induced diabetic mices 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 MELC inhibited hepatic lipid peroxidation in diabetic mices as revealed by the reduction of TBARS levels toward normal, suggesting MELC's ability to improve the pathologic condition of diabetes by inhibiting lipid peroxidation in diabetic mices, restoring balance between prooxidant and antioxidant states. It has been shown that in diabetes, that oxidative stress occurs because of body's inability to scavenge the excess production of free radicals (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 (O_2^-) and hydrogen peroxide (H_2O_2) in the biological systems, which in turn genemicee 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 peroxidise (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 mices. 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 MELC contain a free radical scavenging activity, which could exert a beneficial action against pathological altemiceion caused by the presence of

superoxide radicals and hydrogen peroxide radical. On the basis of this evidence it is possible that these activities of MELC are due to the presence of the above said phytoconstituents . Therefore, it can be concluded that the methanol extract of *Litsea cubeba* leaves is remarkably effective against high fat diet/ streptozotocin-induced diabetes in Wistar mices plausibly by virtue of its augmenting the endogenous antioxidant mechanism, lipid lowering ability, and serum glucose lowering ability.

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Conclusion

CONCLUSION:

This is a significant study investigating the effect of methanol effect of *Litsea cubeba* in STZ induced diabetic mices exploring different mechanism underlying their anti-diabetic potential exposed to 100, 200 &300 mg/kg body weight show decrease in blood glucose level.

In this research work phytochemical screening showed the presence of flavonoid, phenol, tanin, alkaloid, saponin and glycoside due to that they may show antioxidant activity, evaluation of blood glucose level control along with serum biochemical parameters, histopathological evaluation were performed.

From the above conducted study it may be concluded that the methanol extract of *Litsea cubeba* have been useful for restoring the enzyme parameters of the test subject as compare to control. It has also been helpful in restoration of the serum biochemical parameters (SGOT, SGPT, ALP, Total Protein) of the test subject. Lipid profile parameter (Total Cholesterol, HDL, LDL, Triglyceride), glycosylated hemoglobin (HbA1c) have showen good antihyperglycemic effect also.

Moreover, histopathological slides further reveald that the reduced islet cells were restored to near normal condition on treatment . Hence plant extract shows considerable potential as an antidiabetic drug.

However, just drawing interferences from phtoconstituent to be responsible for antidiabetic activity is not enough and extensive isolation characterization technique need to be employed to identify and isolate the molecule responsible for anti-diabetic activity. Also research could be further extended in more specific diabetic model involving genetic manipulation, tissue culture and other obesity induced diabetic model to confirm its potential.