Evaluation of Nephroprotective effects of *Ficus rumphii* in streptozotocin-induced diabetic animal models

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CERTIFICATE

This is to certify that Jayashree Mondal has carried out the research on the project entitled **"Evaluation of Nephroprotective effects of *Ficus rumphii* in streptozotocin-induced diabetic animal models"** under my supervision, in the division of Pharmacology and Toxicology, Department of Pharmaceutical Technology, Jadavpur University, Kolkata-700032.

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

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DECLARATION

I declare that **"Evaluation of Nephroprotective effects of *Ficus rumphii* in streptozotocin-induced diabetic animal models"** 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.

Signature of the student:

Full Name:

Date:

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I thank God almighty for giving me patience, courage and abundant blessing for conducting the study and helping me in every walk of my life with all that I have got.

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DATE: SIGNATURE

PREFACE

The present study entitled **"Evaluation of nephro-protective effects of *Ficus rumphii* in streptozotocin-induced diabetic animal models"** covers original research work conducted by the author for the award of Master of Pharmacy in the Department of Pharmaceutical Technology, Jadavpur University, Kolkata.

The enormous diversity of the plant kingdom has become a target for drug companies looking for Novel medications and lead compounds. Their widespread availability, low toxicity, and little or non-existent adverse effects have motivated us to use medicinal herbs in the treatment of a variety of ailments. Traditional applications necessitate background in order to be a of proper value, and as a result, today they are a significant component of study. As a result, the thesis covered the above-mentioned study in a coherent way in respect to the other research-related aspects.

In conclusion, the detailed study has been put together in a way that justifies the work's relationship to establishing pharmacological activities, notably anti-diabetic activity and Nephroprotective effect.

Jayashree Mondal

CONTENTS

DEDICATED to MY FAMILY & MY MENTOR

Chapter 1: Introduction

1.1: INTRODUCTION:

The burden of diabetes is disproportionately high in India, a nation that is rapidly urbanizing and improving its socioeconomic situation. Because of the urbanization of lifestyle characteristics, studies conducted in various regions of India have shown that diabetes prevalence is rising among both urban and rural people. In 2007, there were an estimated 246 million adults worldwide who had diabetes; by 2025, that figure is expected to reach close to 380 million. According to Sicree et al. (2006), India currently has 41 million diabetics and is projected to have 70 million by 2025. A major rise in the incidence of type 2 diabetes, brought on by record rates of urbanization and the resulting environmental and lifestyle changes, is most likely to blame for the rising number of diabetics in India. According to projections from the World Health Organization (WHO), there will be 3.9 billion urban residents in developing nations by 2030, up from 1.9 billion in 2000. According to Ramachandran and Snehalatha (2009), type 2 diabetes accounts for 85–95% of all instances of the disease and is a significant financial and medical burden on society.

In addition to insulin, synthetic medications such sulphonylurea, biguanides, and thiazolidinediones (TZD) are successfully used to treat diabetes. All of these medications, however, have unfavorable side effects, and they all fall short of restoring glycemic control (Laville and Andreelli, 2000). Such side effects deter patients from following pharmaceutical regimens completely and correctly. This makes it extremely desirable to discover new anti-diabetic drugs that, unlike TZD or insulin, do not cause obesity or other negative side effects while stimulating glucose uptake by adipose or muscle cells.

In the current target-rich, lead-poor environment, ethnopharmacology and drug development employing natural products continue to be significant concerns. Numerous plant-derived therapeutic compounds have been made available by medicinal plants to modern medicine (Evans, 2002). These medications are either entirely natural extractives, semi-synthetic compounds produced from natural precursors, or agents derived from model (prototype) substances. A few examples of historical medicinal plant discoveries include aspirin, atropine, artimesinin, colchicine, digoxin, ephedrine, morphine, physostigmine, pilocarpine, quinine, quinidine, reserpine, taxol, tubocurarine, vincristine, and vinblastine. Worldwide, some 1200 botanicals are used to treat Type 2 diabetes empirically. Only 350 of them, however, have been shown to exhibit hypoglycemic action (Alarcon-Aguilar et al., 2002). For plant extracts, a variety of mechanisms of action have been suggested. Some theories center on how the plant extracts affect the action of pancreatic beta cells, the enzyme insulinase, insulin sensitivity, and insulin-like activity.

1.2: DIABETES MELLITUS:

According to Sharma & Sharma (2017), diabetes mellitus (DM) (meli-honey) is a set of heterogeneous illnesses in which protein and lipid metabolism is elevated while carbohydrate metabolism is decreased. Hyperglycemia, glycosuria, hyperlipidaemia, a negative nitrogen balance, and occasionally ketoacidosis are the hallmarks of this metabolic condition. The thickening of the capillary basement membrane, an increase in the vessel wall matrix, and cellular proliferation are common pathological changes that cause vascular complications like lumen narrowing, early atherosclerosis, glomerular capillary sclerosis, retinopathy, neuropathy, and peripheral vascular insufficiency (KD Tripathi, 2001). Because to impaired insulin secretion, poor insulin action, or both, the body is unable to create enough insulin to meet its own needs. Diabetes continues to be the third greatest cause of death, the second leading cause of blindness, and the second leading cause of renal failure, despite the fact that insulin treatment has significantly extended the life expectancy of diabetic patients. Three "polys"—excessive urination (polyuria), excessive thirst (polydipsia), and excessive eating (polyphagia)—are indicative of DM (Sharma & Sharma, 2017).

More than 422 million people worldwide have diabetes, and if current trends continue, the prevalence would likely continue to rise, according to the WHO Global Report 2016 on the disease. Since 1980, the prevalence of diabetes (age-standardized) in the adult population has increased by nearly twofold, from 4.7% to 8.5%. This reflects an increase in risk factors like obesity and excess weight. Diabetes prevalence has increased more quickly in low- and middle-income nations than in high-income nations over the past ten years.

The pathogenic mechanisms that lead to the development of diabetes include defects that lead to resistance to insulin action as well as autoimmune destruction of the pancreatic B-cells, which results in insulin insufficiency. Diabetes' distinctive clinical features, micro and macrovascular complications, and increased risk of cardiovascular disease are caused by abnormalities in carbohydrate, fat, and protein metabolism, which are caused by inadequate insulin action on target tissues and hyperglycemia (Malecki et al., 2005).

1.2.1: PANCREAS:

A light-grey gland called the pancreas is located in the left hypochondriac and epigastric portions of the abdominal cavity, below the stomach. It performs both exocrine and endocrine functions. The exocrine pancreas is made up of secretory cells that secrete the pancreatic amylase, trypsin, and chymotrypsin, as well as other digestive enzymes, and the endocrine pancreas is made up of distributed, specialized cell clusters known as pancreatic islets. The three primary types of cells in the pancreatic islets are a-cells (25%) that secrete glucagon, which raises blood glucose levels (Waugh et al., 2010).

Insulin is secreted by B cells, lowering blood glucose levels. Somatostatin, which regulates the secretion of insulin and glucagon, is secreted by 8-cells (5–10%). Pancreatic polypeptide (PP) cells secrete this substance.

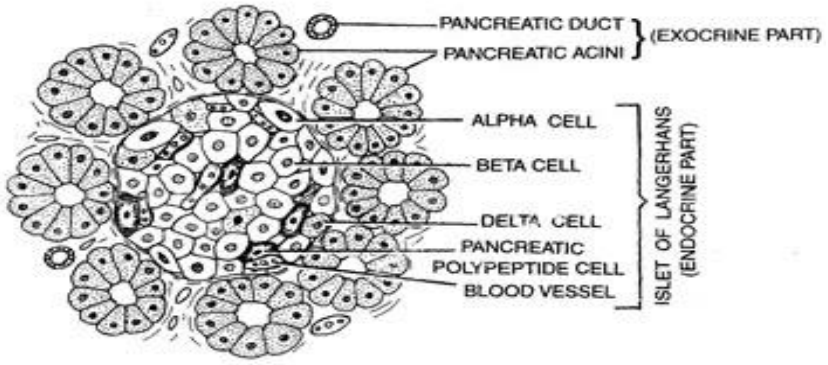

Figure: Different secretory cells of pancreas

1.2.2: REGULATION OF BLOOD GLUCOSE:

The release of insulin and glucagon is the primary mechanism used to control blood glucose levels. This mechanism is based on a negative feedback loop. Proinsulin is broken down by proteases in the golgi apparatus, where it is converted into insulin. These enzymes produce insulin and C-peptide when they are active. The islet of Langerhans in the pancreas releases insulin when the blood sugar level is above 70 mg/dL. The metabolism of glucose produces ATP, which inhibits the activity of ATP-sensitive K channels. Inhibiting the K channel lowers the beta cell membrane's c conductance, which causes depolarization of the membrane, the opening of voltage-dependent Ca channels, and an increase in the production of insulin (Sharma & Sharma, 2017).

Tyrosine kinase insulin receptors bind to insulin, and this association encourages the beta subunit's autophosphorylation. Insulin instructs the liver to store the extra glucose as glycogen. By moving the glucose transporter (GLUT4) to the cell surface, it also stimulates the body's adipose and skeletal muscle cells to absorb more glucose. This aids in restoring normal glucose concentrations in the blood. The pancreatic alpha cells are activated to release glucagon when blood glucose levels are low. In order to achieve homeostasis, glucagon tells the liver to release glucose into the blood from stored glycogen.

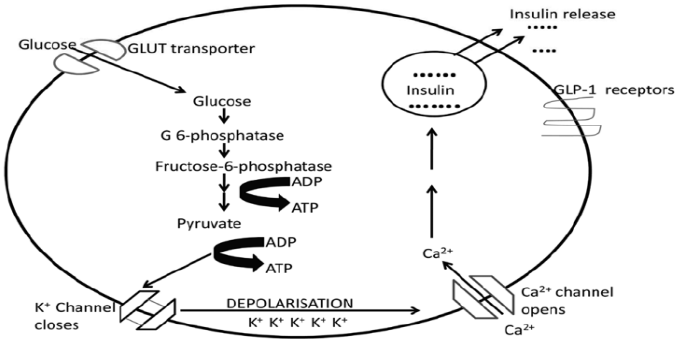

Figure: Insulin Release from the pancreatic β-cells.

1.3: TYPES OF DIABETES MELLITUS:

1.3.1: Type 1 Diabetes Mellitus (TIDM)

TIDM, also known as insulin-dependent diabetes or juvenile onset diabetes, is an autoimmune illness in which the pancreatic islets are invaded by macrophages and activated CD4+ and CD8+ T lymphocytes, which then kill B cells. The first signs of TIDM typically appear between the ages of 35 and 40. It is recognized that both genetic and environmental variables affect this diabetes' vulnerability. An autoimmune response against the B cells occurs in TIDM as a result of an alteration in the HLA (human leukocyte antigen) proteins. A nearby HLA gene called DR also has a significant impact on TIDM. There is evidence that suggests specific viruses may be the cause of TIDM.

Some patients experience idiopathic diabetes, a different type of TIDM that doesn't include autoimmune. The actiology and pathophysiology are poorly understood, it is less frequent than autoimmune TIDM, and individuals with it are more likely to develop ketoacidosis if they don't have antibodies against ß cells (J. Larry Jameson, 2018).

1.3.2: Type 2 Diabetes Mellitus(T2DM)

Insulin resistance, followed by the pancreatic beta cells' failure to counteract insulin resistance (pancreatic beta cell dysfunction), characterizes T2DM as a diverse illness. The primary associations between insulin resistance and compensatory hyperinsulinemia are resistance to insulin-mediated glucose elimination at the periphery and overt cell dysfunction. Insulin resistance is a metabolic defect that typically occurs before these conditions manifest themselves. In order to maintain glucose homeostasis, the cells routinely secrete more insulin to counteract insulin resistance. However, over time, this cell function becomes compromised, which worsens glucose homeostasis and causes the emergence of impaired glucose tolerance and insulin resistance (Gisela Wilcox, 2005). Age, obesity,

and a dietetic/sedentary lifestyle are the main risk factors for the development of type 2 diabetes, notwithstanding the involvement of genetic predisposition. Obesity is present in the majority of people with type 2 diabetes (Yanling Wu, 2014).

Diabetes with obesity and diabetes without obesity are the two subsets of T2DM. Due to changes in cell receptors, obese T2DM patients frequently acquire resistance to endogenous insulin; this is linked to the distribution of abdominal fat. Along with a shortage in insulin synthesis and release, non-obese T2DM also exhibits some insulin resistance at the post receptor levels.

1.3.3: Gestational Diabetes Mellitus

Gestational diabetes mellitus (GDM) is the term used to describe the development of diabetes throughout pregnancy and its remission at the conclusion of the gestation. Females go through a lot of glucose fluctuation and frequently endure accelerated hunger throughout pregnancy and the gestational period. This leads to a temporary state of insulin resistance along with an increase in placental insulin production and a loss in insulin sensitivity at the end of the first trimester. Despite the fact that this type of diabetes resolves at the end of the gestation, it is possible for problems to arise that may be irreversible. For instance, gestational diabetes is a possible teratogen and significantly raises the risk of mortality in both the mother and the fetus. A multitude of anomalies in the development of the fetus, including intrauterine growth retardation (IUGR), premature delivery, and stillbirth, are linked to pre-eclampsia, which can occur as a result of diabetic nephropathy in GDM.

1.3.4: Maturity Onset Diabetes of the Young (MODY)

The monogenic kind of diabetes is known as Maturity Onset Diabetes of the Young (MODY). It accounts for a relatively tiny fraction of diabetic patients and is typically identified during the patient's second decade of life. The two most prevalent forms of the condition are MODY 2 and MODY 3. MODY 3 is distinguished by a significant impairment in insulin secretion. Despite its rarity, it is crucial to correctly diagnose MODY and identify the source of diabetes in order to administer the best possible care.

Hyperthyroidism, Cushing's disease, Kline felters syndrome, and pancreatitis are all known to cause other secondary kinds of diabetes. a few medications and substances, including B-blockers and thiazide diuretics. Secondary diabetes can also be brought on by calcineurin, protease inhibitors, and atypical antipsychotic medications (Anne Waugh & Allison Grant, 2018).

1.4: COMPLICATIONS OF DIABETES MELLITUS:

Diabetes is linked to an increased risk of vascular problems, which raise patient morbidity and death. Vascular problems that affect large (macrovascular), tiny (microvascular), or both types of arteries are caused by poor glycemic and blood pressure control.

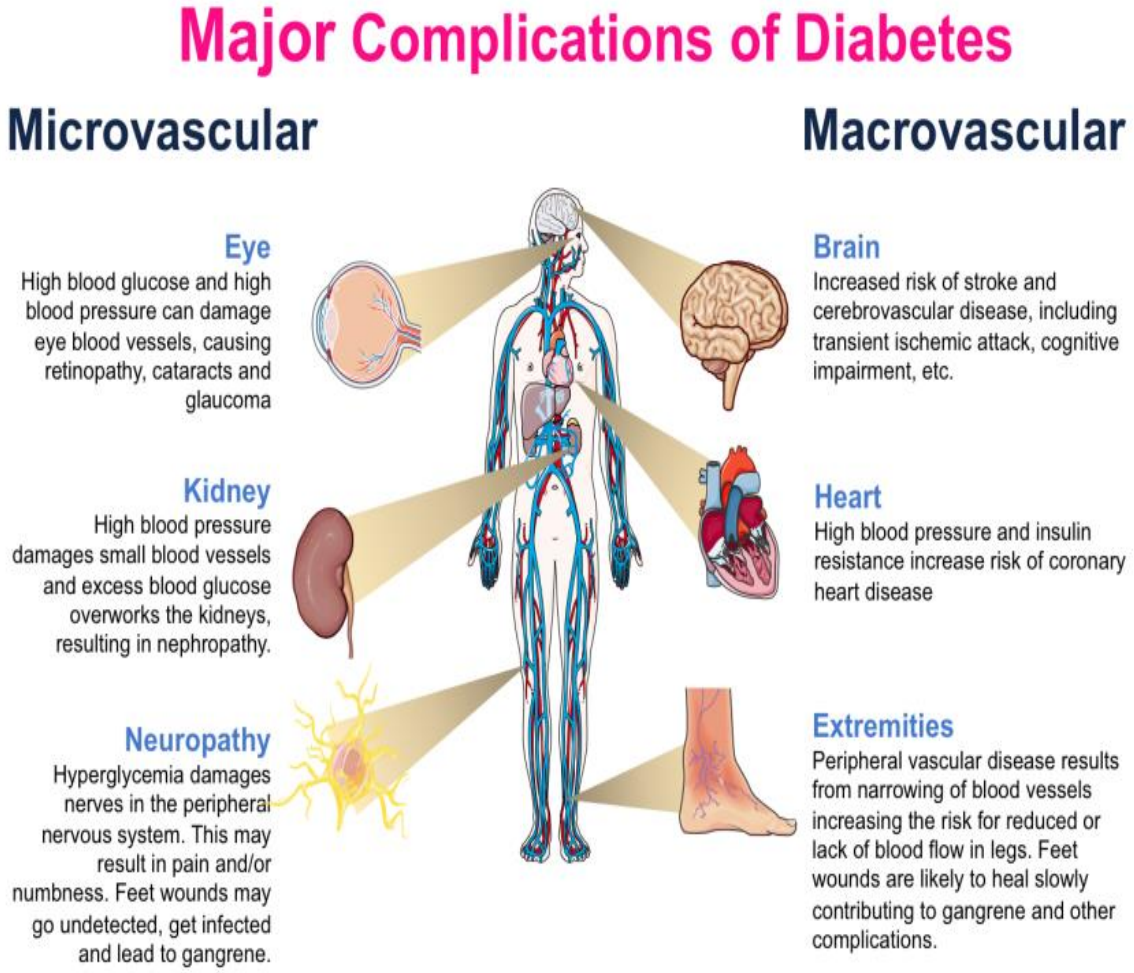

Figure: Microvascular and macrovascular problems that are brought on by Type 2 Diabetes Diabetes is a chronic condition that has a strong link to the onset of microvascular (retinopathy, neuropathy, and nephropathy) and macrovascular (coronary and cerebrovascular disorders) issues in the capillaries and arteries, respectively.

1.4.1: MACROVASCULAR COMPLICATIONS

The macrovascular problems, which affect the big vessels of the circulatory system, may cause a 2–4 times higher incidence of peripheral vascular disease, coronary heart disease, and stroke (cerebrovascular), which can result in ulcers, gangrene, and lower extremity amputations. The migration of leukocytes to the site of arterial injury characterizes these macrovascular complications, which are basically accelerated forms of atherosclerosis.

1.4.2: MICROVASCULAR COMPLICATIONS

Damage to the small blood arteries, which results in diabetes microvascular problems, contributes to diabetic neuropathy (nerve damage), nephropathy (kidney disease), and retinopathy (eye disease).

Diabetic Neuropathy

The majority of long-term consequences of diabetes are peripheral, proximal, focal, and autonomic diabetic neuropathy, which affects the nerves in almost 60% of patients. Amputations of limbs may be necessary as a result of this degenerative condition, which causes pain, weakness, and loss of sensation.

Diabetic Nephropathy

Interpapillary glomerulonephritis, another name for diabetic nephropathy, is a clinical syndrome characterized by arterial hypertension, permanent and irreversible decreases in glomerular filtration rate (GFR), and albuminuria (>300 mg/day or >200 mcg/min) confirmed on at least two occasions 3–6 months apart. According to Vrhovac B et al. (2008), both type 1 diabetes mellitus (beta cell death-complete lack of insulin) and type 2 diabetes mellitus (insulin resistance and/or decreased insulin output) are chronic complications.

There are five stages in the development of diabetic nephropathy:

Stage I: hyperfiltration of hypertrophic tissue. GFR is either enhanced or normal at this point. Stage I has an average lifespan of five years when the sickness first manifests. Although albuminuria and blood pressure remain within the normal range, the size of the kidneys grows by about 20%, and renal plasma flow rises by 10% to 15%.

Stage II: a calm period. This stage, which begins about two years after the disease first manifests, is marked by kidney injury, thickening of the basement membrane, and mesangial proliferation. The disease still shows no clinical symptoms. GFR readings return to normal. Up until their death, many patients are still in this condition.

Stage III: Initial nephropathy or the microalbuminuria stage (albumin 30-300 mg/dU). This is the earliest indication of glomerular injury that may be seen clinically. It often happens five to ten years after the disease first manifests. Both high and normal blood pressure are possible. About 40% of patients progress to this stage.

Stage IV: Chronic kidney failure (CKF) is the final stage and is irreversible. GFR falls below 60 mL/min/1.73 m2, proteinuria (albumin>300 mg/dU) develops, and blood pressure rises above normal levels.

Stage V: End-stage renal disease (TKF) (GFR 15 mL/min/1.73 m2). According to Mogensen CE (1999), kidney replacement therapy, including hemodialysis, peritoneal dialysis, and kidney transplantation, is necessary for about 50% of TKF patients.

Increased kidney size and altered Doppler indicators may be the earliest morphological indications of renal damage in the early stages of diabetic nephropathy, while proteinuria and GFR are the best indicators of the severity of the damage (Buchan IE, 1997).

PATHOLOGY

The glomerular filtration barrier performs the role of a sophisticated biological sieve. Glomerular capillaries differ from other capillaries in the body in that they are generally impermeable to big molecules while being extremely permeable to water (hydraulic conductivity). This permeability is made possible by the distinctive three-layer structure of the glomerular filtration membrane, which is made up of podocytes (glomerular visceral epithelial cells), glomerular basement membrane, and endothelial glycocalyx. Before microalbuminuria manifests in patients with long-duration DM, pathological alterations begin to occur in the glomeruli. According to Solini et al. (2002) and Rudberg et al. (2000), the severity of glomerular injury is proportional to GFR value, DM duration, and blood glucose regulation. The thickening of the glomerular basement membrane (GBM), mesangial expansion, diffuse glomerular sclerosis, nodular sclerosis-Kimmelstiel-Wilson change, tubular interstitial fibrosis, and arteriosclerosis and hyalinosis of kidney blood vessels are the main pathohystological changes in diabetic nephropathy.

Glomerular sclerosis can also appear in various clinical situations in patients with DM, in addition to diabetic nephropathy.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

Although other growth factors are anticipated to replace albuminuria in the future, albuminuria is still the only approved biomarker for diagnostic reasons. It is well-known that individuals with diabetic nephropathy have higher levels of TGF beta, vascular endothelial growth factor (VEGF), and CTGF in their plasma and urine (Nguyen et al., 2008; Pfeiffer et al., 1996).

Nephrosclerosis

In addition to primary glomerular disorders, patients with DM may also develop proteinuria and kidney failure from other illnesses. In elderly type 2 DM patients, atherosclerotic vascular disease (nephrosclerosis) is the most common cause. (2003) Myers et al. Without a kidney biopsy, this condition cannot be clinically distinguished from diabetic nephropathy. However, since the proper diagnosis in this patient group is not clinically significant, a kidney biopsy is typically not required. The considerable rise in blood creatinine levels with the use of ACEIs (angiotensin-converting enzyme inhibitors) or ARBs (angiotensin Il receptor blockers) to treat hypertension or halt the progression of chronic kidney disease is evidence in favor of nephrosclerosis. When there is bilateral renal artery stenosis, the same thing happens.

TREATMENT

Strict Glycemic Control

The impact of rigorous glycemic control is determined on the stage of DM at when it was initiated and the subsequent normalization of glucose metabolism. The following effects of intensified insulin therapy on the kidney are:

a. It reduces glomerular hypertrophy and hyperfiltration (both while fasting and after a protein-rich meal), two major risk factors for long-term glomerular damage.
b. According to an EDIC research from 2003, it delays the onset of albuminuria. The formation or progression of diabetic nephropathy is reduced by intensified insulin therapy that maintains glucose levels within normal ranges.
c. In individuals with severe proteinuria, it stabilizes or lowers protein excretion. Patients who are not comparatively normogycemic throughout the course of two years do not experience this effect. Additionally, type 1 DM patients who underwent combined kidney and pancreas

d. transplantation benefit from re-established normoglycemia since it reduces the risk of nephropathy reoccurring after kidney transplant (Fioretto P et al., 2006).
e. When proteinuria has already occurred and been verified by a semiquantitative approach (test strip), it reduces the progression of kidney disease.
f. It reduces the quantity of mesangial cells and the mesangial matrix.
g. In some individuals, the number of mesangial cells and the thickness of the tubular and glomerular basement membranes return to normal, and the glomerular nodules vanish.
h. Tubular atrophy's progression is slowed.

Strict Blood Pressure Control

In individuals with type 2 DM, strict blood pressure control is crucial for halting the progression of diabetic nephropathy and associated problems. A drop in systolic blood pressure of 10 mm Hg reduces the chance of developing diabetic complications by 12%, according to the UKPDS study (Ferrario CM, 2006). The risk is lowest when systolic blood pressure values are below 120 mm Hg. According to the Irbesarta Diabetic Nephropathy Trial, lowering systolic blood pressure to the lower limit of 120 mm Hg lowers the risk of heart failure and cardiovascular mortality (but not myocardial infarction), as well as the risk of a double increase in serum creatinine or progression to terminal kidney failure (Berl et al., 2005).

Inhibition of Renin-Angiotensin-Aldosterone System

The most potent component of the renin-angiotensin-aldosterone system (RAAS), angiotensin II is produced by a number of proteolytic processes beginning with the catalytic conversion of angiotensinogen to angiotensin I (ATI) by rennin. Blood pressure control, body fluid volume, and vascular reaction to injury and inflammation are all intimately correlated with RAAS. This system's inappropriate activation raises blood pressure and has anti-inflammatory, prothrombotic, and proatherogenic actions, all of which eventually cause irreparable harm to the target organs. Angiotensin II(AT2), which is created by kinase and endopeptidase activity in the heart, brain, and kidneys through alternate pathways, is more efficient than angiotensin II produced in the bloodstream (Cooper ME, 2004). AT1 and AT2 receptors are ligands for angiotensin II. According to Hilgers and Mann (2002), AT1 receptor activation causes vasoconstriction, aldosterone release, vascular remodeling, oxidative stress, and has effects that are anti-inflammatory, proatherogenic, and prothrombotic. By showing that enalapril reduces structural glomerular damage, proteinuria, and glomerular capillary hypertension in diabetic rats, Zatz et al. established in 1986 that RAAS plays a role in the development and progression of diabetic nephropathy. Later research has verified that angiotensin II is crucial for the structural and functional alterations that relate proteinuria to the onset of diabetic nephropathy. Albuminuria can be reduced by increasing ACEI and ARB dosages above those advised for the treatment of hypertension,

or by combining them (Jacobsen et al., 2003). In patients with DM, aldosterone receptor antagonists and renin inhibitors also reduce albuminuria, but a significant randomized study is required to assess whether they have any advantages over ACEI and ARB when used alone or in combination (Estacio RO, 2009).

Dyslipidemia

All patients with DM experience dyslipidemia, and as diabetic nephropathy progresses, the likelihood of this condition increasing Due to the increased risk of cardiovascular disease in DM patients, aggressive plasma lipid lowering is a crucial therapeutic step. Additionally, diabetic nephropathy is facilitated by dyslipidemia. Statin therapy for dyslipidemia decreases the development of diabetic nephropathy (Tonolo et al., 2006).

The Role of Other Factors

The effects of transforming growth factor beta (TGF-beta) include enhanced collagen production and cell hypertrophy. In an experimental DM model, TGF-beta inhibition stopped the onset and progression of diabetic nephropathy (Benigni et al., 2003). Diltiazem, a non-dihydropyridine calcium channel blocker, inhibits the advancement of the majority of morphological alterations in diabetic nephropathy, according to experimental studies (Gaber et al., 1994). However, diltiazem monotherapy causes global rather than segmental glomerulosclerosis and enhanced tubulointerstitial fibrosis. By using ACEI therapy, this side effect of diltiazem can be reversed. In addition to controlling adipogenesis, lipid metabolism, insulin sensitivity, inflammation, and blood pressure, peroxisome proliferator-activated receptors (PPAR) also appear to be crucial in the development of diabetic nephropathy in people with type 2 diabetes (Guan Y, 2004). Tiazolidinedones (oral hypoglicemic drugs), a PPAR gamma agonist, have been demonstrated to lessen fibrosis, mesangial proliferation, and inflammation in an experimental animal model of diabetic nephropathy (Weissgarten et al., 2006).

New Treatment Strategies

Not all patients have responded well to the current course of treatment. As a result, researchers are looking at novel therapeutic options. In animal studies, it has been demonstrated that high doses of thiamine and its derivative benfotiamine (S- benzoylthiamine Omonophosphate) can delay the onset of microalbuminuria. This is most likely because they reduce PKC activation, protein glycation, and oxidative stress (Babaci-Jadidi et al., 2003). Blood pressure and renal damage were reduced in experimental mice given ALT-711, which metabolizes AGES (advanced glycation endproducts). In experimental animals, the PKC-beta inhibitor ruboxistaurin normalizes GFR, lowers or lowers albuminuria, and improves kidney function (Kelly et al., 2003). In patients with type I diabetes and

proteinuria, the second-generation AGE inhibitor pimagedin lowers albuminuria and GFR decline (Bolton et al., 2004).

DIABETIC RETINOPATHY:

In people of working age, diabetic retinopathy, which is brought on by harm to the retinal vasculature, is a prevalent cause of blindness and visual impairment. By receiving proper and prompt care, diabetic retinopathy can be delayed or even avoided.

IMPAIRMENT OF IMMUNE SYSTEM

The insulin-producing B-cells in the islet of langerhans are gradually killed in TIDM, an autoimmune illness, which prevents the generation of insulin. Autoimmunity is a complicated condition that is influenced by both hereditary and environmental factors. Due to recent research emphasizing the important involvement of T cells in TIDM, B and T cells of the immune system play critical roles in autoimmunity (Jameson, 2018).

PERIODONTAL AND FOOT DISEASES

The ability of the body to protect itself from viral, bacterial, fungal, and protozoal diseases can be negatively impacted by diabetes because immunity can be severely diminished in this condition (P Pozzilli, 2009). The most significant of these infections are periodontal diseases, which can badly harm teeth if care is not followed (Genco RJ, 2004). Additionally, people with diabetes are more likely than non-diabetics to experience fungal infections of the feet, particularly between the toes. Therefore, it may take a very long time to discover an infection, during which time it may spread widely, become more difficult to treat, frequently degenerate into gangrene, and ultimately necessitate amputating the foot (Eckhard M et al., 2007).

1.5: MANAGEMENT OF DIABETES:

The main goals of the treatment are symptom relief and life preservation. Secondary goals include preventing long-term diabetic problems and lengthening lifespan by reducing risk factors. While food and lifestyle changes are taken into consideration for the treatment and maintenance of type 2 DM, insulin replacement therapy is the backbone for individuals with type I DM. Oral hypoglycaemic medicines are also helpful in the treatment of type 2 DM. Insulin is needed in type 2 DM when blood glucose levels cannot be maintained by diet, weight loss, exercise, and oral drugs. These medications' primary goal is to treat the underlying metabolic condition, which may include insulin resistance and insufficient insulin production. They ought to be prescribed along with suitable dietary modifications and lifestyle adjustments. The optimal methods for controlling diabetes are either diet and exercise

alone (non-pharmacological), or diet combined with insulin or herbal or oral hypoglycemic medications (pharmacological) (Bastaki S, 2005).

1.6: TREATMENTS FOR DIABETIC KIDNEY DISEASE:

DKD (Diabetic Kidney Disease) is caused or promoted by a variety of circumstances. Hyperglycemia as well as altered gene expression are initiators. Promoters of diabetes include high blood sugar, high blood pressure, dyslipidemia, smoking, race, sex, and age. Targeted initiators and promoters are those who can be changed (Macisaac et al., 2014; Kim et al., 2017).

1.6.1: CONTROL OF GLUCOSE LEVELS:

Insulin

There are numerous insulin preparations available. To prevent potential hypersensitivity reactions, recombinant insulin preparations that are highly purified are available. In order to improve basal control of blood sugar postprandial and between meals, a variety of short acting and long acting conventional preparations are also available. In order to change the pharmacokinetics, modulate the release, and improve stability, other insulin analogues are also produced. The administration of insulin can complicate insulin therapy with pharmacological issues, weight gain, and hypoglycemia. Therapeutic insulin formulations' pharmacokinetic and pharmacodynamic characteristics are still being studied for their ability to control these effects. Inhaled insulin, insulin pumps, and implanted pumps are just a few of the innovations that have been produced to facilitate the accurate and precise administration of insulin while maintaining strict glycemic control. When it comes to type 1 diabetes, diabetic ketoacidosis, and hyperosmolar non-ketotic hyperglycemic coma, all of which are linked to type 2 diabetes, insulin is crucial. Both alone and in combination with oral hypoglycemic medications, insulin is used. If some B cell function is still present, basal insulin augmentation treatment is beneficial. If Bom depletion develops, basal-bolus insulin replacement is required (Mayfield JA, 2004).

Use of Oral Hypoglycaemic agents

These medicines are orally effective at reducing blood glucose levels and are primarily used in type 2 diabetes.

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 ca2u 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, and promote peripheral glucose utilization by enhancing anaerobic glycolysis thereby overcoming insulin resistance.	Long acting, improve lipid profile by reducing plasma.	Lactic acidosis, Vit 612 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, of most be avoided in liver disease.

Thiazolidinedione	Rosiglitazone, Troglitazone, Pioglitazone	Selective agonist of peroxisome proliferator activated receptor gamma (PPAR gamma), which enhances transcription insulin responsive genes e.g. GLUT-4, entry of glucose into muscle and fat cell is improved, increase lipogenesis.	Improve glycemic control, lower in circulation HbA1C, lowering of serum triglyceride and increment in serum HDL level.	Weight gain, liver toxicity, fluid retention leading to heart failure.
Alpha Glucosidase inhibitor	Agarose, Milglitol (more potent in inhibiting sucrose)	Inhibition of alpha Glucosidase the final enzyme for the digestion of carbohydrate inthe brush border of sgiall intestine mucosa.	Lower post gltremia, Lower HbAk, body weight, serum triglyceride.	Gm, bloating and diarrhea.
DPP-4 inhibitors	Sitagliptin, Vildgliptin, Samliptin	Inhibit the DPP-4 enzyme, increasing the level of endogenous plasma glucagon like peptide- I, which promotes insulin secretion but inhibits glucagon secretion.	Decrease Alburninuria, fibrosis and thickening of GBM.	Nasophatyngitis, GITdis and diarrhea.
SGUI4 inhibitor	Dapagliflozin, Canagliflozin	Inhibits renal glucose reabsorption.	Lower BP and reduce body weight.	Because of more polyuria, there would be more polydipsia, urinary bacterial/fungal infection.

1.6.2: CONTROL OF BLOOD PRESSURE(BP)

Blood

pressure regulation is often advised to prevent stroke, cardiovascular disease, and albuminuria. BP management has been proven in numerous trials to be renoprotective (Muskiet et al., 2014). According to the UKPDS, a 10-mmHg decrease in systolic blood pressure was related with lower levels of diabetic microvascular sequelae, including nephropathy (Alder et al., 2000). To avoid the development and progression of DKD, the ADA (adenosine deaminase) recommends that treatment strive to lower blood pressure below 140/90 mmHg (ADA, 2016). To manage blood pressure, angiotensin t receptor blockers (ARBS) or angiotensin-converting enzyme (ACE) inhibitors are recommended (Taler et al., 2013).

1.7: EMERGING THERAPEUTIC TARGETS

Autophagy

Autophagy is the process by which mammalian cells degrade their own macromolecules and organelles in order to maintain intracellular homeostasis, and it protects organisms from a variety of pathologies such as infections, cancers, neurodegeneration, aging, and heart disease (Shintani & Klionsky, 2004). Autophagy has recently been linked to the pathology of several diseases, including cancer and diabetes (White E, 2012; Masini M, 2009). However, growing evidence suggests that autophagy has cytoprotective properties in the kidney. Renal autophagy is induced under certain stress conditions, such as oxidative stress and hypoxia, and in diabetic animal models' kidneys, but it is repressed in obese type 2 diabetic patients (Yamahara K, 2013; Mizushima N, 2004). Podocytes require autophagy at a basal level to maintain cellular homeostasis, whereas proximal tubular cells use autophagy as a coping mechanism when exposed to nephrotoxic stress. As a result, if a treatment for diabetic nephropathy sought to regulate autophagy, the specificity of such treatment could be problematic (Kume S & Koya D, 2015).

Sre family kinases

Src family kinases (SFKs) are non-receptor tyrosine kinases that are activated by autophosphorylation of their Tyr416 residues and are stimulated by TGF-B1 and epidermal growth factor (Bromann et al., 2004). Src, Fyn, Yes, Yrk, Blk, Fgr, Hck, Lck, Lyn, and the Frk subfamily proteins are all members of the SFKs, and they all have a distinct amino-terminal region, highly conserved kinase domains, and carboxy-terminal tails carrying the (negatively) regulatory tyrosine residue. Src, Fyn, and Yes are found in most tissues, but their activity vary by tissue; the other SFKs are commonly found in hematopoietic cells.

Recent research suggests that the Src protein may be a possible target for treating renal fibrosis. In mice with streptozotocin (STZ)-induced diabetes, high glucose levels activate the Src of mesangial cells and the glomeruli (Mima A, 2006; Taniguchi K, 2013).

1.8: CONCLUSIONS

The prevalence of DKD is projected to rise in tandem with the prevalence of diabetes. Although glucose and blood pressure control can occasionally avoid the onset of DKD, many people with Diabetes progresses to end-stage renal disease (ESRD).

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CHAPTER 2: PLAN OF WORK

Objectives:

- Literature Review and Selection of plant
- Collection and identification of *Ficus rumphii* bark
- 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
- Induction of Diabetic nephropathy in rats.
- Evaluation of in vivo anti-diabetic potential
  - Blood glucose level
- 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
  - Triglyceride
- Histopathology

CHAPTER 3: LITERATURE REVIEW

3.1: NAME:

Ficus rumphii

3.2: PLANT TAXONOMY: (Chantarasuwan, 2014)

Kingdom: Plantae

Class: Magnoliopsida

Order: Rosales

Family: Moraceae

Genus: Ficus

Subgenus: F. subg. Urostigma

Species: F. rumphii

3.3: SYNONYMS: (Zhang et al., 2019)

Urostigma rumphii (Bl.) Miq.
Urostigma cordifolium (Roxb.) Miq.
Ficus populnea Kunth & Bouche
Ficus populiformis Schott ex Miq.
Ficus damit Gagnep.
Ficus cordifolia Roxb.
Ficus conciliorum Oken
Ficus affinior Griff.

3.4: LOCAL NAME: (K.N. et al,2012)

Hindi: Pilkhan

Bengali: Pakur

English: Rumphf's fig tree

Myanmar: nyaung-phyu

Assamese: Pakhori

3.5: PARTS USED:

Leaves, bark, fruit, roots and latex

3.6: ETHNOBOTANY: (Flora of China, 2003)

Medicinal Uses: Various parts of fig trees have been traditionally used in herbal medicine. The latex or sap derived from the stems and leaves of Ficus rumphii may be used topically to treat skin conditions like wounds, cuts, and sores. It is believed to possess antibacterial properties (Kirtika & Basu, 2001) and aid in the healing process(Murugan, et. al. 2013).

Culinary Uses: Ficus rumphii fruits, commonly referred to as figs, are edible (Murugan, et.al. 2013) and can be consumed either raw or cooked. However, it's important to note that not all fig species produce fruits that are suitable for direct consumption. The fruits of some fig species are used to make jams, jellies, and preserves, while others may be dried or processed into sweet treats.

Cultural and Religious Significance: Ficus rumphii, like other fig trees, holds cultural and religious significance in Southeast Asian communities. In some indigenous cultures, fig trees are considered sacred, and offerings may be made to these trees as a form of respect or as part of traditional rituals.

Timber and Crafts: The wood of Ficus rumphii, along with other fig species, is sometimes used for various purposes, such as construction, furniture making, and crafting. The flexible branches of fig trees are also used for making baskets, woven mats, and other handicrafts

Environmental Uses: Ficus rumphii, like other fig species, is ecologically important as it serves as a food source and habitat for various animals, including birds, bats, and insects. The trees also contribute to soil stabilization and erosion control.

3.7: USES:

The plant is used in wound healing activity, anti-inflammatory activity, antiproliferative activity (Natural Product Research, 2014), antibacterial, antimicrobial activity and antioxidant activity.

3.8: GEOGRAPHICAL DISTRIBUTION: (Chaudhary et. al., 2012, Adrian & Storrs, 1998)

Ficus rimphii can be primilarily found in Southeast Asia such as southern China, India, Bhutan, Nepal, Myanmar, Malaysia, Thailand, Indonesia, Vietnam .

3.9: MORPHOLOGY: (Ajmal and Iqbal, 1988)

Growth form: A deciduous tree, Ficus rumphii, can reach a height of 20 meters. As the plant ages, it sends down aerial roots that, when they reach the ground, swiftly form roots and become much thicker and more vigorous. The plant frequently starts out its existence as an epiphyte, growing in the limb of another tree. They provide the fig with nutrients, enabling it to develop more quickly than the host tree. The aerial roots gradually encircle the host tree, inhibiting further growth of the main trunk, and the host tree's foliage is smothered by the aerial roots. The host eventually passes away, allowing the fig to continue growing unhindered.

Leaves: Ficus rumphii leaves are often big and elliptical or ovate in shape. They frequently have a broad, extended shape with a pointy tip. Although the size of the leaves might vary, they typically measure between 10 and 25 cm (4 and 10 inches) in length, which is greater than ordinary.

Bark: The bark of Ficus rumphii is generally smooth and grayish in color. As the tree ages, the bark may develop slight fissures or become slightly rougher in texture.The size of the stem of Ficus rumphii can vary depending on the age and health of the tree. Mature trees can have substantial trunk diameters, reaching several meters in circumference.Like many fig species, Ficus rumphii produces a milky latex or sap when its stem or branches are cut or injured. The sap can be sticky and may have a white or yellowish coloration.

Flower: The trees produce three different types of flowers: male, female with long and short styles, often known as the gall flower. The structure we typically think of as the fruit actually contains all three types of flowers.The female fig wasp penetrates the fig, pollinates the long-styled female flowers, then deposits her eggs on the short-styled female flowers. First, the wingless male fig wasps emerge, fertilize the newly emerged females, and then the winged females burrow exit tunnels out of the fig. Females emerge, gather pollen from male flowers, and then take flight in search of figs with female flowers that

are open to receiving pollen. Individual Ficus spp. must flower asynchronously in order to sustain a colony of its pollinator.

Fruits: They are generally small to medium-sized fruits, ranging from approximately 1 to 3 centimeters (0.4 to 1.2 inches) in diameter. The figs are typically round or slightly pear-shaped. Immature figs are usually green in color, and as they ripen, they can turn shades of yellow, orange, or red.

Seeds: The figs of Ficus rumphii are edible, and the flesh inside the syconium contains numerous small seeds. The seeds are typically embedded within the soft, sweet, and juicy flesh of the fig. The flavor of the fruit can vary but is generally described as sweet and mildly fruity.

3.10: PHYTOCHEMISTRY:

Polyphenols: Figs are known to contain various polyphenolic compounds, including flavonoids, phenolic acids, and tannins. These compounds possess antioxidant properties and contribute to the potential health benefits associated with fig consumption.

Carbohydrates: Figs are rich in carbohydrates, particularly in the form of sugars, such as glucose, fructose, and sucrose. These sugars contribute to the sweet taste of the fruit.

Organic Acids: Figs may contain organic acids, including citric acid, malic acid, and fumaric acid. These organic acids contribute to the characteristic tangy flavor of some fig varieties.

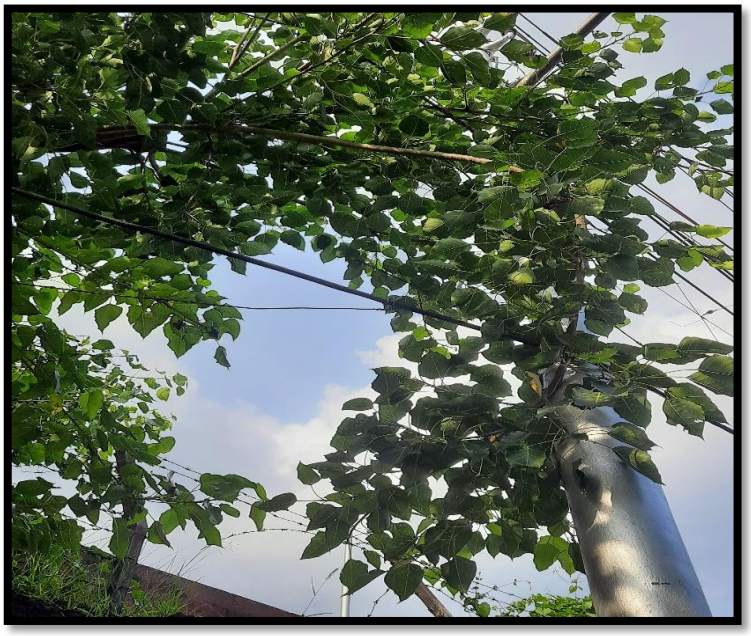
Proteins and Enzymes: Figs contain proteins and enzymes, which are involved in various physiological processes within the fruit. These proteins and enzymes contribute to the ripening, flavor development, and other biochemical changes in figs.

Volatile Compounds: Figs are known to release volatile compounds, contributing to their distinct aroma. These volatile compounds can include various alcohols, esters, aldehydes, and ketones.

3.11: 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.

picture



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Chapter 4: Collection, Extraction and phytochemical screening

4.1: Introduction:

In the healthcare system, medicinal plants are important. Both patients and researchers are interested in herbal medications for the validation of traditional medicine (TM) as well as for drug development from natural sources. Because it is readily available and inexpensive, TM has a significant impact on underdeveloped nations. Comminuted materials or extracts, tinctures, and oils are examples of herbal preparations. These products may be made through extraction, fractionation, or other physical or biological processes (Shinde et al., 2009). The technique of extracting herbs involves using a suitable solvent, known as menstruum, to draw the soluble components out of a crude herbal medication. In natural product research, the extraction method tries to release the therapeutically effective phytoconstituents that are present in the cellular structures of the crude medicine. Depending on the needs, level of production, stability of the secondary metabolites, etc., multiple extraction methods may be used (Okoduwa et al., 2016). The classical methods of solvent extraction of plant materials were founded on careful solvent selection and the use of mechanical forces, such as heat and shaking, to increase secondary metabolite solubility and improve mass transfer (Mandal et al., 2007). The two main types of extraction are cold extraction (such as cold maceration, cold enfleurage, and cold extrusion) and hot extraction (such as infusion, decoction, microwave extraction, soxhlet extraction, distillation, etc.). Cold extraction techniques are appropriate for dealing with plants that contain volatile or thermo-labile chemicals because there are less chances of the secondary metabolites of the plant decomposing in these circumstances. Where the stability of the chemicals in the plant extract is not a major concern, hot extraction methods can be used. In the current study, the bark being studied is hydro-alcoholically extracted utilizing a soxhlet method.

4.1.1 Soxhlet extraction:

One kind of hot continuous extraction method is soxhlet extraction, in which a dried crude drug is subjected to continuous extraction with the same solvent. The soxhlet extractor primarily consists of three parts:

1. At the top- the condenser. Here the solvent vapor gets condensed and thereafter drips in to the main chamber of the Soxhlet extractor.

2. In the middle- the main chamber of the Soxhlet apparatus, which contains the Thimble.The main chamber is loaded with the Thimble, which is actually the powdered crude drug wrapped in a piece of filter paper.

3. At the bottom- a round-bottom flask. It holds the solvent, or menstruum, as it is more popularly known. Sufficient amount of solvent necessary for extraction is loaded in this flask.

The solvent is boiled in the round-bottom flask with the help of a heating mantle. The temperature is set at a temperature around the boiling point of the solvent. There is one side tube originating from the round-bottom flask and opening at the top of the main chamber of the soxhlet apparatus carrying the solvent vapors to the condenser. There is another thin tube known as the siphon tube which is connected to the main chamber. As the level of the solvent in the chamber reaches the top of the siphon tube the entire solvent in the chamber gets transferred into the round-bottom flask. The colour of the solvent in the side tube should be monitored continuously. As long as there is any tinge of colour visible in the siphon tube, the extraction should be continued. When the solvent in the side tube gets colourless, the liquid containing the phytoconstituents should be collected and evaporated in vacuum. If any solid residue is visible, the extraction should be continued. Otherwise, the extraction procedure need not be continued. Importance of this technique is that the process can be done multiple times to achieve effective extraction.

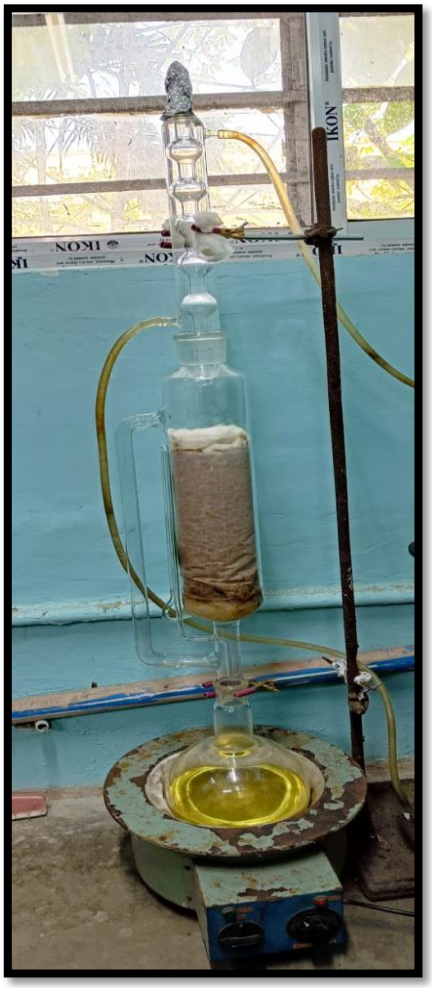

Figure: Soxhlet apparatus

After completion of extraction, through several downstream processing methods, a finished extract is obtained. First, the solvent is removed using a suitable method, which is referred to as concentration. After the concentration procedure, the plant extract is subjected to phytochemical analysis. The preliminary qualitative phytochemical analysis detects the presence secondary metabolites. The quantitative estimations of the total amount of a particular kind of secondary metabolite, such as the TPC and TFC is also under the scope of the present study.

4.2: Collection and identification

In the month of november, the entire plant *Ficus rumphii* (FR) was procured and identified using a reference sample kept at the Jadavpur University Department of Pharmaceutical Technology. Tap water was used to properly clean and sanitize the bark. The bark was dried at room temperature (24–26 °C) in the shade and then powdered in a mechanical grinder. It was then stored in an airtight container.

4.3: Extraction

In a Soxhlet apparatus, the FR bark was extracted using ethanol and water (hydro-alcoholic 70:30). The extract was filtered, and then the previous step was carried out twice more. The solvent was completely removed under reduced pressure in a rotary evaporator. the concentrated extract was obtained by lyophilization and stored in vacuum desiccators (20°C) for further use. The yield value was about 12.44% w/w.

4.4: Qualitative analysis:

Due to the presence of phytochemical components, medicinal plants are helpful for both treating and curing human ailments (Nostro et al., 2000). Plant substances known as phytochemicals are non-nutritive but have protective or disease-preventive qualities. The same molecules that plants make to protect themselves also serve to protect them against disease, according to current research (Ajuru, 2017). Therefore, it is crucial to identify the phytoconstituents in an ant substance or its extract. To determine the kind of component included in the extract, a preliminary qualitative analysis has been created. Alkaloids, flavonoids, saponins, tannins, steroids, glycosides, and carbohydrates were divided into chemical groups.

4.5: Chemical tests:

4.5.1: Test for Steroids

Liebermann- Burchard Test:(Zhou and Yu, 2004)

10 mg of extract was dissolved in 1 ml of chloroform. After adding 2 mL of concentrated H_2SO_4, 1 mL of Acetic Anhydride was added.

- The emergence of a reddish violet tint indicated the existence of steroids. But there is absence.

Salkowski Test:(Bosila and El-Sharabasy, 2009)

1 ml of concentrated H_2SO_4 was added to 10 mg of extract diluted in 1 ml of chloroform. The presence of steroid was shown by the reddish blue hue of the chloroform layer and the green fluorescence of the acid layer. But there is absence.

4.5.2: Test for Flavonoids

Alkaline reagent test:(Solomon et al., 2013)

- To 2 mL of extract, a few drops of 20% sodium hydroxide solution were added. Flavonoids are detected by the production of a bright yellow hue that fades to colorless when mild hydrochloric acid is introduced.

Shinoda test:(Pethappachetty et al., 2012)

- In alcohol, a small amount of extract was dissolved. Two to three pieces of magnesium were added, then powerful hydrochloric acid was added, and the mixture was boated. Appearance The appearance of magenta indicates the presence of flavonoids.

4.5.3: Test for Saponins(Verma et al., 2021)

- 1ml of the extract solution was diluted to 20 ml with distilled water and agitated for 15 minutes in a graduated cylinder. The production of stable foam revealed the presence of saponins.
- 1 mL of extract was treated with a 1% lead acetate solution. The production of white precipitate confirmed the presence of saponins.

4.5.4: Test for Tannins (Segelman et al., 1969)

- 1 mL of 5% ferric chloride solution was combined with 5 mL of extract solution. The greenish black colour suggested the presence of tannins.
- To 5 mL of extract, 1 mL of 10% aqueous potassium dichromate solution was added. The production of a yellowish-brown precipitate revealed the presence of tannins.
- To 5 mL of extract, 1 mL of a 10% lead acetate solution in water was added. The emergence of yellow precipitate indicated the presence of tannins.

4.5.5: Test for Glycoside(Salwaan et al., 2012)

Legal's test:

The extract was dissolved in pyridine and a solution of sodium nitroprusside was added to make it alkaline. The production of pink red to crimson colour indicates the presence of glycosides.

Bontrager's test:

One millilitre of extract solution was treated with a few millilitres of mild sulphuric acid before being filtered and chloroform extracted. The layer was treated with 1 mL of ammonia. The formation of a crimson colour indicates the presence of anthraquinone glycosides.

4.5.6: Test for Carbohydrate

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

The test solution was mixed with a few drops of Benedict's reagent (an alkalino solution containing cupric citrate complex) and heated in a water bath to look for the formation of reddish brown precipitate, which confirmed carbohydrate formation.

Molish test:(Salwaan et al., 2012)

1 mL of a-naphthol solution and 1 mL of concentrated sulphuric acid were added to 2mL of extract via the test tube sides. Carbohydrates are detected by the presence of a purple or reddish violet tint at the junction of the two liquids.

4.5.7: Test for Alkaloids (Bruck de Souza et al., 2020)

Mayer's test:

A 1.2 mL test tube held the extract. When 0.2 mL of weak hydrochloric acid and 0.1 mL of Mayer's reagent are mixed together, a yellowish buff precipitate forms, indicating a positive test for alkaloids.

Dragendroff'stest:

0.1 ml of dilute hydrochloric acid and 0.1 ml of Dragendroff's reagent were added to a test tube containing a 2m1 extract solution. The presence of alkaloids was confirmed by the production of orange brown precipitate.

Wagner's test:

Two millilitres of extract solution were treated with 0.1 millilitres of Wagner's reagent and two millilitres of mild hydrochloric acid. A reddish brown coloration indicated a positive response to alkaloids.

Hager'stest:

The extract was combined with 0.2 millilitres of mild hydrochloric acid and 0.1 millilitres of Hager's reagent. A yellowish precipitate suggested the presence of alkaloids.

4.5.8: Test for Phenols(Saxena et al., 2015)

The test solution received 3-4 drops of FeCl3. The creation of a bluish black colour indicates the presence of phenol.

4.5.9: Test for Triterpenoid

Salkowski test:(Nayak et al., 2010)

The test extract received a few drops of concentrated H2SO4. The emergence of a yellow colour in the lower layer suggested the presence of triterpenoids. But there is absence.

4.6: Results:

Table 1: Preliminary phytochemical screening of *Ficus rumphii* hydro-alcoholic extract

SL NO.	Phytoconstituents	Presence/Absence
1	Flavonoids	+
2	Alkaloids	+
3	Phenolics	+
4	Steroids	-
5	Carbohydrates	-
6	Glycosides	+
7	Tannins	+
8	Proteins	+
9	Saponin	-
10	Triterpenoid	-

['+' indicates the Presence of the particular constituents whereas '-' indicates Absence]

4.7: Conclusion:

During a first phytochemical analysis, the presence of flavonoids, alkaloids, phenolics, glycosides and tannins was found. A wide range of phytochemicals are referred to as polyphenols, which also include flavonoids and phenolic acid. By controlling hepatic enzyme activity, glucose metabolism, and lipid profile, flavonoids help to prevent the genesis and complications of diabetes. Regarding metabolic

diseases including diabetes, cancer, obesity, and cardiovascular disease, flavonoids have a range of health advantages. Additionally, they function as antioxidants, reducing the body's oxidative stress by neutralizing the effects of nitrogen and oxygen species and preventing disease. The anti-diabetic activity of flavonoids helps to control insulin secretion, glucose uptake, and fat deposition. Therefore, *Ficus rumphii* hydro-alcoholic extract (FRHE) has a lot of polyphenols. To develop a deeper understanding of the mechanism of action on diabetic nephropathy, more research is required.

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CHAPTER 5:

EVALUATION OF DPPH FREE RADICAL SCAVENGING ASSAY, TPC & TFC DETERMINATION

5.1: Introduction

Oxidative stress affects a variety of diseases including diabetes mellitus, cardiovascular disease, neurological disorders, chronic renal disease, tissue injuries, and others by damaging DNA and proteins, lipids, and proteins. Diabetes has been associated with a variety of pathological conditions, such as coronary heart disease, stroke, neuropathy, nephropathy, retinopathy, and slow wound healing. In this study, the DPPH radical scavenging method was used to assess the antioxidant potential of FRHE. In order to generate natural antioxidant compositions for food, cosmetics, and other uses, antioxidants extracted from fragrant, spicy, medicinal, and other plants have been studied. Alkaloids, terpenoids, and phenolic metabolites are the three main classes of plant chemicals. Phenolic compounds are the subject of the most research among these three types since they are crucial for nutritional applications. Polyphenols (hydrolyzable and condensed tannins), phenolic acids (hydroxybenzoic and hydroxycinnamic acids), and flavonoids are examples of phenolic compounds. Humans have used these compounds as antioxidants to protect plants from oxidative damage. For use in natural antioxidants, functional foods, and neutraceuticals, it is incredibly interesting to find new and secure antioxidants from natural sources.

5.2: Drugs & Chemicals

Standard Ascorbic acid (Hi-media, Mumbai, Maharashtra, India), Gallic Acid (Sigma-Aldrich Co., USA.), Quercetin (Hi-media, Mumbai, Maharashtra, India) and all other Chemicals of analytical grade were obtained commercially.

5.3: Evaluation of the *in-vitro* antioxidant activity

5.3.1: DPPH free radical scavenging assay

Material and methods:

Preparation of 0.1 mM DPPH (2,2-diphenyl-1-picrylhydrazyl) solution:

4mg DPPH was dissolved in 100ml methanol or ethanol to produce 0.1 Mm DPPH solution.

Preparation of dilutions of the plant extracts:

Ficus rumphii hydro-alcoholic extract 1 mg were dissolved in 1 ml methanol by vortex. The solution was filtered through a 0.45 µ filter (Millipore). From there, the following dilutions were prepared with the help of methanol.

20 µg/ml solution: 20 µl stock sol(1mg/ml) + 980 µl methanol
40 µg/ml solution: 40 µl stock sol(1mg/ml) + 960 µl methanol
60 µg/ml solution: 60 µl stock sol(1mg/ml) + 940 µl methanol
80 µg/ml solution: 80 µl stock sol(1mg/ml) + 920 µl methanol
100 µg/ml solution: 100 µl stock sol(1mg/ml) + 900 µl methanol
120 µg/ml solution: 120 µl stock sol(1mg/ml) + 880 µl methanol
200 µg/ml solution: 200 µl stock sol(1mg/ml) + 800 µl methanol
400 µg/ml solution: 400 µl stock sol(1mg/ml) + 600 µl methanol

The IC50 value(50% inhibitory concentration) was calculated by nonlinear regression using software Graphpad Prism version 5.0. The concentration response curve was difference within and between groups were evaluated by one -way analysis of variance test (ANOVA) followed by a multi comparison Dunnett test compared with the positive control. IC50 were determined through non linear regression of the plots of percentage relative activity = f([concentration]).

% inhibition was calculated using the formula

DPPH scavenging effect=[A1-A0)/A1]*100

Where, A1 was the absorbance of control (DPPH solution without sample at 517; A0 was the absorbance at 517 of sample at different concentrations with DPPH, The antioxidant activity was expressed as IC50 (mg/ml), the concentrations of the sample required to cause a 50% decrease of the absorbance at 517 nm. A lower IC50 value corresponded to a higher antioxidant activity (Jing et al., 2015)

Using a DPPH free radical scavenging experiment developed in our lab, the antioxidant effects of *Ficus rumphii* extract were evaluated. In this test, a 96-well microplate containing 100 µl of the sample and 0.2 mg/ml of the DPPH solution was combined and allowed to stand at room temperature in the dark for 10 minutes. The sample solution's 517 nm absorbance decrease was measured using a spectrophotometer. A control was used, which was ascorbic acid. Free radical scavenging activity was expressed as the IC50 value (g/ml). The following formula was used to obtain the inhibition percentage (Brand-Williams et al., 1995).

Inhibition= A_c-A_s/A_c ×100

Where, A_s is the ‘absorbance of test substance’ and A_c is the ‘Absorbance of Control’

5.4: Determination of TPC:

5.4.1 Principle

Folin cio-calteau phenol’s reagent contains Sodium tungstate (VI) dihydrate (Na_2WO_4 .$2H_2O$) and Sodium molybdate (VI) dehydrate (Na_2MO_4 .$2H_2O$). The mean oxidation state of the metals is between

5 and 6. Tungsten blue and molybdenum blue are formed by reacting with a reductant. The chemical reaction which takes place is as follows:

Na_2WO_4/Na_2MO_4 + Polyphenols→ (Phenol- $MoW_{11}O_{40}$) -4 [Blue coloured complex].

Mo+6 + e-1→ Mo+5; Mo+5 + e-1→ Mo+4 [Blue coloured].

This blue coloured complex shows a λ_{max} of 765 nm. The complex is estimated spectrophotometrically at 765 nm. The spectroscopic estimation of the phenolic compounds is done in alkaline pH. The pH is made alkaline with the help of a weak base, such as $NaHCO_3$ (7.5% w/w). Under these conditions, phenolic compounds are allowed to react with the Folin cio-calteau phenol's reagent at 45ºC for 45 minutes (Blainski et al., 2013).

5.4.2 Materials and methods:

The experiment on determination of TPC of FRHE was performed with some modifications according to the method of(Chan et al., 2007); (Lu et al., 2011);(Stankovic, 2011)

In this study, in vitro antioxidant activity, total phenolic content and concentration of flavonoids of five different extracts, from the whole herb of Marrubium peregrinum L. (Lamiaceae) were determined using spectrophotometric methods. Antioxidant activity of extracts were expressed as percentage of DPPH radicals inhibition and IC 50 values (µg/ml). Values in percentage ranged from 27.26 to 89.78%. The total phenolic content ranged from 27.26 to 89.78 mg/g of dry weight of extract, expressed as gallic acid equivalents. The total flavonoid concentrations varied from 18.72 to 54.77 mg/g, expressed as rutin equivalents. Methanolic exstract of M. peregrinum showed the highest phenolic and flavonoid concentration and strong antioxidant activity. The significant linear correlation was confirmed between the values for the total phenolic content and antioxidant activity of plant extracts. The high contents of phenolic compounds indicated that these compounds contribute to the antioxidant activity. The M. peregrinum can be regarded as promising candidates for natural plant sources of antioxidants with high value.(Stankovic, 2011).

Preparation of Folin cio-calteau phenol's reagent: 100 g Sodium tungstate (VI) dihydrate ($Na_2WO_4 .2H_2O$) and 25 g Sodium molybdate (VI) dehydrate ($Na_2MO_4 .2H_2O$) were dissolved in a mixture of 700 ml distilled water, 100 ml conc. H_2SO_4 and 50 ml 85% H_3PO_4. 150 g of Lithium sulphate hydrate (Li_2SO_4) was then added to this mixture (Agbor et al., 2014); Stankovic et al, 2011).

Preparation of standard Gallic acid dilutions: Gallic acid of 95% purity was purchased from Sigma Aldrich. First, a 1 mg/ml Gallic acid solution was prepared in methanol. From there, with the help of serial dilution, 6 dilutions of Gallic acid- 1000, 500, 250, 125, 62.5, 31.25 µg/ml solutions were prepared.

Preparation of 7.5% w/v NaHCO3 solution: 7.5 g $NaHCO_3$ was dissolved in 100 ml water.

Preparations of 1 mg/ml extract solution: *Ficus rumphii* hydro-alcoholic extract 1 mg were dissolved in 1 ml methanol by vortex. The solution was filtered through a 0.45 µ filter (Millipore).

Methodology: In a 96 well plate as a standard dilution 18 µl Gallic acid dilution,90 µl of 10% FC reagent and 90 µl of 7.5% sodium bicarbonate was added in first row. Then in the next row as a blank dilution 18 µl of methanol, 90 µl of 10% FC reagent and 90 µl of 7.5% sodium bicarbonate was added. In the next row as a test dilution 18 µl of FRHE, 90 µl of 10% FC reagent and 90 µl of 7.5% sodium

bicarbonate was added. Now incubate at 45° C for 45 minutes. Finally measured the absorbance at 765nm in Spectramax ID3. Interpolation done from the calibration curve (mg/ml concentration).Total phenolic content determined and expressed as GAE mg/g.

5.5. Determination of TFC:

5.5.1 PRINCIPLE

The mechanism of the reaction involves nitration of the catechol moiety of a flavonoid at three or four unsubstituted or sterically unhindered positions. This nitrated compound, on reaction with $AlCl_3$ (i.e. Al (III) ion) produces a yellow coloured complex, which is then reacted with NaOH to give a red coloured compound, which is estimated colorimetrically at 518 nm (Lim et al., 2019; Pekal et al, 2014).

5.5.2 Materials and methods

TFC was determined using the method of Jing et al, 2015.

Preparation of 5% w/v $NaNO_2$ solution: 5 g $NaNO_2$ was dissolved in 100 ml water.

Preparation of 10% w/v $AlCl_3$ solution: 10 g $AlCl_3$ was dissolved in 100 ml water.

Preparation of 4% NaOH solution: 4 g NaOH was dissolved in 100 ml water.

Preparation of 1 mg/ml extracts solutions: *Ficus rumphii* hydro-alcoholic extract 1mg were dissolved in 1 ml Methanol with the help of vortexing. The solution was filtered through a 0.45 µ filter (Millipore). The TFC have been expressed as Rutin equivalent (RE), mg/g.

Methodolody: TFC was determined using the method of Jing et al, 2015. In a 96 well plate as standard dilution add 10 µl of $NaNO_2$, 100 µl dilution of Rutin , 10 µl $AlCl_3$ and 50 µl NaOH. In the next row as blank dilution add 10 µl of $NaNO_2$, 100 µl of methanol, 10 µl $AlCl_3$ and 50 µl NaOH was added. Then in the next row as a test dilution add 10 µl of $NaNO_2$, 100 µl dilution of FRHE, 10 µl $AlCl_3$ and 50 µl NaOH. Finally measure the absorbance at 518 nm in Spectramax ID3. Interpolation done from the calibration curve (mg/ml concentration). Total Flavonoid content determined and expressed in terms of Rutin equivalent (RE %w/w).

Result:

5.5: Statistical analysis

Results from statistical analysis are presented as mean SEM. Following a one-way ANOVA, the statistical significance between the groups was examined using Dunnet's multiple comparison test. Statistics were judged significant at $P<0.05$.

Table 1: IC_{50} values of antioxidant assay of AIHE

TYPE OF EXTRACT	DPPH IC_{50} (µg/ml)
FRME	6.37±0.23

Each value expressed as MEAN ± SEM (n=3)

Gallic acid equivalents (GAE) per gram of dry extract were used to assess the number of total phenols present in the dry extracts. The dry extract's total phenolic content was 6.17±0.090 mg GAE/gm of dry extract. The equation for the gallic acid standard curve was Y= 0.002980*x+ 0.018, and the correlation coefficient was $R^2 = 0.9816$. Quercetin equivalents (QE) per gram of dry extracts were used to assess the number of total flavonoids present in the samples. Total flavonoids content of AIE was 0.92±0.041 mg QE/g of dry extract. The standard curve equation of quercetin was Y = 0.001516*X + 0.4766 with a correlation coefficient of $R^2 = 0.9863$.

5.6: Discussion

The purpose of this study was to evaluate the invitro-antioxidant activity of a hydroalcoholic extract of *Ficuus rumphii* stem. The presence of phenolic and flavonoid content ensured this plant's antioxidant activity, which was confirmed by an invitro DPPH radical scavenging assay using ascorbic acid as a reference.

5.7: Conclusion

The current investigation confirmed that the hydro alcoholic extract of *Ficus rumphii* stem portion had substantial invitro antioxidant activity.

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CHAPTER 6: TOXICITY STUDY

6.1. INTRODUCTION:

In the modern human population, there are now practically infinite amounts of chemicals and pharmaceuticals being used (Sperling F, 1979). These may now be found in the form of ingredients in foods, medications, drinks, various domestic and commercial goods, and other industrial and commercial products. These chemicals or pharmaceuticals, however, have the potential to cause acute toxicity when taken in large quantities that can cause an instant harmful effect as well as chronic toxicity in the living system when used over an extended period of time. Depending on the substance, these effects may be minor or severe.

Toxicology is a combination of the words toxicon, which means poison, and logos, which means science. The study of the detrimental effects of chemicals and medications on living systems is known as toxicology. It aids in figuring out the type and amount of chemical that will make it poisonous.

Data from toxicity tests may be utilized in the following ways:

- deciding on a therapeutic dose.
- gaining knowledge of the detrimental impact on particular organs.
- Determination of the harmful action's mode.
- Toxic substance establishment as a future reference (Sperling, F., 1979).

6.2: Acute Toxicity Studies (single dose)

The purpose of this study is to identify the median lethal dosage (LD50), or the dose at which 50% of an animal species will perish. Such investigations may also point to the chemical's likely target organ and its particular harmful effect. An early evaluation of the compound's harmful symptoms offers recommendations for the levels to be employed in extensive research. A program of toxicity testing that serves as the foundation for the creation of additional testing programs includes acute toxicity tests. These investigations involve at least two different animal species, and the medicine is administered in graded dosages to various animal groups by at least two different routes, at least one of which should be a suggested method for use in humans. The typical observation duration is 7 to 14 days.

The Organization for Economic Co-operation and Development (OECD) changed it in 1981 and reduced the number up to 30 for 3 dose-groups. It was originally developed in the 1920s and was known as "classical LD50" and involved 100 animals for 5 dose-groups. We should use alternate techniques that involve fewer animals because animal sacrifice is overdone.

The lethal dose test is viewed by FRAME (Fund for the Replacement of Animals in Medical Experimentation) as unnecessary harsh and unreliable from a scientific standpoint. A number of nations, notably the UK, have taken action to outlaw the oral LD50, The requirement for the oral test was eliminated in 2001 by the OECD, an organization that advises international governments. Three further approaches are as follows: Acute Toxic Class technique (ATC)-OECD TG 4234, Up-and-Down Procedure (UDP)-OECD TG 4255, Fixed Dose Procedure (FDP)-OECD TG 4203. In place of mortality symptoms noted during research, such as increased motor activity, these methods solely take signs of toxicity into account. tremors, rolling, arching, and anesthesia. Alternative techniques reduce the use of laboratory animals (Deora PS et al., 2010).

6.3: Sub-Acute Toxicity Studies (Daily Dose)

Identification of the target organs vulnerable to medication toxicity is the goal. In order to properly design chronic toxicity studies to completely assess the compound's harmful potential, the goal of this test is to ascertain the maximum tolerated dose and to identify the nature of toxic effects.

6.4: Long-term Toxicity Studies (Chronic Toxicity Studies)

In order to imitate more practical alternatives, the animals were repeatedly exposed to the drug's detrimental effects. Study periods might last anywhere between one and two years and up to seven years. The primary goals of these long-term studies are to identify the organs impacted and assess whether or not the medicine is potentially carcinogenic based on data from sub-acute toxicity trials. Phase-I clinical trials, the initial human research, may be undertaken alongside these testing.

6.5: Special toxicity studies

Following the unfortunate thalidomide disaster in 1961, which resulted in the congenital deformity and crippling of more than 10,000 newborns due to phocomelia, toxicological data on teratogenicity (including the effects on reproductive functions), mutagenicity and carcinogenicity, as well as local toxicity (for skin diseases), have now become required.

6.6: VARIOUS METHODS OF LD50 DETERMINATION

6.6.1: Litchfield and Wilcoxon Graphic Method (Litchfield et. al., 1949)

Here, different doses are administered to various animal groups, and animal death needs to be noted. The percentage of mortality is then shown against the log dosage on a graph.

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

Here, any dosage, such as 1 mg, 1 ml, 1 gm, or 1 mg/ml, is ingested. Two mice get the unit dose, and they are watched for 24 hours. If the animals can handle the dose, it is increased by multiplying by 3/2. As two animals were utilized, this must be done until a dose results in the death of one animal, or 50% death. However, until it is confirmed using a larger number of animals, this result is not trustworthy. However, the technique is simple, quick, and inexpensive.

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

This procedure entails giving numerous groups, each with five animals, differing doses of the test drug. The test chemical is given to the first group of animals in the same medium it was dissolved or diluted in (for example, water or saline solution). The second group, however, receives varying dosages of the test drug. The doses administered to the animals in each group are different, and the increment in dose increases from group to group (beginning with group 2, which receives the lowest dose). The critical parameters in this strategy are the dosage differential between the groups and the interval mean of the number of mortalities reported in each group. Using Karber's arithmetical approach, the LD is computed.

Which is as follow:

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

Were,

LD-Median lethal dose

LD100- Least dose required to kill 100%

a= Dose difference

b= Mean mortality

n= Group population

6.6.4: Lorke's method (Lorke D., 1983)

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

Phase 1: Nine animals are needed for this stage. Three sets of three species each comprise the nine animals. Different test drug doses (10, 100, and 1000 mg/kg) are given to each group of animals. The animals are kept under observation for 24 hours to track their behavior and determine whether any of them will die.

Phase 2: Three animals, divided into three groups of one animal each, will be used in this phase. Higher dosages of the test chemical (1600, 2900, and 5000mg/kg) are given to the animals, who are subsequently monitored over 24 hours for behavior and mortality.

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

6.6.5: Up and Down Method (Shetty JA., 2007)

Guidelines established by the OECD (Organisation for Economic Co-operation and Development) for chemical testing are routinely reevaluated in light of new scientific findings or evolving evaluation techniques. The Up and Down testing strategy was first introduced by Dixon and Mood. Bruce first suggested using an up-and-down (UDP) method to assess the immediate toxicity of compounds in 1985. The UDP experimental design can be used in a variety of ways to calculate LD50. The technique is applicable to substances that cause mortality within one or two days. When a significant amount of delayed mortality is anticipated, the procedure is impractical. To determine which substances are most likely to have minimal toxicity, a limit test can be utilized. Up to 5 animals can be used in the experiment. It is possible to utilize a test dose of up to 2000 mg/kg or, in rare cases, 5000 mg/kg. The primary test is a single ordered dosage progression in which each animal is dosed separately at intervals of 48 hours. The dose given to the first animal is a little lower than the best estimate of the LD50. The dose for the following animal is increased to a factor of 3/2 of the initial dose if the first animal survives, but it is dropped by a similar dose progression if the second animal dies. Before deciding on the subsequent dose to be administered to the following animal, each animal should be carefully monitored for 48 hours. An estimate of the LD50 and a confidence interval are produced for the test based on the conditions of all animals at termination, and dosing is halted when one of the criteria is met.

6.7: Method used

Acute Toxicity Study

6.7.1: Animals: Each group consisted of 10 healthy Swiss Albino mice weighing 20–25g, housed in 38–10–cm poly acrylic cages with a maximum of six mice per cage. They were maintained under standard laboratory conditions with sufficient food and water ad libitum.

6.7.2: Procedure

Fasted animals of one sex were dosed in a stepwise fashion using the described method (Organization for Economic Co-operation and Development 420) and a fixed dose of 5, 50, 300, 500, 1000, and 2000 mg/kg orally (OECD 2000). For at least 24 hours, all of the animals were watched for any indications of toxicity or demise. For a total of 14 days, all the animals were subjected to intense surveillance. Up to 2000 mg/kg body weight, a methanolic extract of Basella alba did not cause any mortality or harmful effects in mice.

6.7.3: Result

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

6.7.4: Discussion

LD50 is a measure of acute toxicity. Hydroalcoholic extract of *Ficus rumphii* given orally to mice at doses up to 2000 mg/kg did not significantly alter their behavior, respiratory patterns, cutaneous reactions, sensory nerve system responses, or gastrointestinal functions. No deaths were noted during the experiment's run. According to the findings, *Ficus rumphii* hydroalcoholic extract is safe up to a dose of 2000 mg/kg.

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Chapter 7: In-vitro anti diabetic activity

7.1: Introduction

Hyperglycemia, altered lipid, carbohydrate, and protein metabolism are characteristics of diabetes, and they all have an impact on a patient's quality of life in terms of their social and psychological well-being as well as their physical health. Although the pathophysiology of the two types of diabetes is different, both share typerglycemia as a hallmark cardiovascular consequence of diabetes brought on by altered lipoprotein metabolism, which causes atherosclerosis, stroke, etc. Even though there are several distinct pharmacological classes available to address type II diabetes, developing a new molecule that is superior to current medications while having no unfavorable side effects is a difficult undertaking. Observation and assessment of in vivo biological parameters in diabetes-induced mice are now used in diabetes treatment. There are additional in vitro assay techniques to assess the antidiabetic properties of the plant extract in addition to these in vivo measures. Enzyme inhibition and yeast cell uptake of glucose are two rare examples of such in vitro tests. Normally, carbohydrates are broken down into simple sugars (monosaccharides), which the intestines absorb. Reducing gastrointestinal glucose production and absorption is hence one of the treatment strategies for treating diabetes. Following a mixed-carbohydrate dinner, alpha amylase and alpha glucosidase enzymes break down carbs and raise postprandial blood glucose levels. Therefore, by blocking certain carbohydrate-digesting enzymes like alpha amylase and alpha glucosidase, blood glucose can be regulated. An enzyme called alpha amylase is prevalent in saliva and pancreatic juice but also exists in many other tissues. "Ptyalin" is the more popular name for salivary amylase. Large alpha linked polysaccharides like starch and glycogen have alpha bonds that can be hydrolyzed by alpha amylase to produce glucose and maltose. The primary site of action for starch is the alpha (1,4 glycosidic) bond. The breakdown of starch results in simpler monosaccharides like glucose and disaccharides like maltose. In the brush border of the small intestine, alpha glucosidase enzymes also break down carbs. Alpha glucosidase inhibitors limit the enzyme's ability to break down carbs by acting as competitive inhibitors. Intestinal glucosidases that are membrane-bound hydrolyze oligo-, tri-, and disaccharides into glucose and other monosaccharides in the small intestine. Alpha glucosidase inhibitors (Acarbose), which function as intestinal alpha glucosidase's competitive inhibitors, can postpone the digestion and subsequent absorption of high blood sugar levels. The enzyme was pre-incubated with the various extract concentrations before the substrate p-nitrophenyl-a-d-glucopyranoside (PNPG) was added. Using a spectrophotometric approach, the color produced by the release of p-nitrophenol from the hydrolysis of the substrate PNPG by a-glucosidase was used to quantify the activity of alpha glucosidase. Assisted diffusion along the concentration gradient is the basis for glucose transport through the yeast cell membrane. Only until intracellular glucose has been efficiently utilized (reduced) does glucose transfer

take place. Therefore, improving effective glucose utilization should be the goal of our desired plant extract in order to reduce blood glucose levels (Nair SS et al., 2013).

7.2: Materials and methods

7.2.1: In-vitro alpha amylase inhibitory activity (Wickramaratne MN et al., 2016)

The 3,5-dinitrosalicylic acid (DNSA) method was used to conduct the a-amylase inhibition assay (GL Miller, 1959). The MEBA was dissolved in a minimum of 10% DMSO before being added to a buffer (Na2HPO4/NaH2PO4 (0.02 M), NaCl (0.006 M), and pH 6.9) to generate concentrations ranging from 10 to 800 g/ml. The extract was combined with 200 l of a-amylase solution (2 units/ml), which was then incubated for 10 min at 30 °C. After that, each tube received 200 l of the starch solution (1% in water (w/v)), which was then incubated for 3 min. 200 ml of the DNSA reagent (12 g of sodium potassium tartrate tetrahydrate in 8.0 mL of 2 M NaOH and 20 mL of 96 mM 3,5-dinitrosalicylic acid solution) were added to the reaction to stop it, and it was then heated for 10 minutes at 85–90 °C in a water bath. A UV-Visible spectrophotometer was used to measure the absorbance at 540 nm after the mixture had been diluted with 5 ml of distilled water and allowed to cool to room temperature. The plant extract was swapped out with 200 l of buffer to create a blank with 100% enzyme activity. In the absence of the enzyme solution, a blank reaction was similarly constructed using the plant extract at each concentration. Acarbose (100 g/ml-2 g/ml) was used to make a positive control sample, and the reaction was carried out in the same way as the previously reported reaction with plant extract. The following equation was used to compute the percent inhibition of the a-amylase inhibitory activity: The extract concentration was plotted against the percentage of a-amylase inhibition, and the graph's IC50 values were calculated.

% of inhibition of a-amylase = 100* ((Abs 100% control-Abs sample) / Abs 100% Control).

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

Using a modified version of a previously published approach (R.T. Dewi et al., 2007), the inhibition of alpha-glucosidase activity was assessed. Alpha-glucosidase (Saccharomyces cerevisiae, Sigma-Aldrich, USA) was dissolved in 100 mL of bovine serum albumin (Merck, German) and phosphate buffer (pH 6.8). The reaction mixture contained 250 ul of 5 mM p-nitrophenyl a-D-glucopyranoside (Sigma-Aldrich, Switzerland) and 490 ul of phosphate buffer pH 6.8. It also contained 10 ul. of sample at different concentrations (0.52 to 33 g/mL). Alpha-glucosidase was added and pre-incubated at 37°C for 5 minutes before being added and incubated at 37°C for 15 minutes. The reaction was stopped by adding 2000 mL of 200 mM Na2CO3. The amount of p-nitrophenol produced from p-NPG was measured in order to spectrophotometrically calculate the activity of a-glucosidase at 400 nm on a

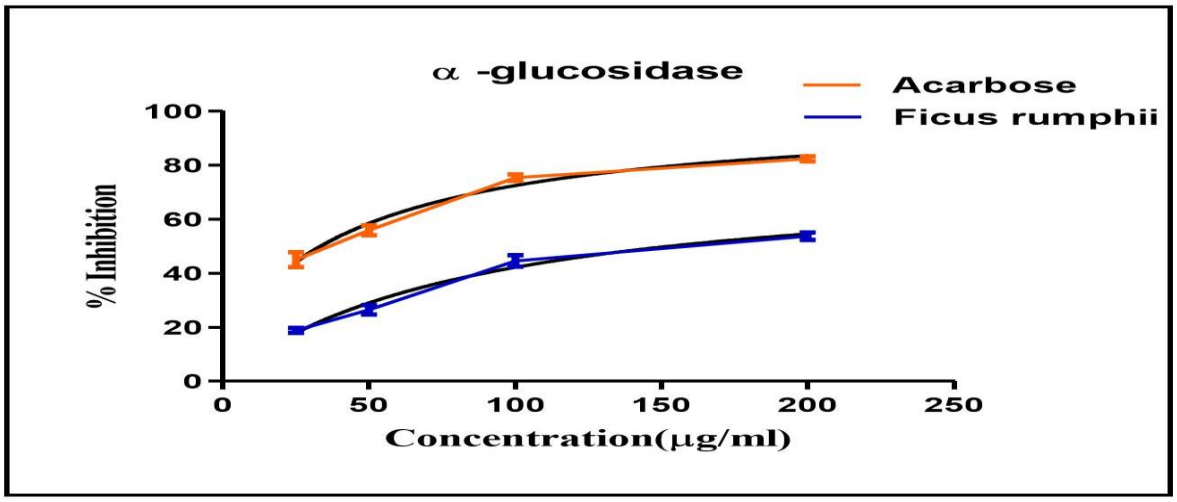
spectrophotometer for UV-Visible Spectroscopy. Acarbose served as the a-glucosidase inhibitor's positive control. The IC50 value was established as the amount of extract needed to inhibit 50% of α-glucosidase activity under the test conditions.

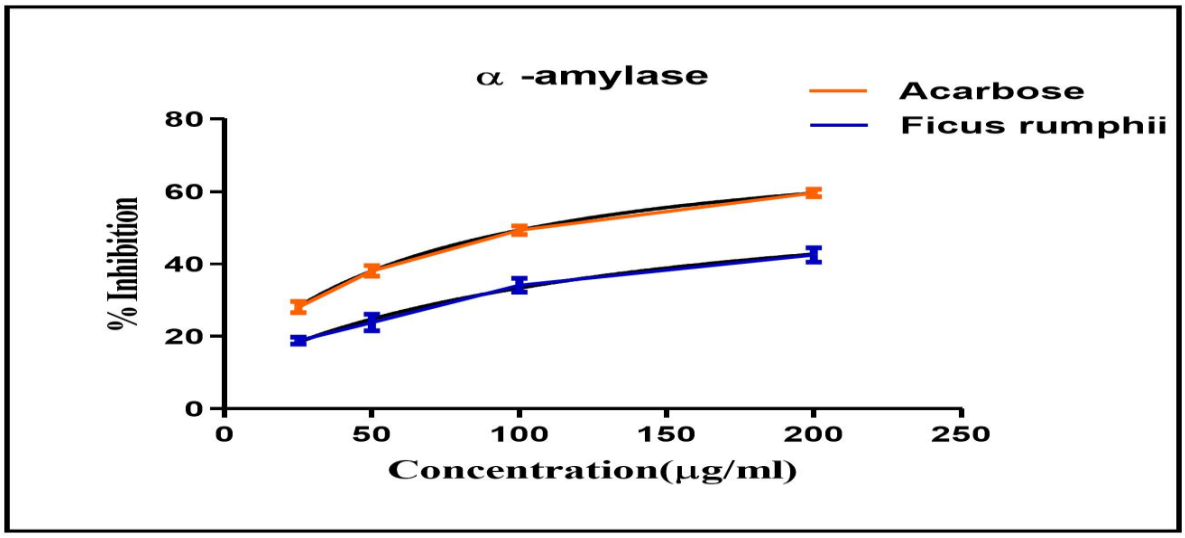
% α glucosidase inhibition=100*((Abs100% control-AbsSample) / Abs100% Control)

7.3: Statistical analysis

The results are all shown as mean + SEM. Graph Pad Prism 5.0 software (Graph Pad Software, USA) was used to do a one-way analysis of variance (ANOVA) and post hoc Dunnett's test to determine whether the results were statistically significant. Statistics were considered significant at p values less than 0.05.

7.4: Result:





TYPE OF EXTRACT	**Alpha-glucosidase inhibition assay** IC_{50} (µg/ml)	**alpha amylase inhibitory activity** IC_{50} (µg/ml)
FRHE	83.15±1.92	130.7±0.91
Acarbose	47.45±1.676	74.23±1.87

The alpha-amylase inhibition study was conducted with different concentrations of FRHE and acarbose (50, 100, 200, 500 µg/ml) and the alpha glucosidase inhibition study was conducted with different concentrations of FRHE and Acarbose (25, 50,100,200 µg/ml). In alpha amylase, IC50 value of FRHE was found to be 130.7±0.91 µg/ml when compared to that of IC50 value of acarbose which was 74.23±1.87 µg/ml. In alpha glucosidase IC50 value of FRHE was found to be 83.15±1.92µg/ml when compared to that of acarbose which was 47.45±1.676µg/ml.

7.5: Discussion:

The current study aims to assess the hydro alcoholic extract of Ficus rumphii bark (FRHE) in vitro anti hyperglycemic efficacy. The aim of treatment for diabetic patients is to keep their glycemic control close to normal, both during fasting and after meals. Regarding the inhibition of glucose synthesis from the carbohydrates in the gut or glucose absorption from the intestine, many natural sources have been researched (Matsui T et al., 2001). The conversion of dietary carbohydrates to glucose by the body's alpha-amylase and alpha-glucosidase enzymes causes postprandial hyperglycemia. Therefore, the extract's inhibition of these enzymes may reduce postprandial hyperglycemia in diabetes. It has been suggested that postprandial hyperglycemia is a separate risk factor for coronary vascular disease. Therefore, it is thought that controlling postprandial hyperglycemia is crucial for the management of diabetes and the avoidance of its consequences. Alpha-amylase and alpha-glucosidase activity were significantly inhibited by the results.

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Chapter 8: In-Vivo Antidiabetic Activity

8.1: Introduction: As part of their metabolic profile, patients with type 2 diabetes frequently experience obesity-related reduced insulin sensitivity as well as activated B-cell compensatory mechanisms, such as excess basal insulin secretion and hyperproinsulinemia (Kahn SE et al., 1997). These pathogenic circumstances appear before the B-cells significantly fail in late stage (insulin-dependent) type 2 diabetes (Prentki M et al., 2006) and early in the disease course of type 2 diabetes (Tabak A G et al., 2009). Long-term nutritional excess causes obesity to develop from a metabolically healthy state (Wang J et al., 2001). The prediabetic condition entails a vicious loop that includes hyperinsulinemia, insulin resistance, dyslipidemia, inflammatory and dysfunctional adipose tissue, ectopic fat deposition in liver and muscle, and B-cell failure (Ravussin E et al., 2002). There is an urgent need for more efficient therapies and treatment plans to tackle type 2 diabetes. To accomplish this goal of testing new and improved therapies, type 2 diabetic animal models that are thoroughly defined and clinically pertinent are needed. There are experimentally created non-spontaneous diabetes models as well as hereditary spontaneous diabetes models. The streptozotocin (STZ) mice model of diabetes is an illustration of an artificially induced animal model. In certain circumstances, sugar is used in this model to cause hyperinsulinemia, insulin resistance, and glucose intolerance, which is then treated with the B-cell toxin STZ, which causes a sharp decrease in the mass of functional B-cells (Skovso S et al., 2014). DNA is alkylated by streptozotocin as it enters the pancreatic cell through the glucose transporter GLUT2. Additionally, STZ causes the release of nitric oxide and the activation of poly adenosine diphosphate ribosylation. Pancreatic cells are killed by necrosis as a result of HFD/STZ action (Mythili et al., 2004). Since STZ may cause renal injury, oxidative stress inflammation, and endothelial dysfunction in addition to its toxic effects on pancreatic cells (Lei Y C et al., 2005), it is important to look for ways to reduce its dosage in order to lessen side effects. These two stresses are intended to work together to replicate the pathophysiology of type 2 diabetes, albeit on a shorter timescale than that of human disease. The in vivo anti-diabetic efficacy of MEBA against streptozotocin-induced rats is the topic of the current chapter. The study that follows focuses on several variables that should be examined as a result of diabetes, including tissue antioxidant, serum variables, blood glucose level, glycosylated hemoglobin, and histopathology of the affected organs.

8.2: Material and Methods:

8.2.1: Animals

Optimal Swiss the study employed albino rats of either sex that weighed 25±5 g. Rats were maintained in a conventional laboratory setting with 12-hour light/dark cycles, a temperature of 25–28 °C, and a

relative humidity of 55–60%. They were fed a standard pellet diet and given access to water as needed. Prior to the trial, the animals spent a week becoming used to the lab environment.

8.2.2: 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.

8.2.3: 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.

8.2.4: 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 28 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 FRHE (200mg/kg body weight) orally daily.

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

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

FBG level was measured on day 0, 7^{th} , 14^{th} , 21^{st} and 28^{th} 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 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.

8.3: Body weight:

Body weight of rats from each group was measured on day 0,7th, 14th, 21st and 28th. Weight was measured using standard digital weight balance to get accuracy.

8.4: 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 28 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.

8.5: 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:

8.5.1: 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 concentmiccions of the samples were calculated using the extinction coefficient of MDA ($1.56 \times 10^5\ M^{-1}cm^{-1}$).

8.5.2: 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.

8.5.3: 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.

8.6: Serum biochemical parameters

8.6.1: 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.

8.6.2: 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.

8.6.3: 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.

8.7: 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.

8.8: Results:

8.8.1. 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: Effect of FRHE on body weight

Groups	Day0	Day7	Day14	Day21	Day28
Normal Control	169±3.26	176±1.79	186.3±4.71	191.3±2.43	195.4±1.98
Diabetic control	235.7±5.48*	229.8±6.81*	229.3±8.23*	217.8±5.61*	219.5±2.35*
200mpk	238.7±5.28	230.6±6.39	230.6±3.76	205.6±3.54	210.1±9.12
400mpk	240.2±5.62	232.3±5.91	232.3±4.23	207.5±3.67	211.4±7.29
Standard control	239.7±6.53	235.7±6.20	235.7±5.82	237.2±9.42	240.4±2.61

8.8.2: 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 FRHE 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.

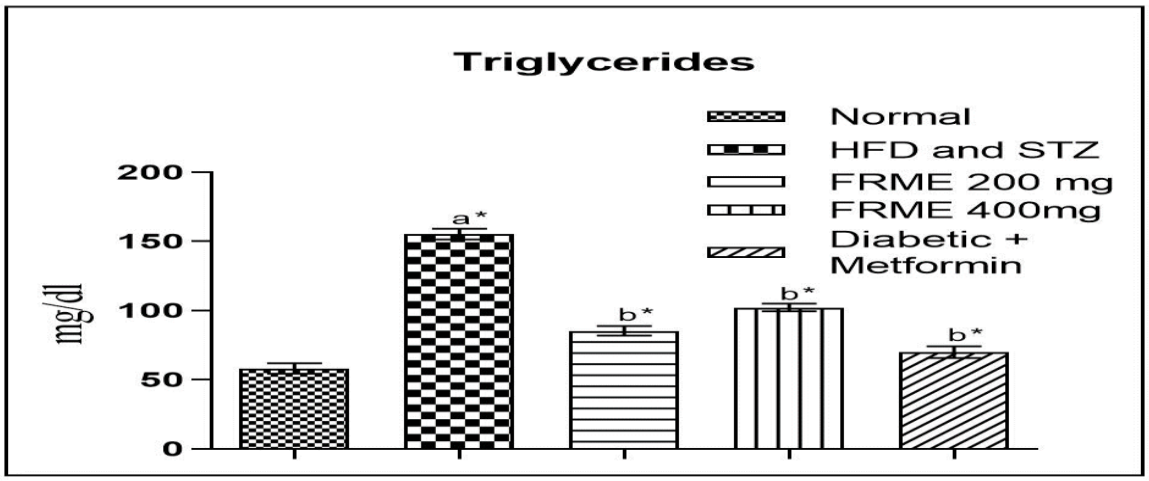
Table: Effect of FRHE on fasting blood glucose(mg/dl)

Groups	Day0	Day7	Day14	Day21	Day28
Normal Control	55.3±2.36	61.4±1.30	60.67±1.95	62.67±2.65	72.76±3.79
Diabetic control	338.7±2.56^{a*}	358.5±1.02^{a*}	378.6±5.62^{a*}	413.2±2.45^{a*}	429.9±2.49^{a*}
200mpk	356.2±8.42^{b}	306.9±7.31^{b}	274.9±7.01^{b}	198.3±5.69^{b}	145.6±2.38^{b}
400mpk	345.5±2.16^{b*}	287.6±5.39^{b*}	165.4±5.28^{b*}	135.3±7.12^{b*}	114.3±4.01^{b*}
Standard control	420.3±6.41^{b*}	200.1±2.95^{b*}	160.7±5.84^{b*}	110.2±0.97^{b*}	74.2±1.23^{b*}

a= comparison of diabetic control with normal control, b= comparison of treated group with diabetic control(*=p<0.05)

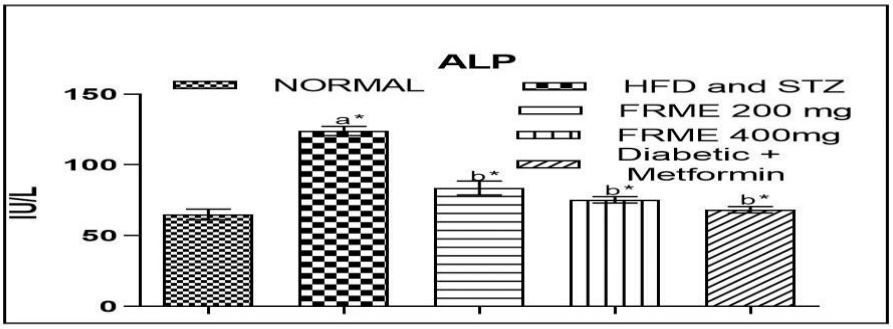
8.8.3: Estimation of serum lipid profiles:

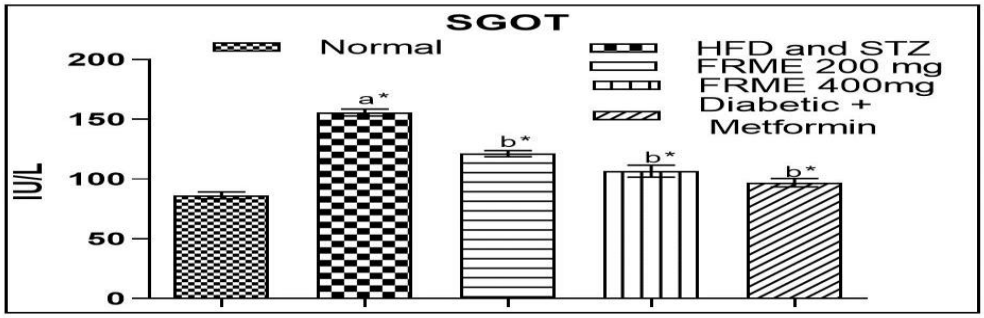
Serum lipid profiles like total cholesterol, triglyceride & HDL cholestrol in STZ-induced diabetic rats were significantly ($p < 0.05$) elevated and the HDL level significantly ($p < 0.05$) decreased compared to normal control group. Treatment with MEBA at the doses of 200 and 400 mg/kg significantly ($p < 0.05$) reduced the total cholesterol, triglyceride level and significantly ($p<0.05$) increased the HDL level when compared to the diabetic control group.

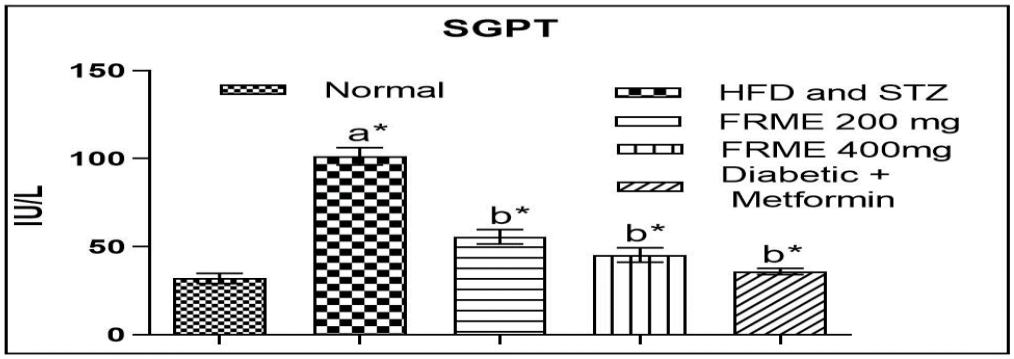


8.8.4: Estimation of serum liver function test parameters:

Biochemical parameters like SGOT, SGPT, ALP, Total Protein 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.

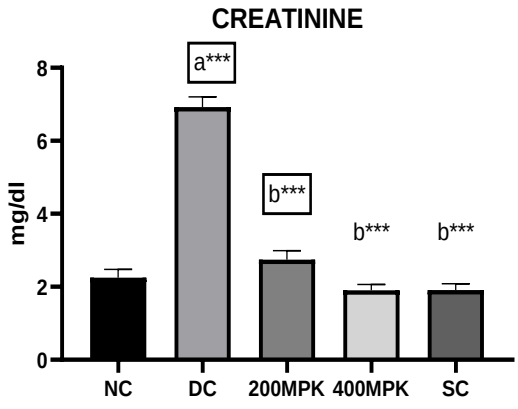


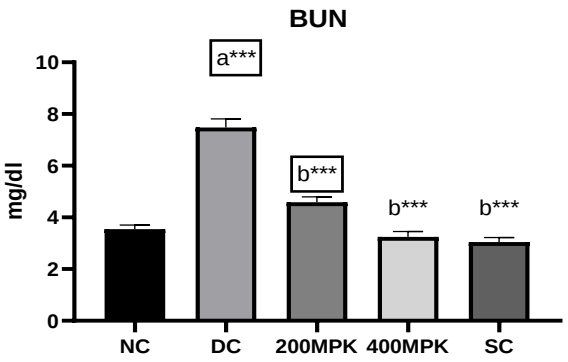


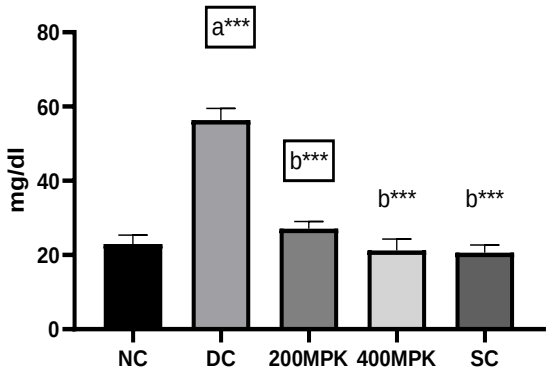


8.8.5: Estimation of serum kidney function test parameters:

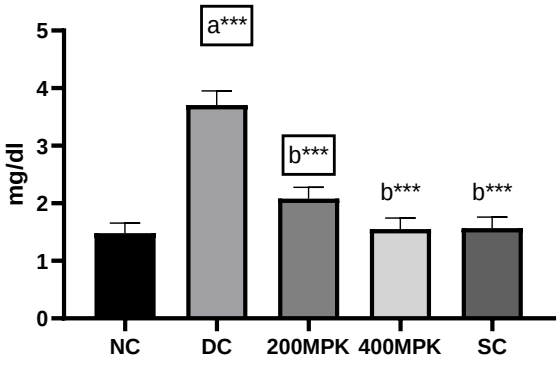
Biochemical parameters like Creatinine, Urea, Uric acid, Blood urea Nitrogen in STZ-induced diabetic rats were significantly ($p < 0.05$) elevated as compared to the normal control group. Treatment with FRHE at the doses of 200 and 300 mg/kg significantly ($p < 0.05$) reduced the Creatinine, Urea, Uric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





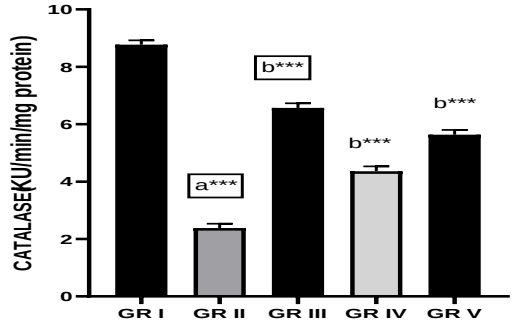


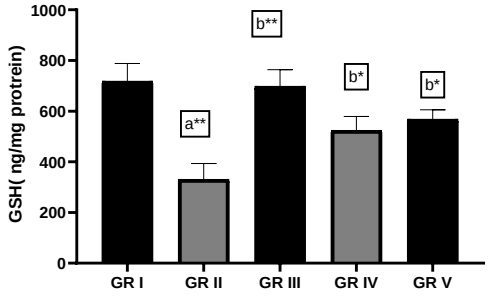
UREA

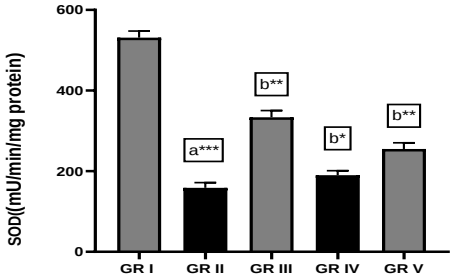


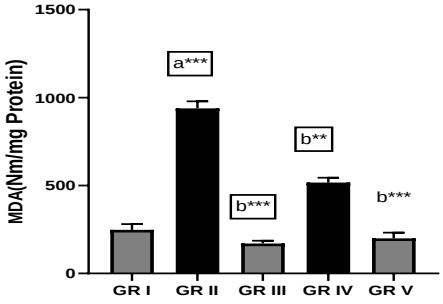
URIC ACID

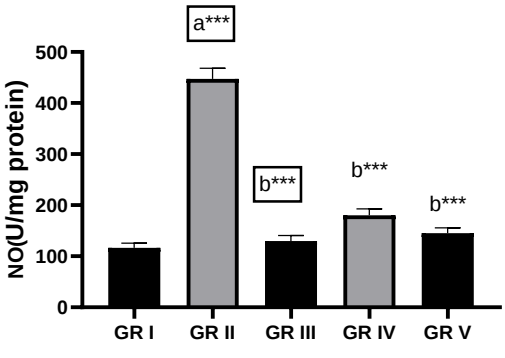
Tissue Antioxidants and free radicals estimation:









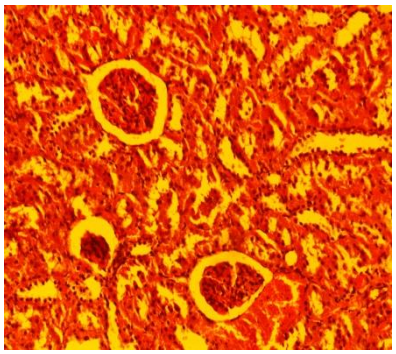


GR 1: Normal control; GR II: Diabetic control; GR III: StdC; GR IV: 200mpk; GR V: 400mpk (a= when Diabetic control is compared with Normal control, b+ when stdc and treated groups are compare with disease control; ***=p,0.0001,=p,0.001,*=p,0.05)**

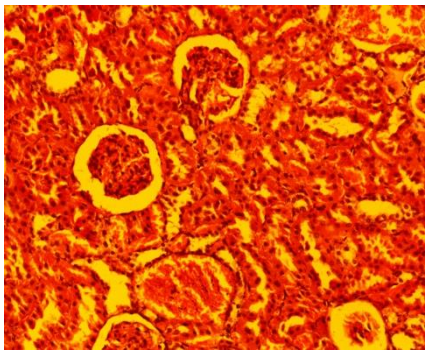
8.8.6: Histopathological Studies:

Kidney:

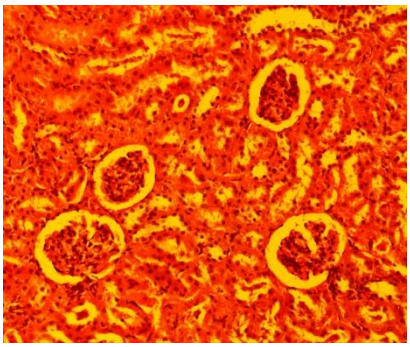
Thickening of Glomerular membrane is seen in disease control (DC) with respect to normal control (NC). But thickness of glomerular membrane is decreasing in a dose gradient manner in 200 mg/kg (200mpk) & 400 mg/kg (400mpk) treatment of FRHE as compare to disease control.



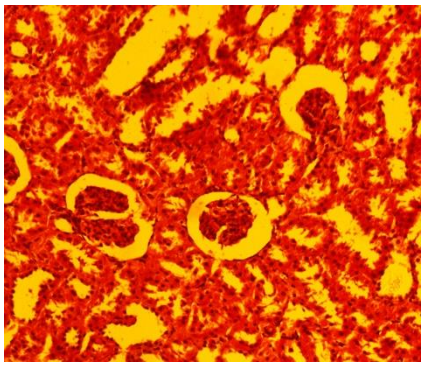
NC



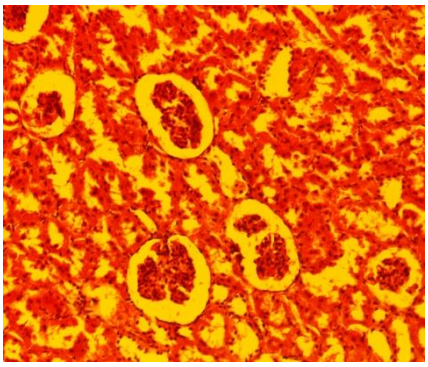
DC



STDC

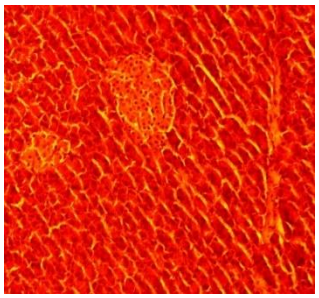


200mpk

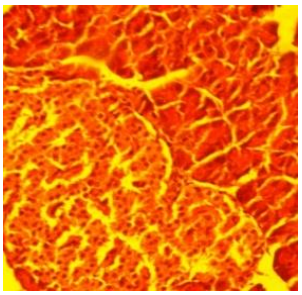


400mpk

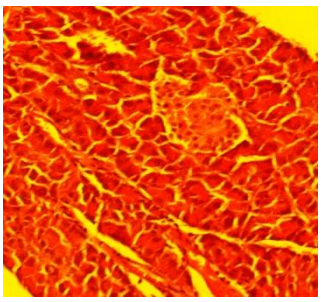
Pancreas: NC- ß cell in normal control rats, STDC - Total destruction of B cells In STZ control rats, 200mpk- remnants of beta cell in FRHE (200 mg/kg) treated rats. 400mpk- Gradual regeneration of ß cells in FRHE (400 mg/kg) treated rats.



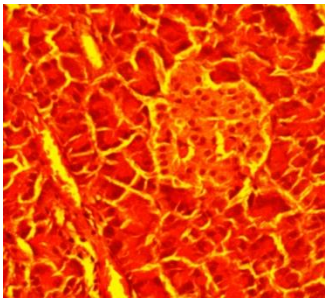
NC



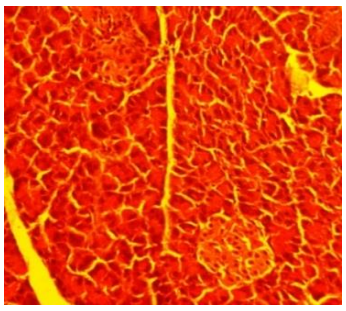
DC



STDC



200MPK



400MPK

Discussion:

The introduction of diabetes in the current study led to a notable decline in the clearance of urea and creatinine. Additionally, it has been noted that rising levels of serum creatinine and blood urea nitrogen in diabetic rats indicate progressive renal impairment.

The current study examines the Hydroalcoholic extract of Ficus rumphii antihyperglycemic and renal protecting properties in diabetic rats. When compared to the normal control group, it was found that the STZ-induced diabetic rats had significantly higher levels of fasting blood glucose (FBG), weight, triglyceride (TG), total cholesterol (TC), blood glycosylated hemoglobin (HbAlc), and lipid peroxidation (MDA) and significantly lower levels of total protein, body weight, and liver antioxidants (GSH, SOD). therapy with FRHE at 400 mg/kg doses considerably normalized increased blood glucose levels, glycosylated hemoglobin, body weight, restored serum, and liver biochemical parameters, however therapy at 200 mg/kg doses was not that dramatically normalizing. In comparison to the diabetic control group, the FRHE treated group's urine volume and protein excretion rate were near normal values.

Due to its extensive biodiversity, India has the most advanced traditional medicine system based on plants. Because of this, underdeveloped nations manage diabetes using traditional ways of medicine. Due to their affordability, potency, and few negative effects on human health, these herbal medications are primarily used in healthcare (Sekar et al., 2010). According to WHO statistics, 80% of people worldwide currently utilize herbal medicines for their primary healthcare. Alkaloids,

flavonoids, and saponin are thought to be abundant in plants with antidiabetic activity and are known to be effective against diabetes (Mishra et al., 2010). When phytochemical screening was done, the FRHE bark extract revealed the presence of alkaloid, flavonoid, phenol, glycoside and tannin.

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. Hepatocytes damaged due to hepatotoxicity cause ALT and GGT enzymes to leak out into blood circulation. Elevated serum biomarker enzymes such as SGOT, SGPT, and ALP were observed in diabetic (STZ induced) rats indicating impaired liver function.

Malondialdehyde (MDA), a thiobarbituric acid reactive substance (TBARS), is typically generated as a result of oxidative stress damage to the membrane's polyunsaturated fatty acids (PUFA) (Venukumar and Latha, 2002). According to several studies, there is an imbalance between the prooxidant and antioxidant states in the body, which results in an imbalance in systemic redox status. This imbalance

is evidenced by an increase in serum TBARS and a decrease in plasma SOD, as well as GSH activity. In the current investigation, we discovered a substantial imbalance between the prooxidant and antioxidant states in diabetic control mice as compared to normal control animals, shown by a decrease in serum SOD, GSH, and higher MDA content activity. According to Sabu and Kuttan (2004), the decreased liver antioxidant status associated with diabetes may be due to the body's heightened defenses against the generation of lipid peroxides. The concentration of TBARS in STZ-induced diabetic rats showed a significant rise, which indicated increased lipid peroxidation resulting in tissue damage and failure of the endogenous antioxidant defense mechanisms to avoid excessive free radical formation. By reducing TBARS levels toward normal, treatment with MEBA prevented hepatic lipid peroxidation in diabetic rats, suggesting that MEBA has the potential to improve the pathologic state of diabetes by preventing lipid peroxidation in diabetic rats and reestablishing the balance between prooxidant and antioxidant states. According to research by Florence et al. (2013), oxidative stress in diabetes is a result of the body's inability to eliminate an excessive amount of free radicals. SOD is an enzyme-based antioxidant that is crucial in the scavenging and removal of free radicals from cells. Insufficient levels of superoxide anion (O2) and hydrogen peroxide (H2O2) in biological systems can result in the generation of hydroxyl radicals (OH), which in turn can cause the beginning and spread of lipid peroxidation (Latha and Pari, 2003). By accelerating the elimination of superoxide radicals, which harm cellular structures and membranes, SOD defends against oxygen free radicals. In the endogenous nonenzymatic antioxidant system, glutathione is crucial. In the presence of the enzyme glutathione peroxidase, it functions primarily as a reducing agent and detoxifies hydrogen peroxide (Biswas et al., 2011). In STZ-induced hyperglycemic mice, the reduced GSH may be caused by a decrease in GSH synthesis or GSH breakdown by oxidative stress (Loven et al., 1986). The hepatic SOD and GSH activities in the diabetic rats in the current study significantly increased in the extract-treated groups. This suggests that the extracts can boost the activities of antioxidant enzymes and decrease the possibility of enzyme glycation. They may also lower reactive oxygen free radicals. This finding provides proof that MEBA have free radical scavenging properties, which may protect against pathological changes brought on by the presence of superoxide and hydrogen peroxide radicals. Based on the research, it is conceivable that the presence of the aforementioned phytoconstituents is what is causing MEBA to engage in these activities.As a result, it is reasonable to conclude that the hydroalcoholic extract of Ficus rumphii bark is remarkably effective against high fat diet/streptozotocin-induced diabetes in Wistar rats due to its enhancement of the endogenous antioxidant mechanism, ability to lower lipids, and capability to lower serum glucose.

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CONCLUSION

Diabetes is a condition of the metabolism of carbohydrates, fats, and proteins that is caused by decreased insulin production or growing insulin resistance. Patients with both insulin-dependent and non-insulin-dependent diabetes, diabetic retinopathy, diabetic peripheral neuropathy, etc. have employed herbal remedies for the disease. The effectiveness of the botanicals in lowering the sugar level has been demonstrated by scientific validation of various Indian plant

One of the main microvascular consequences of diabetes mellitus is diabetic nephropathy. As previously reported by Knoll et al., the development of DN in the present investigation was supported by large increases in kidney/body weight ratio, BUN in diabetic rats.

This thesis is a trailblazer in the field of research for scientific studies on hydroalcoholic extract of Ficus rumphii stem. The notion of diabetes and diabetic nephropathy, natural products in the treatment of diabetes, and a description and literature analysis of the plant under investigation make up the thesis' opening section. The next chapter then discusses the collection, extraction, and chemical characterisation. In the subsequent chapter's acute toxicity research, it is noted that a dose of 2000 mg/kg of plant extract is safe.

Then, in the following chapter, there is a discussion of the antioxidant studies. The last two chapters have talked about the antidiabetic action both in vitro and in vivo. The hydroalcoholic extract of the entire Ficus rumphii plant does exhibit anti-diabetic activities, according to the in vitro and in vivo data. The phytoconstituents in the plant that were discussed in the thesis in an earlier chapter may be responsible for the outcomes. Alkaloids and flavonoids found in plants have been reported to have therapeutic effects for treating conditions including diabetes and nephropathy. Thus, the extract's activity may be attributed to either the alkaloids, flavonoids, or both contained in the extract. The antioxidant studies revealed that the extract has decent free radical scavenging abilities, which may also be a probable explanation for its renal protective properties. Free radicals are a major cause of kidney cell destruction, which may then result in nephroprotective properties, and quenching these free radicals may be able to counteract such negative effects. Extensive isolation and characterization procedures must be used to identify the molecule or molecules in the extract genuinely responsible for the extract's antidiabetic activity because speculative claims that phytoconstituents are the cause of the extract's antidiabetic activities are insufficient such pursuits.