ROLE OF *BASELLA ALBA* LEAVES EXTRACT AGAINST STREPTOZOTOCIN (STZ) INDUCED MICROALBUMINURIA IN RATS

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DECLARATION

I declare that "**Role of *Basella alba* leaves extract against Streptozotocin (STZ) induced microalbuminuria in rats"** 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……..

CERTIFICATE

This is to certify that **Reshma Kumari** has carried out the research on the project entitled **"Role of *Basella alba* leaves extract against streptozotocin(STZ) induced Microalbuminuria in rats"** under my supervision, in the Division of Pharmacology, Department of Pharmaceutical Technology, Jadavpur University, Kolkata – 700032.

She 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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Reshma Kumari

Date:

PREFACE

The present study entitled **"Role of *Basella alba* leaves extract against streptozotocin (STZ) induced microalbuminuria in rats."** covers original research work conducted by the author for the award of Master of Pharmacy in the Department of Pharmaceutical Technology, Jadavpur University, Kolkata for the degree of Master of Pharmaceutical Technology.

The immense wealth of the plant kingdom has become a target for the search of new drugs and lead compounds by drug companies. Their easy availability, low toxicity, lesser or almost minimal side effects has prompted us to apply medicinal plants in therapeutic management of different diseases. The traditional uses need scientific backgroumd 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 antidiabetic activity.

Reshma Kumari

CONTENTS

Sl. no	Description	Page no
1	Introduction	1-24
2	Plan of work	25-26
3	Literature Review	27-35
4	Collection, Extraction and Phytochemical Screening	36-42
5	Toxicity Studies	43-49
7	In vitro Antidiabetic studies	52-57
8	In vivo Antidiabetic studies	58-85
9	Conclusion	86-87


Dedicated
To
My Family &
my Mentor

INTRODUCTION

INTRODUCTION:

India, a country experiencing rapid socioeconomic progress and urbanization, carries a considerable share of the global diabetes burden. Studies in different parts of India have demonstrated an escalating prevalence of diabetes not only in urban populations, but also in rural populations as a result of the urbanization of lifestyle parameters. The estimated number of adults with diabetes in 2007 was 246 million; It is estimated that nearly 380 million adults worldwide will have diabetes by 2025. India has 41 million diabetics and this number is expected to increase to 70 million by 2025 (Sicree et al., 2006). The increased number of diabetics in India is likely to be due to a significant increase in the incidence of type 2 diabetes, caused by unprecedented rates of urbanization, which results in environmental and lifestyle changes. According to World Health Organization (WHO) estimates, the urban population in developing regions will increase from 1.9 billion in 2000 to 3.9 billion in 2030. Approximately 85–95% of all cases of diabetes are type 2 diabetes and the worldwide explosion of this disorder is a major health care as well as economic burden (Ramachandran and Snehalatha, 2009).

For the treatment of diabetes besides insulin, synthetic drugs like Sulphonylurea, Biguanides and Thiazolidinediones (TZD) are used successfully. However, all these agents have undesirable side effects and finally, all of them fail to restore glycemic control (Laville and Andreelli, 2000). Such side effects discourage the correct and complete observance of medication protocols by the patients. For this reason, it is highly desirable to find new anti-diabetic agents that stimulate glucose uptake by adipose or muscle cells but, unlike TZD (de Souza et al., 2001) or insulin (Laville and Andreelli, 2000), do not induce obesity or other side effects.

Ethnopharmacology and drug discovery using natural products remain important issues in the current target-rich, lead-poor scenario. Medicinal plants have provided the modern medicine with numerous plant derived therapeutic agents (Evans, 2002). These drugs are either totally natural extractives, or semi-synthetic derived from natural precursors, or model (prototype)-derived agents. Aspirin, atropine, artimesinin, colchicine, digoxin, ephedrine, morphine, physostigmine, pilocarpine, quinine, quinidine, reserpine, taxol, tubocurarine, vincristine, and vinblastine are few examples of what medicinal plants have given us in the past. Approximately 1200 plants are used worldwide for the empirical treatment of Type2-Diabetes. However, only

about 350 of them are documented to present hypoglycemiant activity (Alarcon-Aguilar et al., 2002). 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 (DM) (meli=honey) is a group of heterogenous disorders in which carbohydrate metabolism is reduced while that of proteins and lipids are increased (Sharma & Sharma, 2017) . It is a metabolic disorder characterized by hyperglycemia, glycosuria, hyperlipidaemia, negative nitrogen balance and sometimes ketonaemia. A wide-spread 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 (KD Tripathi, 2001). It arises because the body is unable to produce enough insulin for its own needs, either because of impaired insulin secretion, impaired insulin action, or both. 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 of renal failure. The hallmarks of DM are three "polys": an excessive urine production (Polyuria), an excessive thirst (Polydipsia), and an excessive eating (Polyphagia) (Sharma & Sharma, 2017).

According to WHO global report 2016, more than 422 million people live with diabetes worldwide, and the prevalence is predicted to continue rising if current trends prevail. The global prevalence (age-standardized) of diabetes has nearly doubled since 1980, rising from 4.7% to 8.5% in the adult population. This reflects an increase in associated risk factors such as being overweight or obese. Over the past decade, diabetes prevalence has risen faster in low- and middle-income countries than in high-income countries.

Several pathogenic processes are involved in the development of diabetes, these range from autoimmune destruction of the β-cells of the pancreas with consequent insulin deficiency to abnormalities that result in resistance to insulin action. Deficient action of insulin on target

tissues and hyperglycemia are the basis of the abnormalities in carbohydrate, fat, and protein metabolism causing diabetes characteristic clinical features, micro and macrovascular complications and increased risk of cardiovascular disease (Malecki *et al*., 2005).

PANCREAS:

Pancreas is a pale grey gland situated in epigastric and left hypochondriac regions of the abdominal cavity below the stomach. It has both exocrine and endocrine function, exocrine pancreas consist of secretory cell which secrets enzyme responsible for digestion of carbohydrate (pancreatic amylase), proteins (trypsin and chymotrypsin) and fats (pancreatic lipase), endocrine pancreas consist of distributed specialized groups of cell called pancreatic islets. There are three main type of cells in pancreatic islets (Waugh *et al*,. 2010). α-cells (25%) secrets glucagon which increases blood glucose level. β-cells secrets insulin which decreases blood glucose level. δ-cells (5-10%) secrets somatostatin which control release of insulin and glucagon. PP (Pancreatic Polypeptide) cells secrets pancreatic polypeptide.

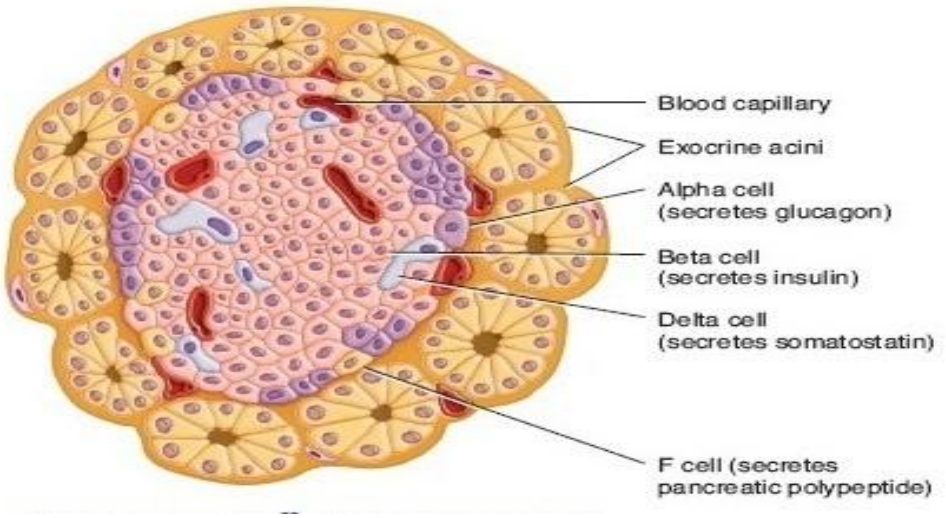

Figure 1.1: Different secretory cells of pancreas

REGULATION OF BLOOD GLUCOSE

Regulation of the levels of glucose in the blood is based on a negative feedback loop and acts via the release of insulin and glucagon. Insulin is synthesised from proinsulin by the proteolytic

degradation of proinsulin, in golgi apparatus by proteases. The action of these enzymes generates insulin and C-peptide . When glucose level is > 70 mg/dL, the β cells of the islet of langerhans in the pancreas are triggered to release insulin, metabolism of glucose generates ATP which inhibits the activity of ATP- sensitive K^+ channels. The inhibition of K^+ channel decreases the outward conductance of beta cell membrane thereby induces depolarization of the membrane and opening of voltage-dependent Ca^{2+} channels and stimulation of insulin secretion (Sharma & Sharma, 2017).

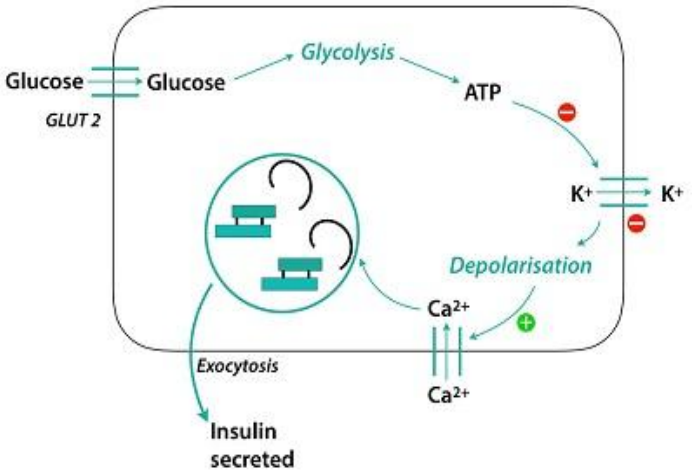

Figure 1.2:- Insulin Release from the Pancreatic β-cells.

GLUT- 2:- Glucose Transporter- 2

Insulin binds to the tyrosine kinase insulin receptor, this binding promotes autophosphorylation of the beta subunit. Insulin signals the liver to convert the excess glucose to glycogen for storage; it also triggers other cells in the body (adipose/ skeletal muscle cells) to take up more glucose by the translocation of glucose transporter (GLUT4) to the cell surface. This helps to bring the circulating glucose concentrations to normal levels. When the glucose concentration in the blood is low, the alpha cells of the pancreas are stimulated to release glucagon. Glucagon signals the liver to convert stored glycogen into glucose which is released into the blood to achieve homeostasis.

TYPES OF DIABETES MELLITUS

Type 1 Diabetes Mellitus(T1DM)

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. In T1DM, an abnormality in the HLA(human leukocyte antigen) 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, 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 (J. Larry Jameson, 2018).

Type 2 Diabetes Mellitus(T2DM)

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 Insulin Resistance (Gisela Wilcox, 2005). 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.

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. 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.

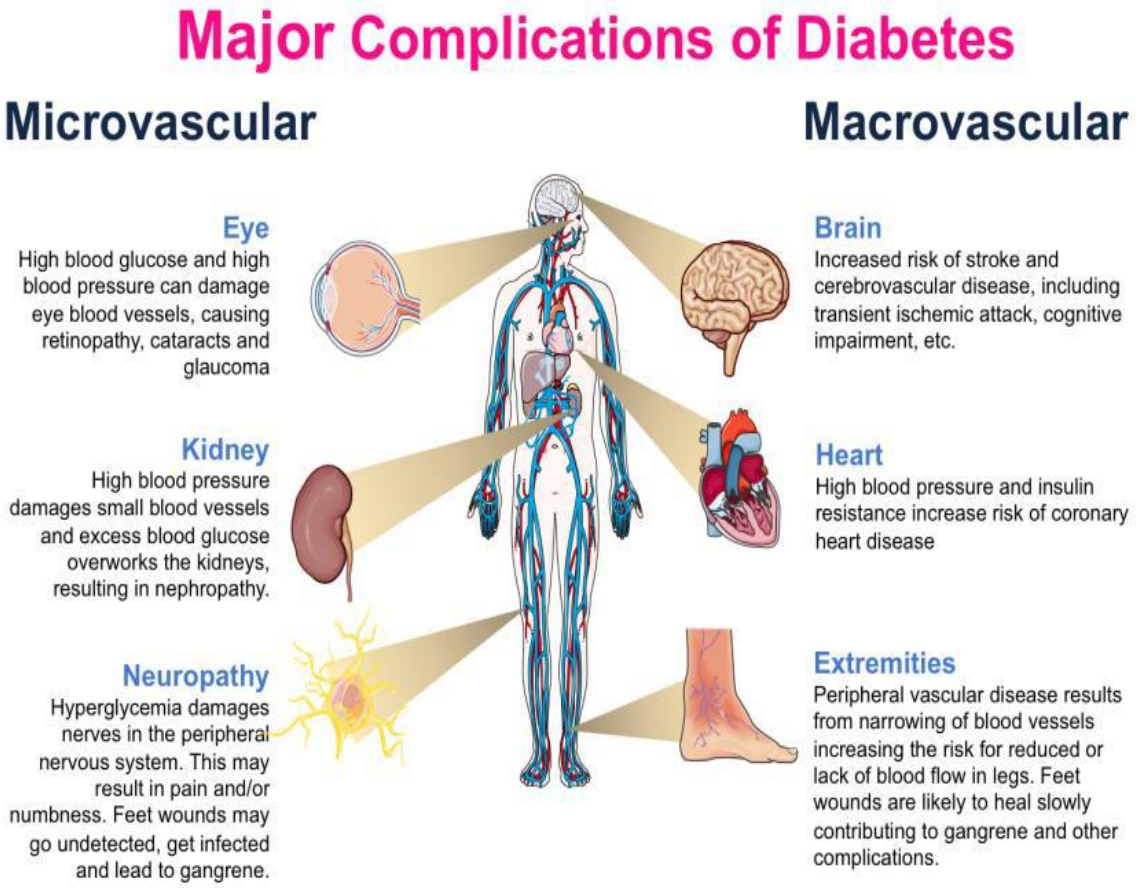

Figure 1.3:- Macrovascular and microvascular complications (secondary to Diabetes Mellitus) Diabetes is a chronic illness closely associated with the development of macrovascular complications (coronary and cerebovascular diseases), in the arteries, and microvascular (retinopathy, neuropathy and nephropathy) complications, in the capillaries.

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, also known as intercapillary glomerulonephritis, is a clinical syndrome characterized by albuminuria (>300 mg/day or >200 mcg/min) confirmed on at least two occasions 3-6 months apart, permanent and irreversible decrease in glomerular filtration rate (GFR), and arterial hypertension. Diabetic nephropathy is a chronic complication of both type 1 DM (beta cell destruction- absolute lack of insulin) and type 2 DM (insulin resistance and/or decreased secretion of insulin) (Vrhovac B *et al.*, 2008).

There are five stages in the development of diabetic nephropathy:

Stage I: Hypertrophic hyper filtration. In this stage, GFR is either normal or increased. Stage

I lasts approximately five years from the onset of the disease. The size of the kidneys is increased by approximately 20% and renal plasma flow is increased by 10%-15%, while albuminuria and blood pressure remain within the normal range.

Stage II: The quiet stage. This stage starts approximately two years after the onset of the disease and is characterized by kidney damage with basement membrane thickening and mesangial proliferation. There are still no clinical signs of the disease. GFR returns to normal values. Many patients remain in this stage until the end of their life.

Stage III: The microalbuminuria stage (albumin 30-300 mg/dU) or initial nephropathy. This is the first clinically detectable sign of glomerular damage. It usually occurs five to ten years after the onset of the disease. Blood pressure may be increased or normal. Approximately 40% of patients reach this stage.

Stage IV: Chronic kidney failure (CKF) is the irreversible stage. Proteinuria develops (albumin > 300 mg/dU), GFR decreases below 60 mL/min/1.73 m2, and blood pressure increases above normal values.

Stage V: Terminal kidney failure (TKF) (GFR < 15 mL/min/1.73 m2). Approximately 50% of the patients with TKF require kidney replacement therapy (peritoneal dialysis, hemodialysis, kidney transplantation) (Mogensen CE, 1999).

In the initial stages of diabetic nephropathy, increased kidney size and changed Doppler indicators may be the early morphological signs of renal damage, while proteinuria and GFR are the best indicators of the degree of the damage (Buchan IE, 1997).

PATHOLOGY

Glomerular filtration barrier functions as a complex biological sieve. As opposed to other capillaries in the body, glomerular capillaries are highly permeable to water (hydraulic conductivity) and relatively impermeable to large molecules. Such permeability is possible because of the unique three-layer structure of glomerular filtration membrane consisting of endothelial glycocalyx, glomerular basement membrane, and podocytes (glomerular visceral epithelial cells). Pathological changes develop in the glomeruli of patients with long-duration DM before the appearance of microalbuminuria. The severity of glomerular damage is proportional to GFR value, DM duration, and blood glucose regulation (Solini *et al.,* 2002; Rudberg *et al.,* 2000*).* The main pathohystological changes in diabetic nephropathy include the thickening of the glomerular basement membrane (GBM), mesangial expansion, nodular

sclerosis – Kimmelstiel-Wilson change, diffuse glomerular sclerosis, tubular interstitial fibrosis, and arteriosclerosis and hyalinosis of kidney blood vessels.

In addition to diabetic nephropathy, glomerular sclerosis can also develop in other pathological conditions in patients with DM. These are:

a. dysproteinemia (amyloidosis and other deposit diseases)

b. conditions with chronic ischemia (cyanotic congenital heart disease)

c. chronic membranoproliferative glomerulonephritis

d. Idiopathic diseases mostly associated with smoking and increased blood pressure (Nasr & Agati, 2007).

Biomarkers of diabetic nephropathy

Albuminuria remains the only biomarker acceptable for diagnostic purposes, although some growth factors are expected to replace albuminuria in future. It is known that values of TGF beta, vascular endothelial growth factor (VEGF), and CTGF are increased in the plasma and urine of patients with diabetic nephropathy (Nguyen *et al.,* 2008; Pfeiffer *et al.,* 1996).

Nephrosclerosis

Proteinuria and kidney failure in patients with DM may also be caused by other diseases apart from primary glomerular diseases. The most frequent cause is atherosclerotic vascular disease (nephrosclerosis) in older patients with type 2 DM (Myers *et al.,* 2003). This disease cannot be clinically discerned from diabetic nephropathy without kidney biopsy. However, kidney biopsy is not necessary in most cases, because the correct diagnosis in this patient group is not clinically important. What speaks in favor of nephrosclerosis is the significant increase in serum creatinine after the introduction of ACEI (angiotensin-converting-enzyme inhibitor) or ARB (Angiotensin II receptor blockers) for the treatment of hypertension or slowing down the progress of chronic kidney disease. The same occurs when there is a bilateral renal artery stenosis.

TREATMENT

Strict Glycemic Control

The effect of strict glycemic control depends on the DM stage in which it was started and consequent normalization of glucose metabolism. Intensified insulin therapy has the following effects on the kidney:

a. It partly decreases glomerular hypertrophy and hyperfiltration (in fasting state and after protein-rich meal), both of which are important risk factors for permanent glomerular damage.

b. It postpones the development of albuminuria (EDIC study, 2003). Intensified insulin therapy that keeps glucose values within normal ranges decreases the development or progress of diabetic nephropathy.

c. It stabilizes or decreases the elimination of proteins in patients with pronounced proteinuria. This effect is not apparent in patients who are not relatively normogycemic during two years. Furthermore, re-established normoglycemia after combined kidney and pancreas transplantation in patients with type 1 DM has preventive effects on recurrence of nephropathy in kidney transplant (Fioretto P *et al.*, 2006).

d. It slows down the progress of kidney disease in case of already developed proteinuria confirmed by semiquantitative method (test strip).

e. It reduces mesangial cell number and mesangial matrix.

f. In some patients, the thickness of glomerular and tubular basement membranes and mesangial cell number become normal and glomerular nodules disappear.

g. The progress of tubular atrophy is slowed down.

Strict Blood Pressure Control

Strict blood pressure control is important in the prevention of progress of diabetic nephropathy and other complications in patients with type 2 DM. According to the UKPDS (United Kingdom Prospective Diabetes Study) study, a reduction in systolic blood pressure by 10 mm Hg decreases the risk of development of diabetic complications by 12%; the risk is the lowest where

systolic blood pressure values are below 120 mm Hg (Ferrario CM, 2006). The Irbesartan Diabetic Nephropathy Trial showed that decreasing systolic blood pressure to the lower limit value of 120 mm Hg reduces the risk of cardiovascular mortality and heart failure (but not of myocardial infarction) and the risk of double increase in serum creatinine or progress to terminal kidney failure (Berl *et al.*, 2005).

Inhibition of Renin-Angiotensin-Aldosterone System

Angiotensin II is the most effective factor of renin-angiotensin-aldosterone system (RAAS), resulting from a range of proteolytic reactions that begin with the conversion of angiotensinogen to angiotensin I (AT1) through the catalytic action of rennin. RAAS is directly associated with blood pressure regulation, body fluid volume, and vascular response to injury and inflammation. Inappropriate activation of this system increases the blood pressure and has anti-inflammatory, prothrombotic, and proatherogenic effects, which in the long run lead to irreversible damage of target organs. Angiotensin II(AT2), which is produced in the heart, brain, and kidneys through alternative pathways by kinase and endopeptidase activity, is more effective than angiotensin II produced in the bloodstream (Cooper ME, 2004). Angiotensin II binds to AT1 i AT2 receptors. AT1 receptor activation is responsible for vasoconstriction, release of aldosterone, vascular remodeling, oxidative stress, and has anti-inflammatory, proatherogenic, and prothrombotic effects (Hilgers & Mann, 2002). In 1986, Zatz *et al.,* provided evidence that RAAS plays a role in the pathogenesis and progress of diabetic nephropathy by proving that enalapril decreases glomerular capillary hypertension, structural glomerular damage, and proteinuria in diabetic rats. Later studies have confirmed that angiotensin II plays the key role in the functional and structural changes linking proteinuria with the development of diabetic nephropathy. Increasing ACEI and ARB dosages above the recommended values for the treatment of hypertension or their combination is very effective in reducing albuminuria (Jacobsen *et al.,* 2003). Aldosteron receptor antagonists and renin inhibitors also decrease albuminuria in patients with DM, but large randomized trial are needed to determine their possible advantage over ACEI and ARB either as monotherapy or combined therapy (Estacio RO, 2009).

Dyslipidemia

Dyslipidemia occurs in all patients with DM, and its occurrence increases with the development of diabetic nephropathy. Aggressive plasma lipid reduction is an important therapeutic intervention, because patients with DM have an increased risk of coronary disease. In addition, dyslipidemia contributes to the development of diabetic nephropathy. Treating dyslipidemia with statins slows down the progression of diabetic nephropathy (Tonolo *et al.*, 2006).

The Role of Other Factors

Transforming growth factor beta (TGF-beta) has effects on cell hypertrophy and increased collagen synthesis. Inhibition of TGF-beta in experimental DM model prevented the development and progression of diabetic nephropathy (Benigni *et al.,* 2003).Experimental studies have shown that non-dihydropyridine calcium channel blocker (diltiazem) slows down the progression of most morphological changes in diabetic nephropathy (Gaber *et al.,* 1994). On the other hand, diltiazem monotherapy leads to the increased tubulointerstitial fibrosis and global, but not segmental, glomerulosclerosis. This negative effect of diltiazem can be corrected by ACEI therapy. Peroxisome proliferator-activated receptors (PPAR) play a significant role in the regulation of adipogenesis, lipid metabolism, insulin sensitivity, inflammation, and blood pressure control; however, they also seem to play a significant role in the development of diabetic nephropathy in type 2 DM patients (Guan Y, 2004). In an experimental animal model of diabetic nephropathy, PPAR gamma agonists, such as tiazolidinedones (oral hypoglicemic agents), were shown to reduce fibrosis, mesangial proliferation, and inflammation (Weissgarten *et al.,* 2006).

New Treatment Strategies

Current treatment has not always been effective in all patients. Therefore, new treatment options are being investigated. High doses of thiamine and its derivative benfotiamine (S-benzoylthiamine Omonophosphate) were shown to slow down the development of microalbuminuria in animal models, most likely by decreasing the activation of PKC, protein glycation, and oxidative stress (Babaei-Jadidi *et al.,* 2003). In experimental animals treated with ALT-711, which metabolizes AGEs (advanced glycation endproducts), a decrease in blood pressure and kidney damage was observed . PKC-beta inhibitor (ruboxistaurin) normalizes

GFR(Glomerular Filtration Rate), reduces or decreases albuminuria, and improves kidney function in experimental animals (Kelly *et al.*, 2003). Pimagedin (second generation AGE inhibitor) reduces albuminuria and GFR decrease in patients with type 1 DM and proteinuria (Bolton *et al.*, 2004).

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 (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 (Genco RJ, 2004) and fungal infections of feet (especially between the toes) are more frequent in patients with diabetes than in nondiabetics. 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 *et al.*, 2007).

MANAGEMENT OF DIABETES:

The aim of the treatment is primarily to save life and alleviate symptoms. Secondary aims are to prevent long term 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) (Bastaki S, 2005).

TREATMENTS FOR DIABETIC KIDNEY DISEASE:

Many factors initiate or promote DKD (Diabetic Kidney Disease). Initiators include hyperglycemia and altered expression of certain genes. Promoters include hyperglycemia, hypertension, dyslipidemia, smoking, ethnicity, sex, age, and a long diabetes duration. The aim is to target modifiable initiators and promoters (Macisaac *et al.*, 2014; Kim *et al.*, 2017).

CONTROL OF GLUCOSE LEVELS:

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. 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).

Use of 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)
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,	Selective agonist of paroxysome	Improve glycemic	Weight gain,liver toxicity,fluid

	Pioglitazone	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	control,lower in circulation HbA1c, lowering of serum triglyceride and increment in serum HDL level.	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.
DPP-4 inhibitors	Sitagliptin, Vildagliptin, Saxagliptin	inhibit the DPP-4 enzyme, increasing the level of endogenous plasma glucagon-like peptide-1, which promotes insulin secretion but inhibits glucagon secretion;	Decrease Albuminuria, fibrosis and thickening of GBM	Nasopharyngitis, GIT distress and diarrhoea.
SGLT-2 inhibitor	Dapagliflozin, Canagliflozin	Inhibits renal glucose re-absorption	Lower BP and reduce body weight.	Because of more polyuria, there would be more polydipsia,urinary bacterial/fungal infection.

CONTROL OF BLOOD PRESSURE(BP)

BP control is generally recommended to prevent stroke, cardiovascular disease, and albuminuria. Many studies have shown that BP control is renoprotective (Muskiet *et al., 2014).* The UKPDS suggested that a 10-mmHg decrease in systolic BP was associated with reduced levels of diabetic microvascular complications, including nephropathy (Alder *et al.,* 2000). Therefore, to prevent

the development and progression of DKD, the ADA(adenosine deaminase) recommends that treatment should aim to reduce the BP below 140/90 mmHg (ADA, 2016). Angiotensin II receptor blockers (ARBs) or angiotensin-converting enzyme (ACE) inhibitors are recommended to control BP (Taler *et al.*, 2013).

EMERGING THERAPEUTIC TARGETS

Autophagy

Autophagy is the process by which mammalian cells degrade their own macromolecules and organelles to maintain intracellular homeostasis, and it protects organisms organisms against diverse pathologies, including infections, cancers, neurodegeneration, aging, and heart disease (Shintani & Klionsky, 2004). Recently, autophagy has been implicated in the pathology of a number of diseases, including cancer and diabetes (White E, 2012; Masini M, 2009). However, accumulating evidence suggests that autophagy plays a cytoprotective role in the kidney . Renal autophagy is activated under certain stress conditions, including oxidative stress and hypoxia, and in kidneys of diabetic animal models, but is suppressed in obese patients with type 2 diabetes (Yamahara K, 2013; Mizushima N, 2004). Podocytes require a basal level of autophagy to maintain cellular homeostasis, whereas proximal tubular cells use autophagy as a coping mechanism when nephrotoxic stress is in play. Therefore, if a treatment for diabetic nephropathy were to seek to regulate autophagy, the specificity of such treatment might be problematic (Kume S & Koya D, 2015).

Src family kinases

The Src family kinases (SFKs) are non-receptor tyrosine kinases activated by autophosphorylation of their Tyr416 residues, and are induced by certain cytokines and growth factors including TGF-β1 and epidermal growth factor (Bromann *et al*., 2004). The SFKs include Src, Fyn, Yes, Yrk, Blk, Fgr, Hck, Lck, Lyn, and the Frk subfamily proteins; all contain a unique amino-terminal region, highly conserved kinase domains, and carboxy-terminal tails containing the (negatively) regulatory tyrosine residue . Src, Fyn, and Yes are expressed in most tissues, but their activities are tissue-dependent; the other SFKs are typically expressed in hematopoietic cells.

Recent studies have suggested that the Src is a potential target when it is sought to treat kidney fibrosis. High glucose levels activate the Src of mesangial cells and the glomeruli of rats with streptozotocin (STZ)-induced diabetes (Mima A, 2006; Taniguchi K, 2013)

CONCLUSIONS

The incidence of DKD is expected to increase as the incidence of diabetes rises. Although control of glucose and BP levels sometimes prevent DKD development, many patients with diabetes progress to ESRD (end-stage renal disease).

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PLAN OF WORK

THESIS TITLE: Role of *Basella alba* leaves extract against streptozotocin (STZ) induced Microalbuminuria in rats.

- Literature Review and selection of plant
- Collection and identification of *Basella alba* leaves.
- Extraction of the selected plant
- Preliminary phytochemical study of the extract
- Determination of LD50 value by OECD guidelines (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 vitro* anti-oxidant activity.
- Induction of Diabetic nephropathy in rats.
- Evaluation of *in vivo* anti-diabetic potential
    - Oral glucose tolerance test (OGTT)
    - Blood glucose level
    - Glycosylated haemoglobin (HbA1c)
- Urine parameters
    - 24 hrs urine volume
    - Urine albumin excretion
    - Creatinine clearance
    - Urea clearance

- Tissue antioxidant parameters
    - Lipid Peroxidation
    - Superoxide dismutase
    - Reduced glutathione
- Serum biochemical parameters
    - SGOT (Serum glutamate oxaloacetate transaminase)
    - SGPT (Serum glutamate pyruvate transaminase)
    - ALP (Alkaline phosphatase)
    - Total Protein
    - Total Cholesterol
    - Triglyceride
    - HDL Cholesterol
- Histopathology

LITERATURE REVIEW

NAME:

Basella alba

PLANT TAXONOMY (Adhikari *et al., 2012)*:

Kingdom	**:**	Plantae
Phylum	**:**	Magnoliopsi
Class	**:**	Magnoliopsida
Order	**:**	Caryophyllales
Family	**:**	Basellaceae
Genus	**:**	Basella
Species	**:**	Alba

SYNONYMS (Kumar *et al.*, 2013)

Basella cordifolia Linn.

Basella lucida Linn.

Basella rubra Roxb .

LOCAL NAME (Deshmukh & Gaikwad, 2014):

Arabic: Malabar spinach,

Chinese: lu luo kui,

French: baselle,

Gujarati: poi-mopal,

Nepalese: poi sagg,

Tamil: pasali,

Telugu: baccali,

Bengali: puishak,

Hindi: lalbachlu,

Oriya: poi saga, **Sanskirt:** upodika,

Telugu: bachhali (Adhikari et al., 2012).

PARTS USED:

Leaves, stem, seed, fruit, flower

ETHNOBOTANY:

Basella alba has been used for many of its useful product from ancient times. Nowadays its properties have been utilized for the extraction of some useful material so that it can be used for the beneficial human activities (Roshan Adhikari *et al.,* 2012). *Basella alba* is a very popular vegetable in many coastal communities of Southern Nigeria and is one of the chief source of major ingredients in the Northern and Northeastern food (Deshmukh and Gaikwad, 2014).

USES:

The plant is used in wound healing activity, antimicrobial activity,antidiabetic, antiviral activity, anti inflammatory activity, anti ulcer, androgenic potential (Deshmukh and Gaikwad 2014), hepatoprotective activity (Adekilekun *et al.,* 2012), anti oxidant activity (Reshmi *et al.,* 2012), steroid production in leydig cells (E. A. Nantia *et al.,* 2011).

GEOGRAPHICAL DISTRIBUTION:

Basella alba is an edible perennial vine, widely distributed in the tropics and often cultivated in warm temperate areas of both the eastern and western hemisphere (Singh M1 *et al.,* 2016). It is a widely cultivated, cool season vegetable with climbing growth habit. It is found in tropical Asia

and Africa. It is native to the Indian subcontinent, Southeast Asia and New Guinea. It is reportedly naturalized in China, tropical Africa, Brazil, Colombia, the West Indies, Fiji and French.

MORPHOLOGY:

Growth form: Short-lived perennial creeping or climbing vine with stems up to 2-6 m long.

Leaves: Leaves are fleshy and ovate or heart shaped,chordate base,dark green in colour , glossy above and glaucous below. . Leaves are axillary dark green, broadly and acute. The size of the leaves varies from 3-9 cm in length and 4-8 cm in width. Taste was found to be bland , with no odour.

Stem: Stem is fleshy, stout at the base with slender upper branches. The stem is green or purplish and quadrangular in shape, about 2-3 cm thick, with prominent nodes and internodes. The taste is bland and mucilaginous with no odour.

Flower: the flowers are inconspicuous, egg-shaped flowers (3-4 mm long) are arranged in a spike inflorescence. The bisexual flowers have 3 styles (female reproductive part) and 5 stamens (male, pollen-bearing part). Flowers are sub sessile, white, pink or red coloured and closed at anthesis. Bracts are scaly and small.

Fruits: Fruits are fleshy, stalk less, ovoid or spherical in shape and enclosed within the persistent fleshy calyx. , 5 to 6 mm and purple when mature.

Seeds: Seeds are black, globose and indehiscent. (Cooke, 1902; Yadav and Sardesai, 2002; Almeida, 2003).

PHYTOCHEMISTRY:

Basella alba on a whole has better phenolic content. Fruits of *Basella alba* contain the highest total phenolic content (5.07 mg/ml).

Flavanoid content of different parts of *Basella* species range between 4.00-9.87 mg/g. Alkaloid content range between 0.33-1.61 mg/g with leaf fraction of *Basella alba* recording the highest alkaloid content .

Fruits contain high percentage of saponin (0.7%-0.9%) whereas the least percentage of saponin in stem (0.02%) .

Tannin content is highest in fruits (5.9-7.27 mg/g) closely followed by the leaf portions (1.46-3.73 mg/g) and the stem fraction records least tannin content.

Terpenoid content of *Basella* plant range between 0.396-0.86 mg/g. Leaves shows the better terpenoid content (0.89 mg/g). *Basella alba* can therefore be described as medicinal plant due to its terpenoid content.

SCIENTIFICALLY PROVEN AND REPORTED ACTIVITIES:

Wound healing activity:

Mohammed et al., (2012) studied wound healing capacity of *Basella alba*, in male albino rats. They created burn wounds on the back of rats and treated them with *Basella alba* leaf extract in glycerin for about 20 days. Their results concluded that, rats treated with aqueous leaf extracts showed a maximum wound healing capacity with significant wound closure and indicated wound healing capacity of *Basella alba*.

Antimicrobial activity:

Oyewole and Kalejaiye (2012) used agar cup method for the determination of antimicrobial effects of *Basella alba* ethanolic extracts against Staphylococcus aureus, Pseudomonas aeruginosa, Escherichia coli and Candida albicans. Their findings reported that *Basella alba* ethanol extracts showed inhibitory activities against all the above bacteria except Candida albicans. Sen et al., (2010) examined the antibacterial activity of the *Basella rubra* leaves by cup plate diffusion method and reported that aqueous, ethanol and petroleum ether extracts of the *Basella rubra* leaves exhibited antibacterial activity against E. coli, Vibrio cholera, Staphylococcus aureus and Staphylococcus typhi.

Antiviral activity:

Bolognesi (1997) isolated single chain (type I) ribosome inactivating proteins from the seeds of Basella rubra and tested them for antiviral activity and inhibited infection of Nicotiana benthamiana by AMVC (Bolognesi, 1997). Liu et al., (2006) studied that the early inoculation of

Basella rubra extract on tobacco plant showed inhibitory effects against tobacco mosaic virus. Dong et al., (2012) reported structures of acidic polysaccharides from Basella rubra and their effects on herpes simplex virus type 2.

Anti inflammatory activity:

Kachchhava (2006) have performed anti inflammatory activities of *Basella alba* extract on rats. He used two different phlogistic agents viz. carageeneen (1%) and formaldehyde (3, 5%) to carry out the activity on two inflammatory models. Phenylbutazone was used as a standard anti inflammatory drug. Aqueous extract of *Basella alba* at the dose of 500mg/kg and 100mg/kg significantly reduced the inflammation. In the carageeneen induced inflammatory method they noted that, the pre ether extract of *Basella alba* did not exhibit significant antiinflammatory activity. Krishna (2012) employed cotton pellet induced granuloma method while Rodda et al., (2012) employed carageeneen along with cotton pellet granuloma induced inflammatory methods in rats, to study the activity on inflammatory models. In both the above studies phenylbutazone was used as a standard. They treated experimental rats with oral suspension of cold macerated 50% ethanol extract of *Basella alba* leaves and resulted that the plant had significant anti inflammatory activity.

Antiulcer activity:

The effect of *Basella alba* as an anti ulcer agent has studied by Venkatalakshmi and Senthamaraiselvi (2012). They treated 7-8 weeks old female albino Wistar rats with aspirin suspended in 1% carboxyl methyl cellulose at a dose of 150 mg/kg to induce the ulcer. The studies resulted that, aspirin altered parameters like ulcer index, percentage of ulcer inhibition, gastric pH, pepsin content, thiobarbituric acid reactive substances, lipid hydro peroxidases, SOD, GPx, CAT, GSH, vitamin C, and vitamin E were restored by the treatment of *Basella alba* leaf extract and indicated its anti ulcer activity. Kumar et al., (2012) evaluated the effects of aqueous and ethanol extracts of *Basella alba* leaves on antiulcer activity in the rats subjected to pylorus ligation and ethanol induced ulcer models. 200 mg/kg and 400mg/kg AEBA were given to the animals in different groups. Fomotidine was used as a standard drug in the ratio of 20mg/kg. The animals treated with AEBA and EEBA showed a dose dependant protection against the action of ethanol and pylorus ligation on gastric mucosa of animals.

CNS Depressant activity:

Anandrajagopal et al., (2011) evaluated the CNS depressant activity of various solvent extracts of *Basella alba* aerial parts on Swiss albino mice of either sex. They concluded that, the methanol extract exhibited CNS depressant activity which is due to the presence of some psychoactive substances like terpenoids in the plant.

Antidiabetic activity in relationship with the Antioxidant property:

Nirmala et al., (2009) studied the hypoglycemic effect of aqueous leaf extract of *Basella alba* with that of streptozotocin (STZ) in two months old male albino rats. After a month of proper treatment they scarified all the experimented animals and analyzed the antidiabetic properties. Their results concluded that the rats treated with *Basella alba* pulp significantly bought back the blood glucose level. Nirmala et al., (2011) studied the antioxidant properties of plant leaf extract and found that the levels of liver enzymatic antioxidants such as catalase, superoxide dismutase, glutathione peroxidases and non enzymatic antioxidants like vitamin C, vitamin E and reduced glutathione greatly increased in the animals treated with the *Basella alba* pulp. Thus their findings suggested that the plant has hypoglycemic and antioxidant properties.

Nephroprotective effect:

S. Alquasoumi, (2011) aimed to investigate the possible protective effect of an ethanolic extract of *Basella alba* L. on gentamycin (GM)-induced nephrotoxicity in Wistar albino rats. In the toxic group, rats were administered GM only (100 mg/kg, i.p.) for 8 days. In drug treated groups, rats were pretreated with *B. alba* (250 and 500 mg/kg per day orally) for 14 days and co-treated with GM for 8 days. After 24 h of the last dose, blood, urine, and tissue samples were collected from the animals. GM when administered induced a marked renal failure, characterized by a significant increase in serum and urine creatinine, urea, uric acid, gamma-glutamyl transferase (GGT), and protein levels. Besides, there were elevation of malondialdehyde (MDA) level and decrease in the concentration of total proteins (TPs) and free -SH in kidney tissue, which are indicators of oxidative stress of kidney. The extract also significantly reduced the GM-induced elevated serum and urine levels of sodium, potassium, calcium, protein, creatinine, urea, uric acid, and GGT. The tissue MDA level was also significantly diminished; the decreased free -SH and TP levels were significantly replenished by an ethanolic extract of *B. alba* treatment. The

experimental results suggest that *B. alba* extract protected GM-induced nephrotoxicity, possibly by enhancing renal antioxidant system. The findings suggest the potential therapeutic use of *B. alba* as a novel nephroprotective agent.

Cytotoxic and antibacterial activity:

The methanolic extract shows the significant growth inhibition on human cancer cell lines and momentous zone of inhibition for microorganisms studied. The overall result of this study indicates that the methanolic extract from B. alba have interesting anticancer and antibacterial properties, and the traditional use of this plant may also derive from its antibacterial and anticancer properties (Rathee et al., 2010).

RATIONAL FOR SELECTION OF PLANT:

Given the wide range of ethnomedicinal uses and also the fact that this plant is easily available has given us sufficient reasons to select this plant for study. Moreover, parts of this plant is edible and hence it is likely to pose fewer side effects if used for therapeutic purposes.

A. Leaves of *Basella alba*

B. Plant of *Basella alba*

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COLLECTION, EXTRACTION AND PHYTOCHEMICAL SCREENING

COLLECTION IDENTIFICATION AND EXTRACTION

The leaves of *Basella alba* plant was collected from the local market of Sealdah, in Kolkata, India in the month of August, 2018. The plant species was identified and authenticated. BY D.K. Agarwala scientist incharge Botanical Survey of India, Gangtok,Sikkim. Air dried leaves (180 g) were powdered in a mechanical grinder and the plant materials was extracted by methanol using Soxhlet extraction apparatus. The solvent was completely removed under reduced pressure in a rotary evaporator. The concentrated extract was obtained by lyophilization and stored in vacuum desiccators (20^{o}C) for further use. The yield of the methanol fraction was about 18.22%.

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 colour 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 colour, which becomes colourless 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 coloured 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 coloured 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 colour 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. Formation of yellow colour at the lower layer suggested the presence of triterpenoids.

RESULTS

S. NO.	PHYTOCONSTITUENT	PRESENCE/ ABSENCE
1.	STEROID	-
2.	FLAVONOID	+
3.	SAPONIN	+
4.	TANNIN	+
5.	GLYCOSIDE	+
6.	CARBOHYDRATE	-
7.	ALKALOID	+
8.	PHENOL	+
9.	TRITERPENOID	+

(+) 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).

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 potentiallity of the compound.

Long-term Toxicity Studies (Chronic Toxicity Studies)

The animals were 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 (phase-1clinical 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 – $\sum(a*b/\text{n})$

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, 2000 mg/kg orally (OECD 2000). All the animals were observed for any signs of toxicity or mortality at least 24 hr. All the animals were subjected for sharp observation for a period of

14 days. Methanolic extract of *Basella alba* showed no mortality or toxic effect up to 2000 mg/kg body weight in mice.

RESULT

No deaths were observed when the animals were given a dose of 2000 mg/kg body.

DISCUSSION

The index of acute toxicity is LD50. Oral administration of methanol extract of Methanolic extract of *Basella alba* in mice at doses up to 2000 mg/kg did not produce any significant change in behavior, breathing, cutaneous effects, sensory nervous system responses or gastrointestinal effects. During the experiment period no deaths were observed. So from the results it is concluded that Methanolic extract of *Basella alba* is safe up to dose of 2000 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 MEBA 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 800 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 IC_{50} values 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-Visible Spectroscopy 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 IC_{50} 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 MEBA and Acarbose

Concentration (µg/ml)	MEBA	Acarbose
50	23±3.06	35.40 ± 1.62
100	26±2.55	46.89 ±2.64
200	35±2.71	55.33± 3.05
500	67.75± 3.07	76.69 ±2.93

Each value expressed as Mean ± SEM

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

Concenration (µg/ml)	MEBA	Acarbose
25	15.17±3.97	44.89±4.79
50	26.75±2.98	56.47±2.72
100	49.58±3.40	77.83±4.54
200	53.25±5.53	82.20±1.27

Each value expressed as Mean ± SEM.

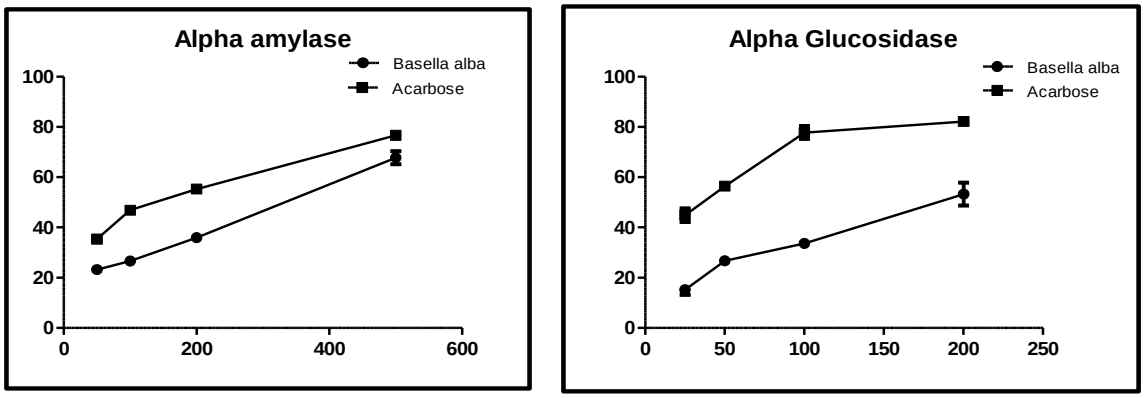

Figure 5.1: % inhibition of alpha amylase and alpha glucosidase shown by different concentrations of MEBA and Acarbose

Table5.3: % Glucose uptake in yeast cells by MEBA and Metformin. Each value expressed as Mean ± SEM.

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

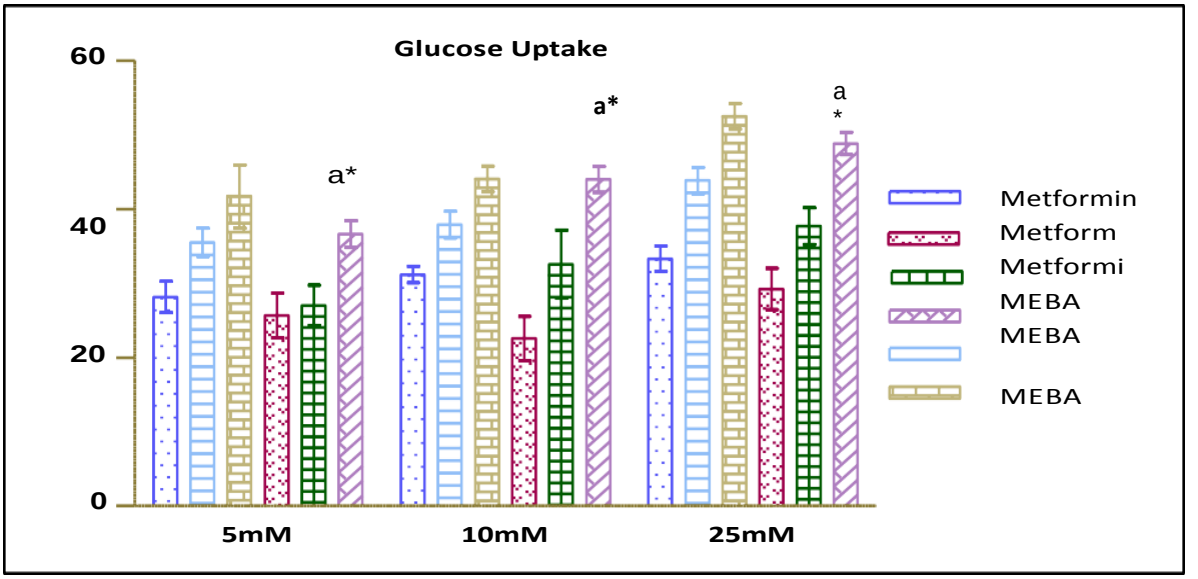

Figure 5.2: % Glucose uptake in Yeast cells as shown by different concentrations of MEBA and Metformin

The alpha-amylase inhibition study was conducted with different concentrations of MEBA and acarbose (50, 100, 200, 500 µg/ml) and the alpha glucosidase inhibition study was conducted with different concentrations of MEBA and Acarbose (25, 50,100,200 µg/ml). In alpha amylase, IC_{50} value of MEBA was found to be 324 ± 10.05 µg/ml when compared to that of IC_{50} value of acarbose which was 154.33 ± 9.08 µg/ml. In alpha glucosidase IC_{50} value of MEBA was found to be 182 ± 7.94 µg/ml when compared to that of acarbose which was 38.63 ± 8.05 µg/ml. The glucose uptake in yeast cell was carried out at different concentrations (5, 10, 25 mM) of glucose by different concentrations of MEBA (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 methanolic extract of *Basella alba leaves* (MEBA). 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 postprandial 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 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 MEBA against streptozotocin induced rats. 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 rats (weighing 25±5 g) of either sex were used in the study. Rats were kept in standard laboratory conditions *i.e.*, 12 hr light/dark cycles at 25-28 °C, relative humidity 55–60 % and were fed with a standard pellet diet and water *ad libitum*. The animals were acclimatized to laboratory condition for one week prior to the experiment.

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.

Induction of diabetes in rats

Streptozotocin, prepared in 0.1 mol/L citrate buffer, pH 4.4, was injected in a single dose of 45 mg/kg, i.v., to rats. Age-matched control rats received citrate buffer and were used in parallel with diabetic animals. Two days after STZ injection, blood samples were collected and plasma glucose levels were estimated using the Accu-check Counter TS diagnostic kit method (Span Diagnostic, Surat, India). Rats having plasma glucose levels > 250 mg/dL after 4 weeks were selected and used in the present study.

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

The rats 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 21 days:

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

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

Group III: Diabetic rats were administered MEBA (200mg/kg body weight) orally daily.

Group IV: Diabetic rats were administered MEBA (400mg/kg body weight) orally daily.

Group VI: Diabetic rats were administered Metformin (150mg/kg body weight) orally daily.

FBG level was measured on day 0^{th}, 7^{th}, 14^{th} , 21^{st} using a one touch glucometer. After 21^{st} hour of last dose and 18 hours of fasting, blood was collected from all rats in each group by cardiac puncture for estimation of glycosylated hemoglobin (HbA1C), serum lipid profile and serum biochemical parameters and then the animals were sacrificed for collection of liver and kidney tissue to check the different endogenous antioxidant parameters.

Body weight

Body weight of rats from each group was measured on day 0^{th}, 7^{th}, 14^{th}, 21^{st} . 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.

Analysis of urine parameters

From the beginning of the fourth week after the induction of diabetes, the rats were kept individually in metabolic cages (8 PM-2 AM) and urine samples were collected for 12-h measurement of urine protein excretion (UPE), urine creatinine and urine urea. The results revealed a significant increase in the urine protein excretion (UPE) rate in the diabetic rats in comparison with the control rats and the animals were considered nephropathic**.**

At the end of the 21 days, the rats were kept individually in metabolic cage (8 PM-2 AM) and 12-h urine samples were collected for biochemical analysis. Then, six rats from every investigated group were sacrificed under ether anesthesia. Moreover, blood and tissue samples were collected.

Estimation of liver biochemical parameters and antioxidant status

Livers and kidneys collected from the sacrificed animals were homogenized separately in 10% w/v 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 Function 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 rats were determined by using commercially available kits from Span Diagnostics Ltd. India.

Statistical Analysis

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

RESULTS

Oral glucose tolerance test

Glucose administration to the normal rats increased blood glucose levels from 81.3±3. to 129±3.60 in first 30 mins and gradually decreased in 60 mins, 120 mins and returned near to normal i.e. 78.33±2.66 at 240mins. Administmiceion of MEBA at the doses of 200 and

400mg/kg significantly (p<0.05) improved glucose tolerance in a dose dependent manner at 120mins with time (Table 7.1).

Table 7.1: Effect of MEBA on OGTT

GROUPS	0 mins	30 mins	60 mins	120 mins
Normal Control	81.3± 3.149	129± 3.60	121 ±3.78	111.33 ±1.85
MEBA 200mg/kg	77.50±1.555	130±3.198*	109±1.915*	73.00±1.915*
MEBA 400mg/kg	86.33±2.963	138.0±2.646*	118.0±1.155*	84.33±1.202*
Standard	79.55±3.510	135.32±1.321*	112±2.568*	78±4.21*

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 MEBA at the doses of 200 and 400 mg/kg significantly ($p < 0.05$) improved of the body weight when compared to the diabetic control group (Table 7.2).

Table 7.2: Effect of MEBA on Body weight

GROUPS	DAY 0	DAY 7	DAY 14	DAY 21
Normal Control(5ml/kg)	169.0±2.08	176.0±2.16	186.3±1.76	191.3±1.75
STZ Control(40mg/kg)	235.7±2.61*	229.3±2.35*	220.9±2.03*	217.8±4.49*
STZ+200mg/kg MEBA	238.7±2.33	230.6±2.19	215.5±1.45	205.6±4.29
STZ+400mg/kg MEBA	240.2±2.31	232.3±2.40	211±3.18	207.5±1.45
STZ+150mg/kg Metformin	239.7±1.48	235.7±2.62	236.5±3.70	237.2±6.42

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 STZ-induced diabetic rats as compared to normal control group. Administration of MEBA in diabetic rats at the doses of 200 and 400 mg/kg significantly ($p < 0.05$) reduced the FBG level towards normal as compared to the diabetic control group (Table 7.3).

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

GROUPS	DAY 0	DAY 7	DAY 14	DAY 21
Normal Control(5ml/kg)	55.3±4.25	61.4±2.51	60.67±1.20	72.67±2.72
STZ Control (40mg/kg)	338.7±4.06[a]*	378.6±2.33[a]*	413.2±2.31[a]*	429.9±0.88[a]*
STZ+200mg/kg MEBA	356.2±2.33[b]	306.9±2.46[b]	274.9±4.01[b]	145.6±2.35[b]
STZ+400mg/kg MEBA	345.5±2.91[b]*	287.6±2.13[b]*	165.4±2.65[b]*	98.6±1.98[b]*
STZ+150mg/kg Metformin	420.3±1.55[b]*	200.1±3.51[b]*	160.7±2.22[b]*	74.2±2.06[b]*

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 rats was significantly ($p < 0.001$) increased compared to normal control group. Treatment with MEBA 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 7.5).

Estimation of glycosylated hemoglobin (HbA1c)

Glycosylated hemoglobin level in STZ-induced diabetic rats was significantly ($p < 0.001$) increased compared to normal control group. Treatment with MEBA at the doses of 200 and 400 mg/kg significantly ($p < 0.05$) reduced the HbA1c level when compared to the diabetic control group (Table 7.5).

Table 7.5: Effect of MEBA on HbA1c

Groups	HbA1c (%)
Normal Control	7.283±1.468
STZ Control (Diabetic)	$10.19 \pm 0.2170^{a*}$
Diabetic + MEBA(200 mg/kg)	9.240 ± 0.6837^{b}
Diabetic + MEBA(400 mg/kg)	8.403 ± 0.1729b*
Diabetic + Metformin (150mg/kg)	7.435 ± 0.3227b*

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).

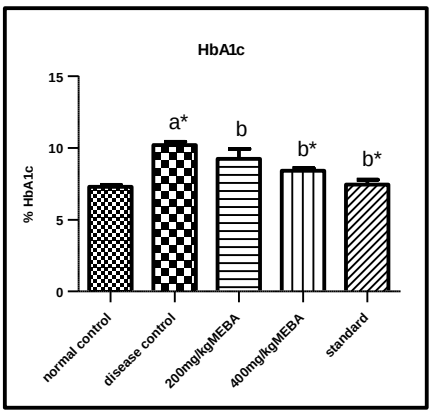


Figure7.1: Effects on MEBA on HbA1c. each calue is expressed as Mean ± SEM where n=6. a* p<0.05 when compared to normal control and b* p<0.05 when compared to diabetic control.

Estimation of serum lipid profiles

Serum lipid profiles like total cholesterol, triglyceride & HDL cholestrol in STZ-induced diabetic rats were significantly ($p < 0.05$) elevated and the HDL level significantly ($p < 0.05$) decreased compared to normal control group. Treatment with MEBA 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 7.4) .

Table 7.4: Effect of MEBA on Lipid Profiles

Groups	Total Cholesterol (mg/dl)	HDL Cholesterol (mg/dl)	Triglycerides (mg/dl)
Normal Control	32.81±4.292	73.99± 4.579	66.20 ± 13.39
STZ Control (Diabetic)	114.8.1±4.079[a]*	27.42±5.817[a]*	152.5± 6.23[a]*
Diabetic + MEBA (200mg/kg)	51.90±10.78[b]*	46.05±4.279[b]*	98.14± 9.415[b]*
Diabetic + MEBA (400mg/kg)	44.14± 10.46[b]*	64.35±3.658[b]*	91.08±9.415[b]*
Diabetic + Metformin (150mg/kg)	33.09±2.72 [b]*	71.00±2.704[b]*	80.14± 9.933[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* $p<0.05$).

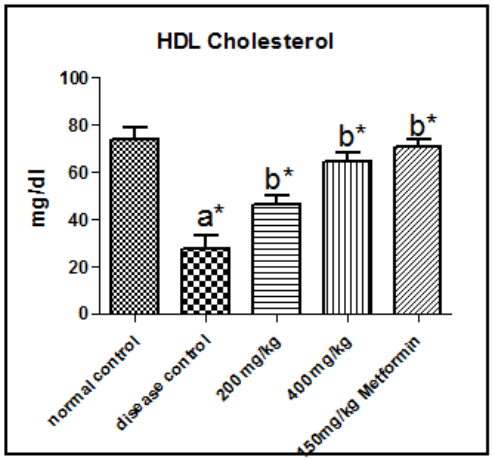


Figure 7.2:Effect of MEBA on HDL Cholesterol . Each value is expressed as Mean ±SEM where n=6. a* p<0.05 when compared to normal control and b* p<0.05 when compared to diabetic control.

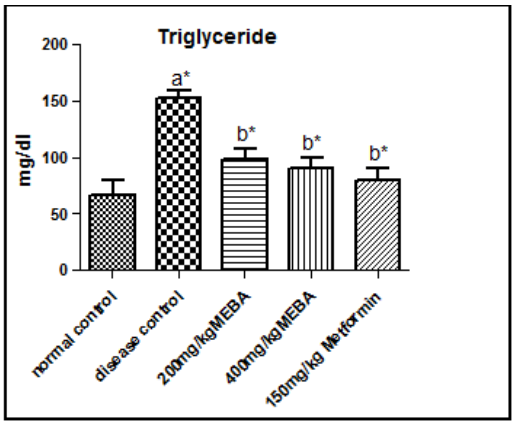


Figure 7.2:Effect of MEBA on Triglyceride. Each value is expressed as Mean ±SEM where n=6. a* p<0.05 when compared to normal control and b* p<0.05 when compared to diabetic control.

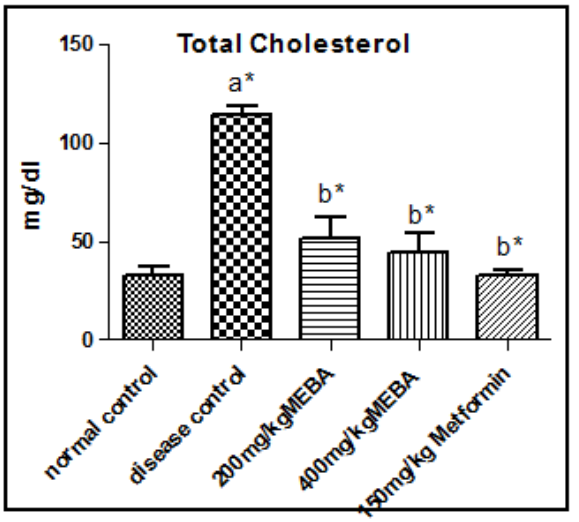


Figure 7.2:Effect of MEBA on Total Cholesterol . Each value is expressed as Mean ±SEM where n=6. a* p<0.05 when compared to normal control and b* p<0.05 when compared to diabetic control.

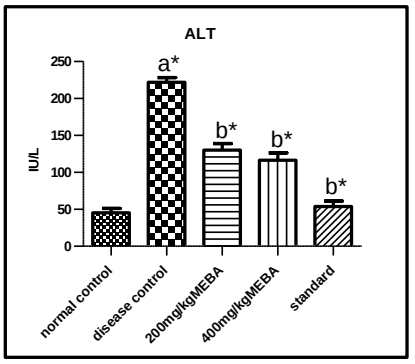
Estimation of serum liver function test parameters

Biochemical parameters like SGOT, SGPT, SALP, Total Protein, Total Bilirubin & Albumin in STZ-induced diabetic rats were significantly ($p < 0.05$) elevated and the total protein content was significantly ($p < 0.05$) decreased compared to the normal control group. Treatment with MEBA 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 7.6).

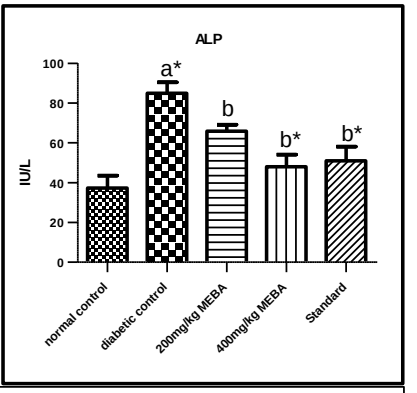
Table 7.6: Effect of MEBA on Serum Liver Parameters.

Groups	SGOT (IU/L)	SGPT (IU/L)	ALP (IU/L)	Total Protein (g/dl)	Total Bilirubin (mg/100mL)	Albumin (g/dl)
Normal Control	82.71 ± 8.87	81.19 ± 2.745	37.26±6.267	9.00 ± 0.4128	0.744±0.159	7.531±0.536
STZ Diabetic Control	176.2 ± 5.366	141.5 ± 2.763[a]*	84.83±508[a]*	4.431 ± 0.437*	3.845±0.3157[a*]	3.493±0.6314[a*]
Diabetic + MEBA (200mg/kg)	157.7 ± 8.268	68.61 ± 2.532[b]*	65.84±3.345[b]*	6.22 ± 0.56*	2.004±0.2929[b*]	5.157±0.602[b]
Diabetic + MEBA (400mg/kg)	110.1 ± 6.290	42.18 ± 1.456[b]*	47.92±6.237[b]*	7.542 ± 0.43*	1.355±0.2632[b*]	6.579±0.633[b*]
Diabetic + Metformin (150mg/kg)	95.86 ± 5.641	36.53 ± 1.009*	50.99±7.174*	8.327 ± 0.41*	1.056±0.248[b*]	7.360±0.454[b*]

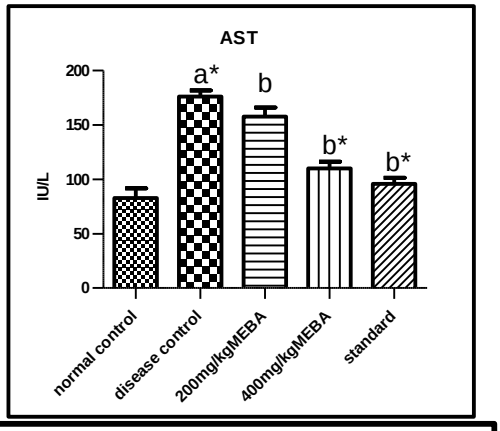
Values are expressed as mean±SEM (n=6). a* p<0.05 when compared to normal control, b* p<0.05 when compared to diabetic control.



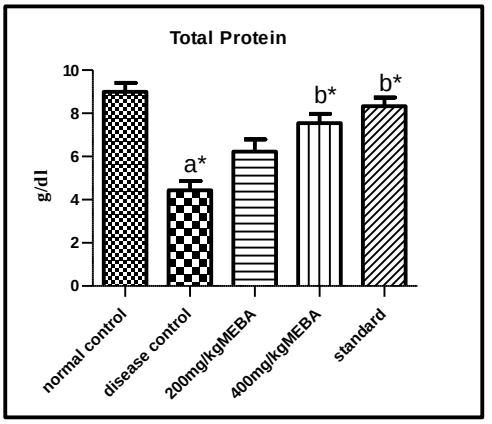
Effect of MEBA on ALT. Each value is expressed asMean ± SEM where n=6. a* p< 0.05 when compared to normal control and b* p< 0.05 when compared to diabetic control.



Effect of MEBA on ALP. Each value is expressed asMean ± SEM w here n=6. a* p< 0.05 w hen compared to normal control and b* p< 0.05 w hen compared to diabetic control.



Effect of MEBA on AST. Each value is expressed asMean ± SEM w here n=6. a* p< 0.05 w hen compared to normal control and b* p< 0.05 w hen compared to diabetic control.



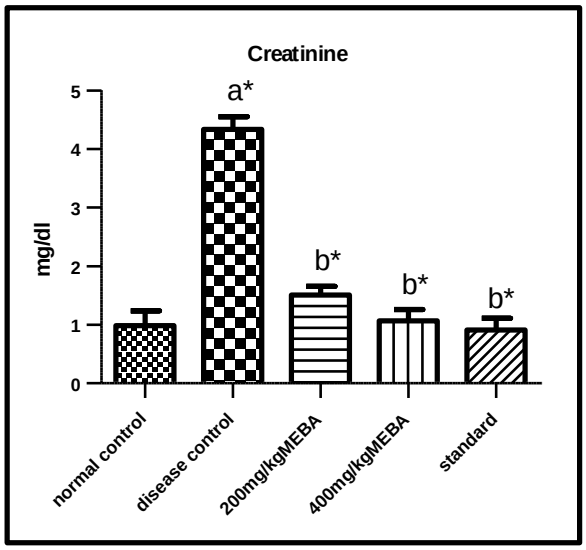
Effect of MEBA on AST. Each value is expressed asMean ± SEM where n=6. a* p< 0.05 when compared to normal control and b* p< 0.05 when compared to diabetic control.

Estimation of serum kidney function test parameters

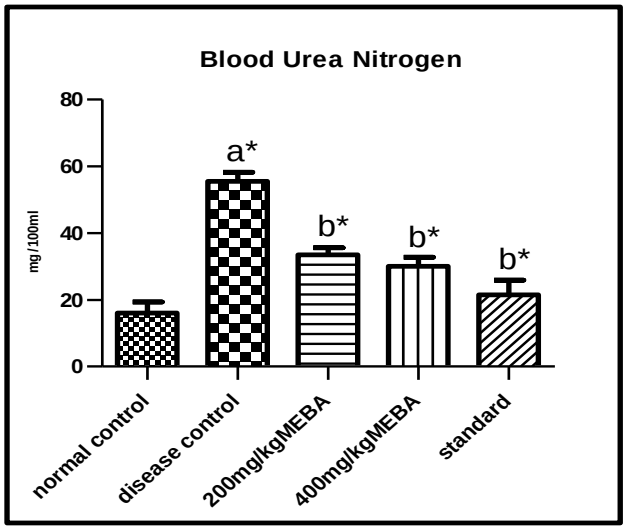
Biochemical parameters like Creatinine, Urea, Uric acid in STZ-induced diabetic rats were significantly ($p < 0.05$) elevated as compared to the normal control group. Treatment with MEBA at the doses of 100, 200 and 300 mg/kg significantly ($p < 0.05$) reduced the Creatinine, Urea, Uric acid levels (Table 7.7).

Table 7.7: Effect of MEBA on Serum Kidney Function Parameters.

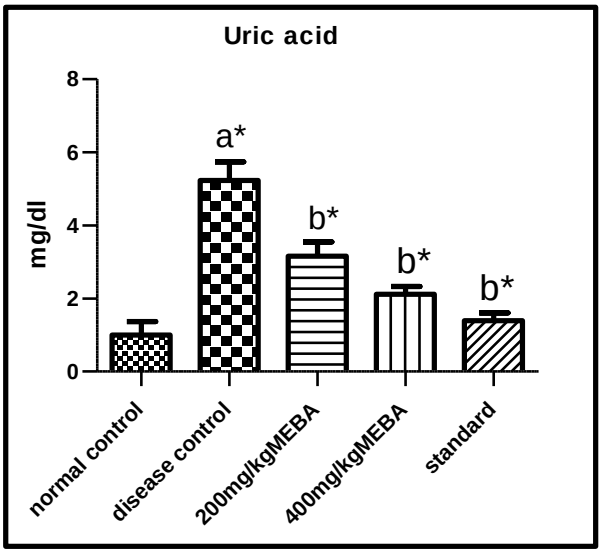
Groups	Creatinine	Urea	BUN	Uric Acid
Normal Control	2.026 ± 0.595	20.94 ± 1.72	3.54 ± 0.569	1.423 ± 0.265
STZ Control (Diabetic)	6.783 ± 0.714	53.23 ± 4.109	7.414 ± 0.448	3.462 ± 0.209
Diabetic + MEBA(200mg/kg)	2.703 ± 0.350	28.18 ± 1.674	4.607 ± 0.510	2.151 ± 0.238
Diabetic + MEBA (400mg/kg)	1.931 ± 0.454	22.74 ± 3.67	3.42 ± 0.392	1.517 ± 0.183
Diabetic + Metformin (150mg/kg)	1.961 ± 0.414	20.37 ± 3.464	3.01 ± 0.519	1.409 ± 0.242



Effect of MEBA on Creatinine. Each value is expressed asMean ± SEM where n=6. a* p< 0.05 when compared to normal control and b* p< 0.05 when compared to diabetic control.



Effect of MEBA on BUN. Each value is expressed asMean ± SEM where n=6. a* p< 0.05 when compared to normal control and b* p< 0.05 when compared to diabetic control.



Effect of MEBA on Uric Acid. Each value is expressed asMean ± SEM where n=6. a* p< 0.05 when compared to normal control and b* p< 0.05 when compared to diabetic control.

Analysis Kit

Protein & Glucose present in urine Analysis Kit

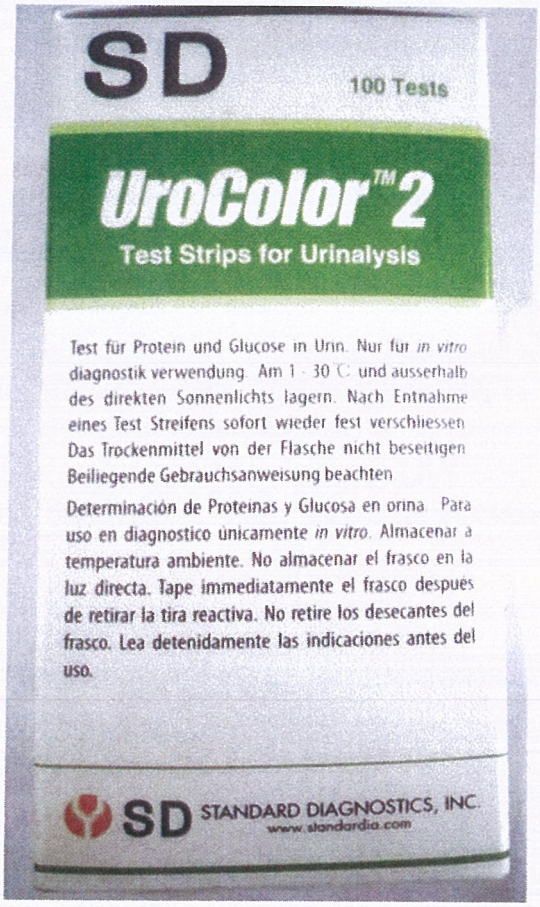


Image 1: Brand Name UroColor 2 Test strips for urinalysis

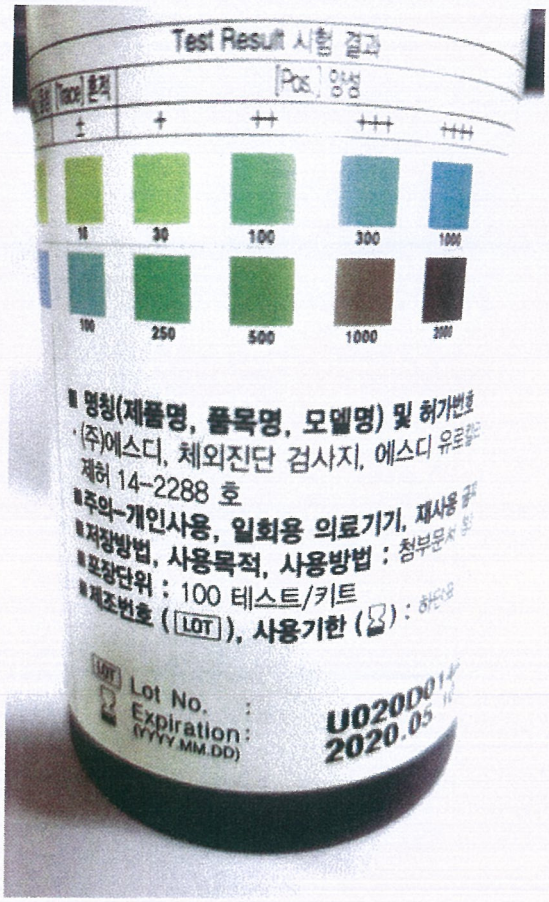


Image 2: Range of protein & glucose concentration in mg/dl .

Protein and glucose concentration range:

	-	±	+	++	+++	++++
Protein (mg/dl)	0	10	30	100	300	1000
Glucose (mg/dl)	0	100	250	500	1000	2000

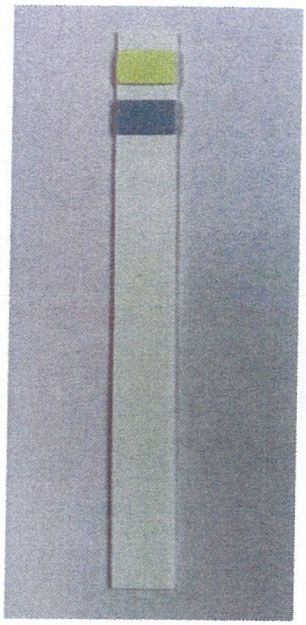


Image 3: Dip Stick color after immersing in Normal Control rat urine.

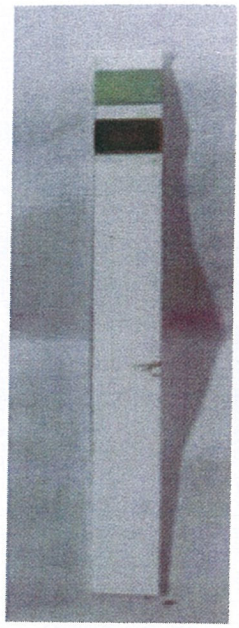


Image 4: Dip Stick color after immersing in Diabetic control rat urine.

Image 5: Dip Stick color after immersing in 200 mg/kg treated rat urine.

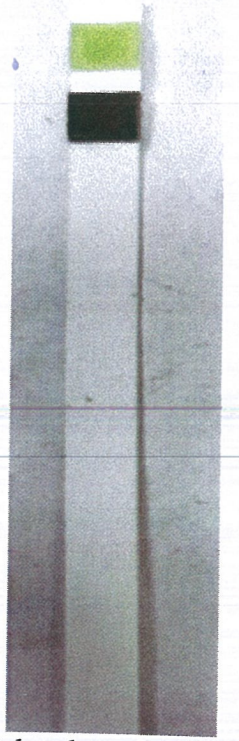


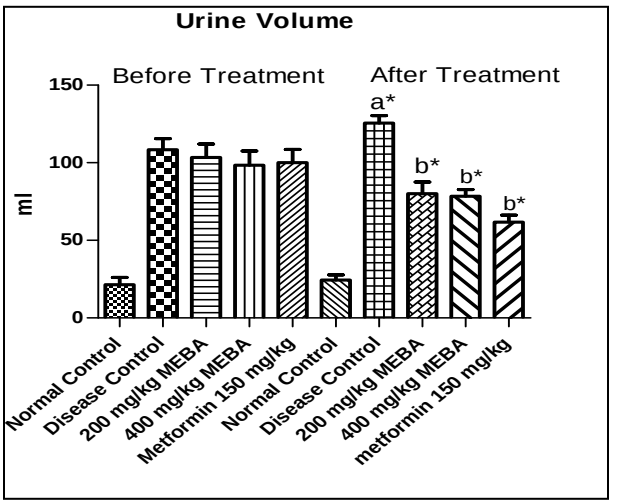
Image 6: Dip Stick color after immersing in 400 mg/kg treated rat urine.

Estimation of urine parameters

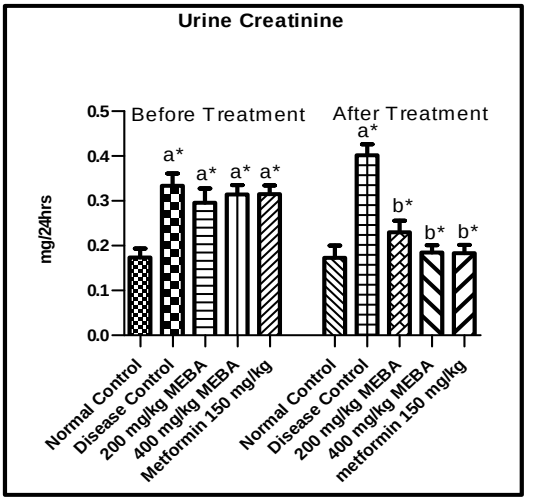
STZ-diabetic rats showed significantly ($p < 0.05$) elevated 24-hour urine volume, accompanied by increase in urine protein excretion. After three weeks of treatment, 24-hour urine volume and 24-hour urine protein excretion for STZ-diabetic rats were significantly ($p< 0.05$) less than those of their vehicle-treated counterparts. In addition, Scr and serum urea levels in STZ-diabetic rats were obviously higher than in rats from the normal control group. These levels were significantly ($p < 0.05$) reduced after the treatment.

Figure 5.2: Urine volume and urinary protein levels after 21 days of daily methanoli extract of *Basella alba* leaves.

Groups	Normal control	Disease control	200 mg/kg MEBA	400 mg/kg MEBA	150 mg/kg Metformin
Urine volume (ml)	24.33 ± 3.48	125.7 ±4.70	80 ±7.63	78.33 ± 4.41	61.67 ± 4.63
Urinary protein (mg/dl)	Negative (-)	Positive (++)	Trace (+)	Negative (-)	Negative (-)



Effect of MEBA on Urin Volume. Each value is expressed asMean ± SEM where n=6. a* p< 0.05 when compared to normal control and b* p< 0.05 when compared to diabetic control.



Effect of MEBA on AST. Each value is expressed asMean ± SEM where n=6. a* p< 0.05 when compared to normal control and b* p< 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 rats. In the present study the MDA level was significantly (p < increased in HFD/STZ-induced diabetic rats compared to normal control group. Interestingly, treatment with MEBA 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 rats compared to normal control group. Administmiceion of MEBA at the doses of 200 and 400 mg/kg significantly ($p < 0.05$) increased GSH, SOD antioxidant enzyme levels in the liver of HFD/STZ- induced diabetic rats compared to the diabetic control group (Table 7.7, 7.8, 7.9).

Table 7.7: Effect of MEBA on MDA.

Groups	MDA (µM/100gm Tissue Homogenate) Liver	MDA (µM/100gmTissue Homogenate) Kidney
Normal Control	1.507±0.11	1.387±0.1357
STZControl(Diabetic)	3.017±0.11^{a*}	3.060±0.10^{a*}
MEBA (200mg/kg)	2.263±0.16^{b*}	2.107±0.11^{b*}
MEBA (400mg/kg)	1.800±0.10^{b*}	1.547±0.12^{b*}
Diabetic+ Metformin (150 mg/kg)	1.405±0.06*	1.104 ± 0.02*

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

Table 7.8: Effect of MEBA on SOD

Groups	SOD LIVER (U/mg)	SOD KIDNEY (U/mg)
Normal Control	3.437 ± 0.14	3.750 ± 0.17
STZControl(Diabetic)	1.107 ± 0.10^{a*}	1.207 ± 0.10^{a*}
MEBA (100mg/kg)	1.760± 0.12^{b*}	1.573 ± 0.12
MEBA (200mg/kg)	2.543 ± 0.23^{b*}	2.040 ± 0.11^{b*}
Diabetic+ Metformin (150 mg/kg)	3.007 ± 0.09*	3.241 ± 0.08*

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

Table 7.9: Effect of MEBA on GSH

Groups	GSH (µg/mg tissue) Liver	GSH(µg/mg tissue) Kidney
Normal Control	3.663±0.07	3.570±0.16
STZControl(Diabetic)	1.717±0.05^{a*}	1.827±0.05^{a*}
MEBA (200mg/kg)	1.927±0.05^{b*}	2.040±0.04^{b*}
MEBA (400mg/kg)	2.513±0.08*	2.520±0.08*
Diabetic+ Metformin (150 mg/kg)	3.124 ± 0.07*	3.077 ± 0.08

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

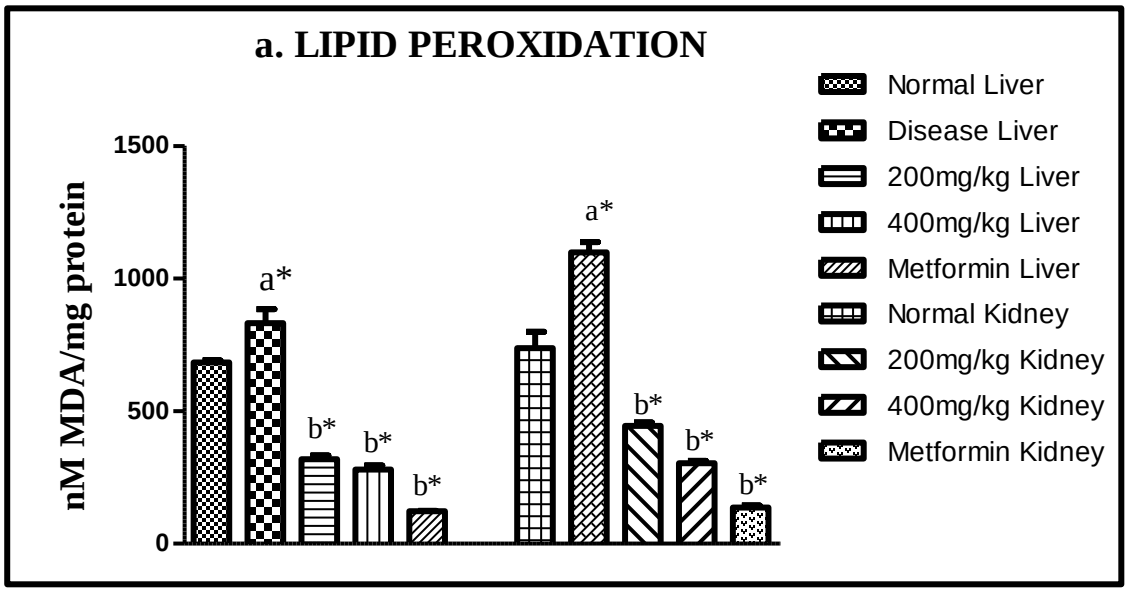


Figure 8.2 (a): Effects of different concentrations of MEBA on Lipid peroxidation. The values are represented as mean ± SEM. a*p<0.05 when Disease control compared to normal liver and kidney, b*p<0.05 when treated groups compared to Disease control liver and kidney.

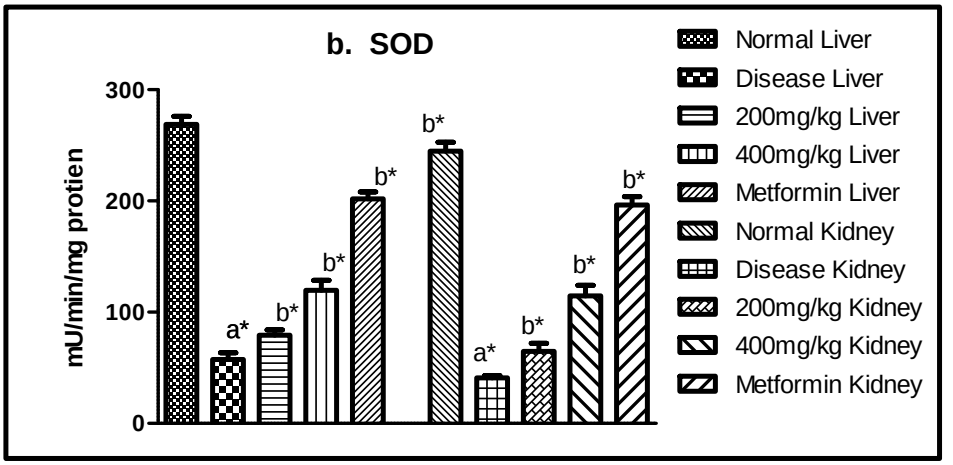


Figure 8.2 (b): Effects of different concentrations of MEBA on SOD. The values are represented as mean ± SEM. a*p<0.05 when Disease control compared to normal liver and kidney, b*p<0.05 when treated groups compared to Disease control liver and kidney.

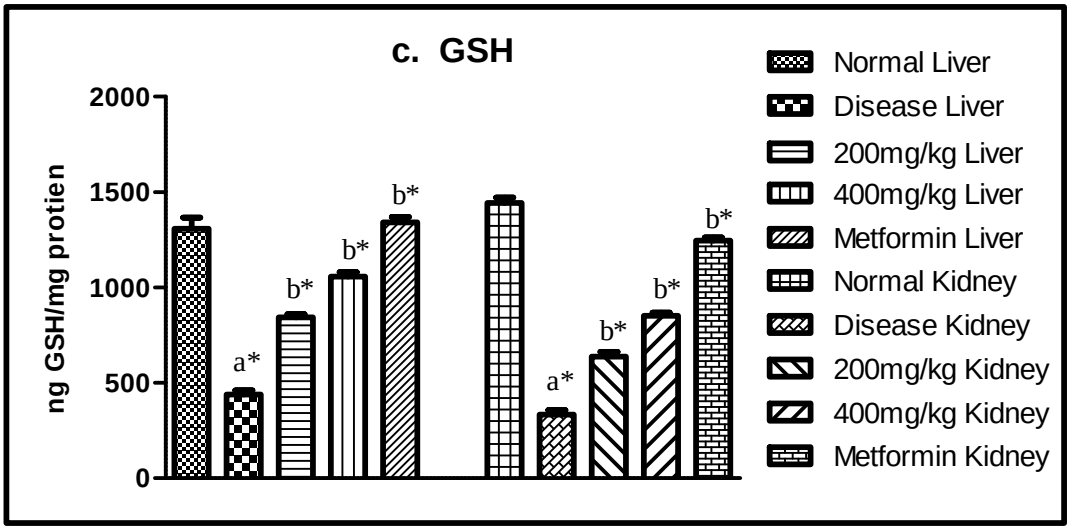
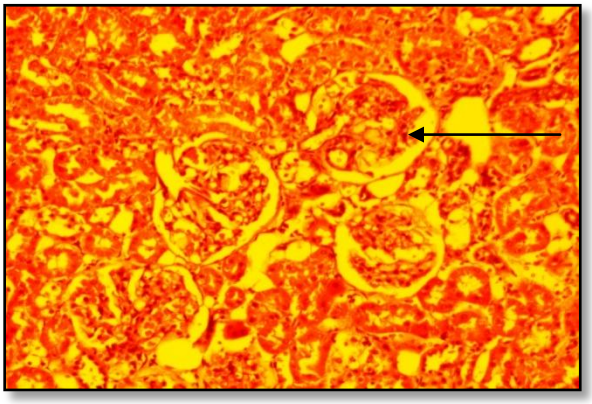
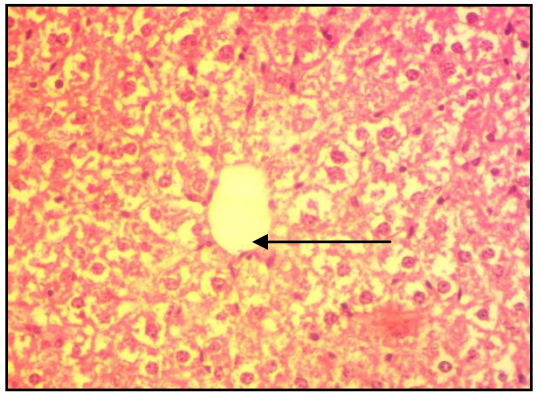


Figure 8.2 (c): Effects of different concentrations of MEBA on GSH. The values are represented as mean ± SEM. $^{a*}p<0.05$ when Disease control compared to normal liver and kidney, $^{b*}p<0.05$ when treated groups compared to Disease control liver and kidney.

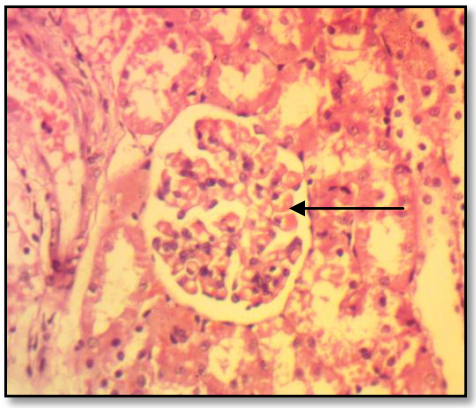
Histopathology:



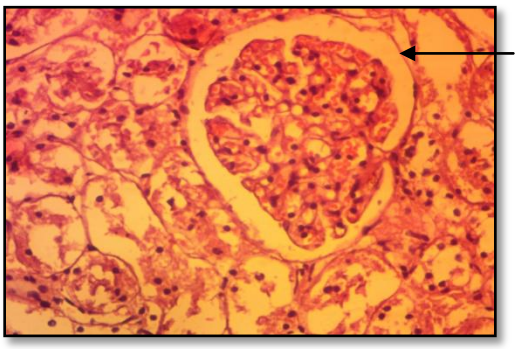
A1.NORMAL CONTROL KIDNEY



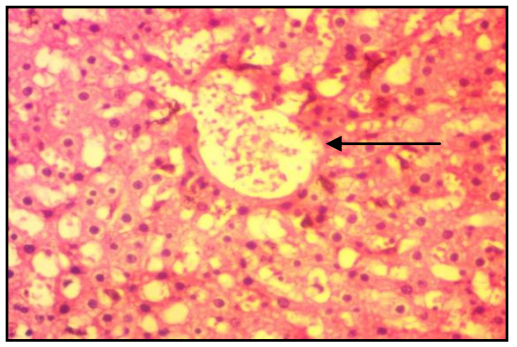
A2- NORMAL CONTROL LIVER



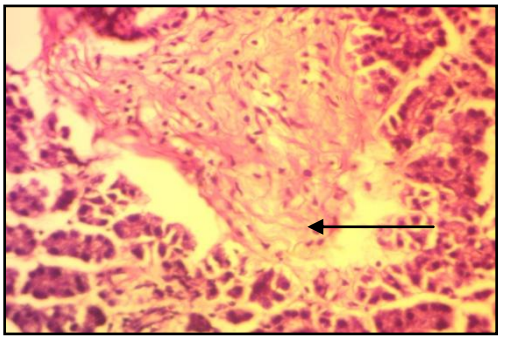
A3- NORMAL CONTROL PANCREASE



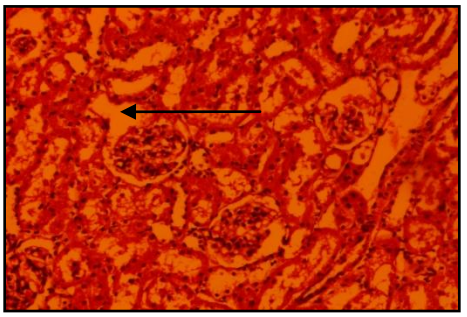
B1-DISEASE CONTROL KIDNEY



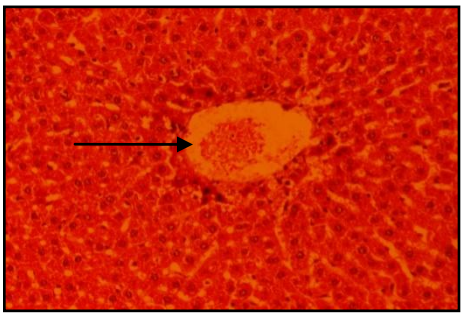
B2-DISEASE CINTROL LIVER



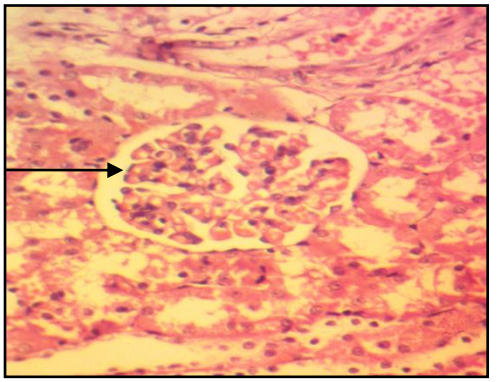
B3- DISEASE CONTROL PANCREASE



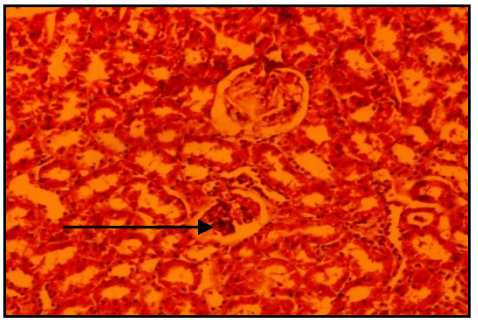
C1-200 Mg/kg of MEBA KIDNEY



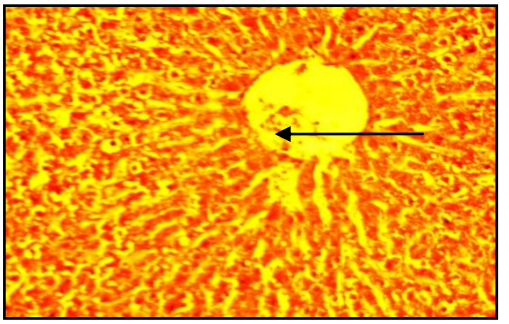
C2-200 Mg/kg of MEBA LIVER



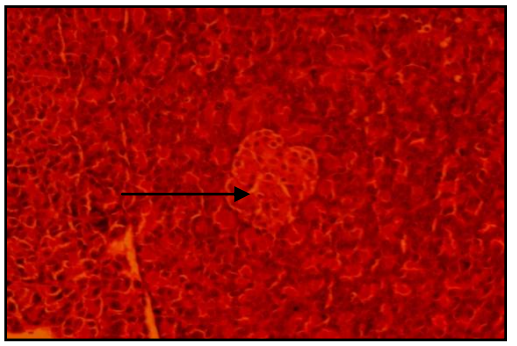
C3-200 Mg/kg of MEBA PANCREAS



D1-400 Mg/kg of MEBA KIDNEY



D2-400 Mg/kg of MEBA LIVER



D3-400 Mg/kg of MEBA PANCREAS

A3- β cell in normal control rats, B3- Total destruction of β cells I n STZ control rats, C3- remnants of beta cell in MEBA (200 mg/kg) treated rats. D3- Gradual regeneration of β cells in MEBA (400 mg/kg) treated rats.

Thickening of Glomerular membrane is seen in disease control (B1) with respect to normal control (A1). But thickness of glomerular membrane is decreasing in a dose gradient manner in 200 mg/kg (C1) & 400 mg/kg (D1) treatment of MEBA as compare to disease control.

Cells around the portal vein is destroyed in Disease control (B2) with respect to normal control (A2) But it is seen that cells are recovering in a dose gradient manner in 200 mg/kg (C2) & 400 mg/kg (D2) treatment of MEBA as compare to disease control.

DISCUSSION

In the present study, the induction of diabetes resulted in a significant decrease in creatinine and urea clearance. It has also been observed that increased blood urea nitrogen and serum creatinine in diabetic rats indicates progressive renal damage.

The present study discusses the antihyperglycemic as well as renal protective activity of methanol extract of *Basella alba* leaves in diabetic rat. 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, total protein, body weight and liver antioxidants (GSH, SOD,) levels in the STZ induced diabetic rats when compared to normal control group. The treatment with MEBA at doses of 400 mg/kg was significantly but 200 mg/kg was not that significant and dose dependently normalization of elevated blood glucose level, glycosylated hemoglobin, body weight , restored serum and liver biochemical parameters. The urine volume,urine protein excretion rate was towards normal values in MEBA 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 MEBA 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 rats than MEBA treated rats while studying oral glucose tolerance test (OGTT). Oral administration of MEBA significantly improved the impaired glucose tolerance in the glucose loaded rats in a dose dependent manner. From the above result, the OGTT showed an increased glucose utilization triggered by insulin production from the beta cells. It was also evident from the result that MEBA significantly lowered FBG level in STZ-induced diabetic rats.

zdiabetes mellitus has caused hyperlipidaemia in diabetic patient. Thus, diabetic rats thathave

been induced by HFD with combination of low dose STZ also showed similar situations, hyperlipidaemia. MEBA significantly reduced serum triglycerides, total cholesterol and increased level of HDL in STZ induced diabetic rats.

Streptozotocin mediated persistent hyperglycemia is due to beta cells destruction, mediated and complicated by the enhanced formation of free radicals (Kaneto *et al.*,2005) . 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 MEBA to diabetic rats 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) rats indicating impaired liver function. Therefore, increase 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 rats also showed high cholesterol level, so it supports the idea that high cholesterol level leads to liver damage. Treatment with MEBA restored all the above mentioned biochemical parameters toward the normal values in a dose-dependent manner. These observation indicates the MEBA 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 rats indicated enhanced lipid peroxidation leading to tissue injury and failure of the endogenous antioxidant defence mechanisms to prevent over production of free radicals. Treatment with MEBA inhibited hepatic lipid peroxidation in diabetic rats as revealed by the reduction of TBARS levels toward normal, suggesting MEBA's ability to improve the pathologic condition of diabetes by inhibiting lipid peroxidation in diabetic rats, 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 rats. This indicate that the extracts can reduce the potential glycation of enzymes or they may reduce reactive oxygen free radicals and improve the activities of antioxidant enzymes. This result shows evidence that MEBA contain a free radical scavenging activity, which could exert a beneficial action against pathological alteration caused by the presence of superoxide radicals and hydrogen peroxide radical. On the basis of this evidence it is possible that these activities of MEBA are due to the presence of the above said phytoconstituents . Therefore, it can be concluded that the methanol extract of *Basella alba* leaves is remarkably effective against high fat diet/ streptozotocin-induced diabetes in Wistar rats plausibly by virtue of its augmenting the endogenous antioxidant mechanism, lipid lowering ability, and serum glucose lowering ability.

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CONCLUSION

CONCLUSION

Diabetes is a disorder of carbohydrate, fat and protein metabolism attributed to diminished production of insulin or mounting resistance to its action. Herbal treatments for diabetes have been used in patients with insulin-dependent and non-insulin-dependant diabetes, diabetic retinopathy, diabetic peripheral neuropathy, etc. Scientific validation of several Indian plant species has proved the efficacy of the botanicals in reducing the sugar level.

Diabetic nephropathy is one of the major microvascular complications of diabetes mellitus. In the present study, the development of DN was confirmed by significant elevations of fasting blood glucose, kidney/body weight ratio, Scr, and BUN, as well as Upro in diabetic rats, as earlier reported by Knoll et al.

This thesis is a pioneer of scientific investigations on methanol extract of the leaves of *Basella alba* in the research field. The introductory part of the thesis is designated by the concept of diabetes and diabetic nephropathy, natural products in diabetic treatment, description and literature review of the investigated plant. After that the collection, extraction and chemical characterization has been mentioned in the next chapter. Then acute toxicity study has been written in the next chapter where it is observed that the plant extract is safe at dose of 2000mg/kg . Then the antioxidant studies have been mentioned in the next chapter. Finally the *in vitro* antidiabetic and *in vivo* antidiabetic activity has been written in last two chapters. Considering the *in vitro* and *in vivo* results it can be concluded that the methanol extract of the whole plant of *Basella alba* does have anti diabetic properties. The results may be attributed to the phytoconstituents present in the plant as mentioned in the thesis in an earlier chapter. Phytoconstituents like alkaloids and flavonoids of reported therapeutic benefits against various ailments including antidiabetic and nephropathy. So the alkaloids of flavonoids or both present in the extract could be responsible for its activity. The antioxidant studies elucidated that the extract has fairly good free radical scavenging activities which could also be a probable reason for its renal protective activities because free radicals are a major cause of destruction of kidney cell which could subsequently lead to nephroprotective and quenching of these free radicals could counteract such ill effects.

However vague speculations asserting phytoconstituents to be responsible for the extract's antidiabetic activities are not enough and extensive isolation and characterization techniques must be employed to find out the molecule or molecules in the extract actually responsible for such activities.