EVALUATION OF *IN-VITRO* AND *IN-VIVO* ANTI-DIABETIC POTENTIAL OF *ACROSTICHUM AUREUM* LINN. WITH SPECIAL EMPHASIS ON DIABETIC NEPHROPATHY

Thesis submitted for the partial fulfillment of the Masters of Pharmaceutical Technology in the Faculty of Engineering and Technology, Jadavpur University, 2024

By

ANIRBITA GHOSH

Exam Roll no.- M1PHL23002

Reg No.- 163647 of 2022-23

Class Roll No.- 002211402003

Under the Supervision of

PROF. PALLAB KANTI HALDAR

Professor, Department of Pharmaceutical Technology, Jadavpur University

Department of Pharmaceutical Technology

Jadavpur University

Kolkata- 700032

India

CERTIFICATE

This is to certify that Anirbita Ghosh has carried out the research on the project entitled "**Evaluation of the *in-vitro* and *in-vivo* antidiabetic potential of *Acrostichum aureum* Linn. with special emphasis on diabetic nephropathy in rats**" under my supervision, in the Division of Pharmacology and Toxicology, Department of Pharmaceutical Technology, Jadavpur University, Kolkata-700032.

She has incorporated her findings into 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 her thesis with proper care and confidence to my entire satisfaction.

Supervised by-

Prof. Pallab Kanti Haldar
Professor
Division of Pharmacology & Toxicology
Department of Pharmaceutical Technology
Jadavpur University
Kolkata- 700032

Forwarded by-

Prof. Amalesh Samanta
Head of the Department
Department of Pharmaceutical Technology
Jadavpur University
Kolkata- 700032

Prof. Dipak Laha
Dean
Faculty of Engineering and Technology
Jadavpur University
Kolkata- 700032

DECLARATION

I declare that, "**Evaluation of in-vitro and in-vivo anti diabetic potential of *Acrostichum aureum* Linn. with special emphasis on diabetic nephropathy on rats**" is my own work that it has not been submitted for any degree or examination in any other university, and that all the sources I have used or quoted have been indicated and acknowledged by complete references.

Signature of the student:

Full name:

Date :


Dedicated to
my Family
& Guide

ACKNOWLEDGEMENT

I am immensely grateful to God Almighty for providing me with the forbearance, tenacity, and lots of blessings needed to carry out the study.

First and foremost, I want to express my sincere gratitude to my guide Prof. Pallab Kanti Haldar for his constant inspiration, suggestions, and insightful advice during the project. He also provided me all the resources and scope I needed to complete this project successfully, for which I am extremely grateful.

I extend my sincere gratitude to the Head of the Department, Prof. Amalesh Samanta to provide access to all the amenities and help needed for the thesis work.

I am also grateful to AICTE for funding this project.

My sincere gratitude is also extended to my seniors and labmates, who have helped me with my work: Barun Dasgupta, Srijon Gayen, Sandipan Jana, Suparna Ghosh, Monihar Pervin, Agnipravo Naskar and Ishani Mukherjee.

I would like to thank my beloved Father in heaven from whom I have derived strength and determination which were required to complete the study. My beloved Mother had been a source of constant support, love and motivation to sincerely carry out the study. Last but not the least my best friend Arghya Paul for motivating me throughout this project.

Date:

PREFACE

The current study, "*Evaluation of in-vitro and in-vivo anti-diabetic potential of Acrostichum aureum* Linn. *with special emphasis on diabetic nephropathy*," is a compilation of the author's original research for the completion of Master of Pharmacy from the Department of Pharmaceutical Technology, Jadavpur University.

Drug companies now want to find new medications and lead compounds by scouring the vast riches of the plant kingdom. We have begun using medicinal plants in the therapeutic management of several ailments due to their easy availability, low toxicity, and negligible or practically negligible adverse effects. Currently, conventional uses constitute a significant portion of research since they require a scientific foundation for appropriate valuation. Hence, the aforementioned study was thus handled in the thesis in a logical order in connection to the other study-related aspects.

In conclusion, the whole investigation has been connected in a way that supports the work's relationship to determining the pharmacological actions—particularly the activity against diabetes induced kidney damage.

Anirbita Ghosh

CONTENTS

CHAPTER 1: INTRODUCTION

1. Introduction

1.1 Diabetes

Diabetes mellitus, commonly referred to as diabetes, is a chronic metabolic disorder characterized by high blood sugar levels over a prolonged period. This condition occurs when the body either does not produce enough insulin or cannot effectively use the insulin it produces. Insulin is a hormone produced by the pancreas that helps regulate blood sugar levels and facilitates the uptake of glucose by cells for energy (Banday et al., 2020).

1.2 Types of Diabetes

There are several types of diabetes, including:

- **Type 1 diabetes:** This type occurs when the body's immune system mistakenly attacks and destroys the insulin-producing beta cells in the pancreas. As a result, the body produces little to no insulin. Type 1 diabetes often develops in children and young adults, although it can occur at any age. Individuals with type 1 diabetes require lifelong insulin therapy to survive. (Lucier et al., 2023)
- **Type 2 diabetes:** Type 2 diabetes is the most common form of diabetes, accounting for the majority of cases worldwide. In type 2 diabetes, the body either becomes resistant to the effects of insulin or fails to produce enough insulin to meet its needs. This type of diabetes is often associated with lifestyle factors such as obesity, physical inactivity, and poor diet. While type 2 diabetes typically develops in adults, it is increasingly being diagnosed in children and adolescents due to rising obesity rates. (Galicia-Garcia et al., 2020)
- **Gestational diabetes:** Gestational diabetes occurs during pregnancy and usually resolves after childbirth. It occurs when the body cannot produce enough insulin to meet the increased demands of pregnancy, leading to high blood sugar levels. Gestational diabetes can increase the risk of complications for both the mother and the baby if not properly managed (Buchanan et al., 2007).
- **Maturity onset diabetes:** Maturity-onset diabetes of the young (MODY) is a form of diabetes that typically presents in adolescence or early adulthood, though it can be diagnosed at any age. Unlike type 1 and type 2 diabetes, MODY is caused by a genetic mutation that affects the function of the pancreas. It is an autosomal dominant condition, meaning a single copy of the mutated gene from an affected parent can cause the disorder in offspring (Fajans et al., 2001).There are several different types of MODY, each associated with mutations in different genes. The most common types include MODY 1 (HNF4A gene mutation), MODY 2 (GCK gene mutation), and MODY 3 (HNF1A gene mutation). These genetic defects lead to a reduction in insulin production or secretion, which causes elevated blood glucose levels (Fajans et al., 2001; Murphy et al., 2008).
  The clinical presentation of MODY can vary depending on the specific genetic mutation involved. Common symptoms include mild to moderate hyperglycemia, which is often asymptomatic or may present with mild symptoms such as increased thirst or urination. Unlike type 1 diabetes, patients with MODY typically do not require insulin therapy

initially and can often be managed with lifestyle modifications and oral hypoglycemic agents (Murphy et al., 2008).

Diagnosis of MODY is confirmed through genetic testing, which identifies the specific gene mutation responsible for the disease. This genetic confirmation is crucial for differentiating MODY from other types of diabetes, as the treatment and management strategies can differ significantly (Hattersley et al., 2018).

Understanding MODY is important for providing accurate diagnosis and appropriate treatment, which can improve patient outcomes and reduce the risk of complications associated with mismanagement of the disease.

1.3 Complications of Diabetes

Diabetes, in any form, raises the risk of long-term consequences. These Usually appear after a number of years (10-20), but they may be the initial symptom in those who have not yet been diagnosed. Damage to blood vessels is one of the most serious long-term effects. Diabetes doubles the risk of cardiovascular disease, and coronary artery disease is responsible for roughly 75% of diabetes deaths in India. Stroke and peripheral vascular disease are two more "macrovascular" illnesses. Damage to the eyes, kidneys, and nerves are the most common microvascular consequences of diabetes. Damage to the eyes, known as diabetic retinopathy, is caused by damage to the blood vessels in the retina of the eye, and can lead to vision loss and blindness over time. Diabetic nephropathy, or kidney damage, can cause tissue scarring, urine protein loss, and eventually chronic kidney disease, needing dialysis or a kidney transplant. Diabetic neuropathy, or damage to the body's nerves, is the most prevalent consequence of diabetes. Numbness, tingling, discomfort, and altered pain feeling are some of the symptoms, which might cause skin damage. Diabetic foot problems (such as diabetic foot ulcers) can emerge and can be difficult to cure, necessitating amputation in certain cases. Proximal diabetic neuropathy also results in severe muscular atrophy and weakening. A relationship exists between cognitive impairment and diabetes. Those with diabetes have a 1.2 to 1.5-fold higher rate of loss in cognitive function than those without the disease. (World Health Organization, Fact Sheet of Diabetes, 2022).

1.4 Diabetic nephropathy: A brief overview

Diabetes can lead to various complications affecting different organ systems in the body. One significant complication is diabetic nephropathy, which refers to kidney damage caused by diabetes. Diabetic nephropathy is a leading cause of chronic kidney disease (CKD) and end-stage renal disease (ESRD) worldwide.

The exact mechanisms underlying diabetic nephropathy are complex and involve various factors such as prolonged hyperglycemia, abnormal activation of certain pathways within the kidneys, inflammation, and genetic predisposition. The probable pathophysiology has been depicted schematically in fig 1. Over time, these factors can lead to damage to the small blood vessels (glomeruli) in the kidneys, impairing their ability to filter waste products and excess fluids from the blood effectively. Early stages of diabetic nephropathy

may not cause noticeable symptoms. However, as the condition progresses, individuals may experience symptoms such as swelling (edema) in the legs, ankles, feet, or hands; increased blood pressure; proteinuria (protein in the urine); reduced urine output; fatigue; nausea; and shortness of breath. In some cases, diabetic nephropathy may progress to end-stage renal disease (ESRD), where the kidneys fail to function adequately. In such cases, treatment options may include kidney dialysis or kidney transplantation to replace lost kidney function.

Early detection and intervention, along with comprehensive management of diabetes and related risk factors, can help delay or prevent the progression of diabetic nephropathy and improve outcomes for affected individuals. (Sagoo et al., 2020)

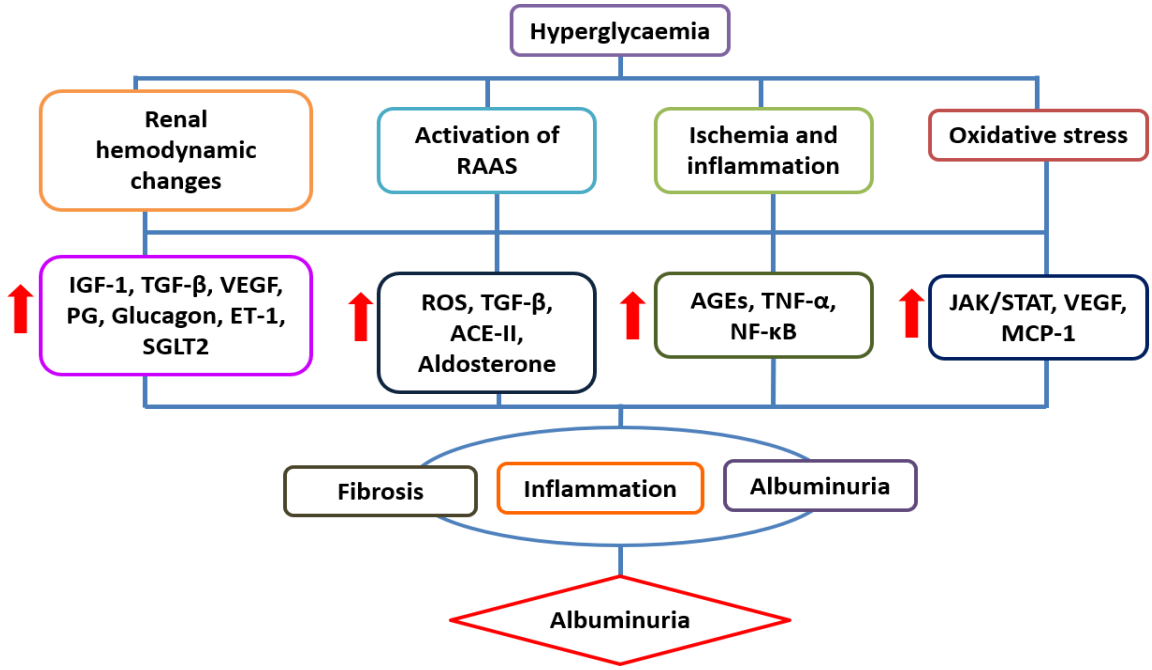


Figure 1: Pathophysiology of Diabetic nephropathy

1.5 Management of Diabetic Nephropathy

The treatment of diabetic nephropathy aims to slow the progression of kidney damage, manage symptoms, and reduce the risk of complications. Here are some key treatment options:

- **Blood sugar control**: Tight control of blood sugar levels is essential in managing diabetic nephropathy. This often involves modifications in medications (such as insulin or oral hypoglycemic agents). Maintaining near-normal blood glucose levels can help slow the progression of kidney damage (Hahr et al., 2015).

- **Blood pressure management**: Controlling high blood pressure is crucial in slowing the progression of diabetic nephropathy and reducing the risk of cardiovascular complications. Medications such as angiotensin-converting enzyme (ACE) inhibitors or angiotensin II receptor blockers (ARBs) are commonly used to lower blood pressure and help protect the kidneys. (Ruggenenti et al., 2010)

- **Medications to reduce proteinuria**: Proteinuria, or the presence of excess protein in the urine, is a common feature of diabetic nephropathy. Medications called ACE inhibitors or ARBs can help reduce proteinuria and protect the kidneys from further damage. Other medications, such as mineralocorticoid receptor antagonists (e.g., spironolactone), may also be used in some cases (Remuzzi et al., 2005).

- **Lifestyle modifications**: Adopting a healthy lifestyle can help manage diabetic nephropathy and reduce the risk of complications. This includes maintaining a healthy weight, following a balanced diet low in salt and saturated fats, limiting alcohol consumption, quitting smoking, and getting regular exercise (Onyenwenyi et al., 2015).

- **Dietary modifications**: A dietitian can provide guidance on dietary modifications to help manage diabetic nephropathy. This may include controlling portion sizes, monitoring carbohydrate intake, limiting sodium and protein intake, and ensuring an adequate intake of nutrients (Evert et al., 2019).

- **Smoking cessation**: Smoking can worsen kidney damage and increase the risk of complications in individuals with diabetic nephropathy. Quitting smoking is important in managing the condition and improving overall health (Gündoğdu et al., 2022).

- **Regular monitoring and follow-up:** Regular monitoring of kidney function through blood and urine tests is essential for individuals with diabetic nephropathy. This helps track the progression of the disease and allows for timely adjustments to treatment plans as needed (Narva et al., 2015).

1.6 Biomarkers of Diabetic nephropathy

Biomarkers play a crucial role in the early detection, diagnosis, prognosis, and monitoring of diabetic nephropathy. These biomarkers can provide insights into the pathophysiological processes underlying diabetic kidney disease and help guide treatment decisions. Here are some commonly studied biomarkers for diabetic nephropathy:

- **Albuminuria:** Albuminuria, or the presence of excess albumin (a protein) in the urine, is one of the hallmark features of diabetic nephropathy. It is often the earliest detectable sign of kidney damage in individuals with diabetes. Albuminuria is typically quantified using the urinary albumin-to-creatinine ratio (UACR) or urine protein-to-creatinine ratio (UPCR). (Selby et al., 2020)

- **Serum Creatinine:** Serum creatinine levels are routinely measured to assess kidney function. An increase in serum creatinine levels may indicate impaired kidney function and is associated with diabetic nephropathy progression (MacIsaac et al., 2014).

- **Markers of Inflammation and Oxidative Stress:** Inflammation and oxidative stress play key roles in the pathogenesis of diabetic nephropathy. Biomarkers such as C-reactive protein (CRP), interleukin-6 (IL-6), tumor necrosis factor-alpha (TNF-alpha), and malondialdehyde (MDA) are commonly studied to assess inflammation and oxidative stress levels in diabetic nephropathy (Rapa et al., 2019).

- **Estimated Glomerular Filtration Rate (eGFR):** eGFR is a measure of kidney function that estimates the rate at which the kidneys filter waste products from the blood. A decline in eGFR is indicative of reduced kidney function and is commonly used to monitor the progression of diabetic nephropathy. (Zsom et al., 2022)

- **Kidney Injury Molecule-1 (KIM-1):** KIM-1 is a protein that is expressed in the kidney in response to injury. Elevated levels of KIM-1 in the urine may indicate kidney damage and have been associated with diabetic nephropathy progression (Edelstein et al., 2011).

1.7 Epidemiology

The epidemiology of diabetic nephropathy provides valuable insights into the prevalence, incidence, risk factors, and burden of the condition. Here are some key points regarding the epidemiology of diabetic nephropathy:

- **Prevalence:** Diabetic nephropathy is a common complication of both type 1 and type 2 diabetes. It is estimated that approximately 40% of individuals with Type II diabetes and 30% with Type I diabetes will develop diabetic nephropathy during their lifetime. However, the prevalence varies depending on factors such as the duration of diabetes, glycemic control, and genetic predisposition. (Alicic et al., 2017)

- **Incidence:** The incidence of diabetic nephropathy has been increasing globally, mirroring the rising prevalence of diabetes. With the increasing prevalence of obesity and type 2 diabetes, particularly in low- and middle-income countries, the incidence of diabetic nephropathy is expected to continue to rise in the coming years. (Koye et al., 2018)

- **Risk factors:** Several factors increase the risk of developing diabetic nephropathy, including: (Wang et al., 2019)

  - Duration of diabetes: The longer a person has diabetes, the higher their risk of developing diabetic nephropathy.

  - Poor glycemic control: Uncontrolled high blood sugar levels over time can increase the risk of kidney damage.

  - High blood pressure: Hypertension is a significant risk factor for diabetic nephropathy and can accelerate the progression of kidney damage.

  - Genetic predisposition: Some individuals may have a genetic susceptibility to developing diabetic nephropathy.

  - Smoking: Smoking is associated with an increased risk of kidney damage in individuals with diabetes.

- **Burden:** Diabetic nephropathy contributes significantly to the burden of chronic kidney disease (CKD) worldwide. It is a leading cause of end-stage renal disease (ESRD) and accounts for a considerable proportion of individuals requiring dialysis or kidney

transplantation. The burden of diabetic nephropathy extends beyond healthcare costs, impacting quality of life, productivity, and mortality rates. (Hoogeveen et al., 2022)

- **Health disparities:** Certain populations, such as racial and ethnic minorities, socioeconomically disadvantaged groups, and individuals with limited access to healthcare, may have a higher prevalence and burden of diabetic nephropathy. Health disparities in the incidence, diagnosis, and management of diabetic nephropathy highlight the importance of addressing social determinants of health and ensuring equitable access to care. (Hoogeveen et al., 2022)

- **Prevention and management:** Given the significant burden of diabetic nephropathy, prevention strategies targeting modifiable risk factors such as blood sugar control, blood pressure management, and lifestyle modifications are essential. Early detection through regular screening and comprehensive management of diabetes and related comorbidities can help prevent or delay the onset and progression of diabetic nephropathy, ultimately improving outcomes for affected individuals. (Kumar et al., 2023)

  Understanding the epidemiology of diabetic nephropathy is crucial for informing public health policies, guiding clinical practice, and implementing strategies to mitigate the growing burden of this condition globally.

1.8 Importance of amelioration of oxidative stress in diabetic nephropathy

The common denominator that unites the main routes contributing to the onset and progression of diabetic micro- and macrovascular problems is oxidative stress**.**

Oxidative stress results from an imbalance between reactive oxygen species (ROS) production and antioxidant defenses, leading to cellular and tissue damage. In diabetic nephropathy:

- Hyperglycemia induces the overproduction of ROS, contributing to glomerular and tubular damage.
- ROS activate various signaling pathways, including those involving nuclear factor-kappa B (NF-κB), which promote inflammation and fibrosis.
- Oxidative stress impairs endothelial function and exacerbates microvascular complications.

Research indicates that antioxidants can mitigate the effects of oxidative stress in diabetic nephropathy:

- **Vitamin E and C:** Studies have shown that these vitamins reduce markers of oxidative stress and improve renal function in diabetic patients.
- **Nrf2 Activators:** Nuclear factor erythroid 2–related factor 2 (Nrf2) is a transcription factor that regulates the expression of antioxidant proteins. Nrf2 activators, such as

bardoxolone methyl, have demonstrated renal protective effects by reducing oxidative stress.

- **Polyphenols:** Compounds like resveratrol and curcumin have shown potential in reducing oxidative stress and improving renal function in experimental models of diabetic nephropathy.

Mechanistic Insights and Therapeutic Approaches treating DN are discussed below;

- **Mitochondrial Dysfunction**: Mitochondria are a primary source of ROS in diabetic nephropathy. Therapeutic strategies targeting mitochondrial ROS production, such as mitoquinone (MitoQ), have shown promise in preclinical studies.
- **Enzyme Modulation:** Enhancing the activity of antioxidant enzymes like superoxide dismutase (SOD) and catalase can reduce oxidative damage. For instance, SOD mimetics have shown renal protective effects in diabetic models.
- **Reducing Inflammation and Fibrosis:** Antioxidant therapy can attenuate the inflammatory and fibrotic responses driven by oxidative stress, thereby slowing the progression of diabetic nephropathy**.**

Mitigating oxidative stress is a key therapeutic strategy in managing diabetic nephropathy. Antioxidants and compounds targeting oxidative pathways show promise in reducing renal damage and improving outcomes in diabetic patients. Ongoing research and clinical trials continue to enhance our understanding of the mechanisms involved and the potential of these therapeutic approaches.

1.9 Regulation of inflammatory mediators

Inflammation is a key mediator in the development and progression of diabetic nephropathy. Chronic hyperglycemia leads to the activation of various inflammatory pathways, which in turn contribute to renal injury. Inflammatory cytokines such as interleukin-1 (IL-1), interleukin-6 (IL-6), and tumor necrosis factor-alpha (TNF-α) have been implicated in promoting glomerular and tubular damage (Lim et al., 2014).

Regulating these inflammatory factors can help mitigate kidney damage and slow the progression of diabetic nephropathy. For instance, the use of anti-inflammatory drugs and lifestyle modifications that reduce inflammation, such as dietary changes and regular exercise, have shown promise in reducing renal inflammation and preserving kidney function (Navarro-González et al., 2011).

Additionally, targeting specific inflammatory pathways has the potential to improve therapeutic outcomes. For example, blocking the effects of TNF-α with specific inhibitors has been shown to reduce albuminuria and inflammation in patients with diabetic nephropathy (Dandona et al., 2013). Thus, controlling inflammatory factors is not only crucial for preventing further renal damage but also for improving the overall quality of life in diabetic patients.

1.10 Importance of the inhibition of α-amylase

Inhibition of α-amylase enzyme is one of the major approaches in managing hyperglycemia. Acarbose, miglitol and voglibose are some of the currently marketed drugs which inhibit a-amylase. The inhibitors delay carbohydrate digestion and consequently the rate of glucose absorption. Nevertheless, acarbose and similar drugs have reported to have some undesired s effects such as flatulence, diarrhoea and abdominal and liver deurders. Thus, the need of the hour is to develop a new and more effective amylase inhibitor with fewer side effects (Sales et al, 2012: Cardulo 2019)

Barrett & Co-Researcher 2011 studied with the Phase 2 α-amylase inhibitor obtained from beans to show weight loss and reduce the high glycemic index when taken with meals containing carbohydrates. Reports sugger ellagitannins to be actively inhibiting the a-amylase enzyme (Cardullo 2019) Red and brown algae extract have been reported to show promising inhibitory activity towards a-amylase (Nasab et al., 2020).

Reduction of postprandial hyperglycemia is the main focus in the treatment paradigm of Diabetic complications and this can be achieved by inhibition of combined salivary and pancreatic a-amylase.

Different plants well-known for a-amylase inhibitory potential are as follows: *Agrimonia pilosa, Amaranthus spinosus, Anthocleista schweinfurthii, Aralia elata, Areca catechu, Kielmeyera coriacea, Levisticum officinale, Melia dubia, Memecylon umbellatum, Polygonatum odoratum, Pouteria ramiflora, Punica granatum, Rhus coriaria, Salvia acetabulosa, Syzygium cumini, Vaccinium arctostaphylos, Vitex negundo, Zhumeria majdae and Ziziphus spinachristi*.

1.11 Importance of the inhibition of α-Glucosidase

Alpha glucosidases are essential enzymes in the carbohydrate digestion pathway, releasing monomeric glucose molecules into the bloodstream. As a result, post-prandial blood glucose levels rise. In Type 2 diabetes, inhibiting the function of such enzymes can considerably lower post-prandial glucose levels. HPA and other glucosidases in the starch digestion pathway are promising targets for treating Type 2 diabetes with high blood glucose levels.

Since these products of amylase hydrolysis cannot be absorbed by the colon, small membrane-bound intestinal enzymes break them down further into simpler sugars or monosaccharides (e.g., glucose, galactose, and fructose), such as

- Maltase-glucoamylase (MGAM) releases D-glucose by cleaving non-reducing a-(1,4) glycosidic bonds.
- Alpha (1,4) glycosidic links can also be cleaved by sucrase-isomaltase (SI) to liberate a -D-glucose. The N-terminal domain of sucrase-isomaltase (NtSI) may also cleave the a (1,6) linkage of amylopectin, while the C-terminal domain of sucrase-isomaltase (CtSI) can break the a -(1,2) linkage of sucrose (also known as common table sugar).

Some important plants well-known for both a-amylase and a-glucosidase inhibitory potential are as follows: *Bergenia ciliata, Bougainvillea spectabilis, Chiliadenus iphionoides, Centella asiatica, Chaenomeles sinensis, Cinnamomum cassia, Cinnamomum verum, Citrullus lanatus, Dioscoreg bulbifera. Ficus exasperata, Ficus lutea, Gynura divaricata, Mukla mederaspatana, Morinda lucida, Morus alba, Polyalthia longifolia, Rubus fructicosis, Sclerocarya birrea, Swertia corymbosa, Telfairia occidentalis, and Terminalia paniculata*.

1.12 Role of medicinal Plants in treating DN

Several medicinal plants have been studied for their potential to lower diabetic nephropathy progression or mitigate its effects. It's important to note that while some of these plants have shown promising results in preclinical studies, further research, including clinical trials, is needed to confirm their efficacy and safety in humans. Here are some medicinal plants that have been investigated for their potential in managing diabetic nephropathy:

Plant Name	Family	Parts Used	Mechanism of Action	References
Gymnema sylvestre R.Br.	Apocynaceae	Leaves	Inhibits glucose absorption and renal damage	Patel et al., 2012
Berberis vulgaris L.	Berberidaceae	Roots, bark	Anti-inflammatory, antioxidant, and nephroprotective effects	Imenshahidi et al., 2018
Curcuma longa L.	Zingiberaceae	Rhizomes	Anti-inflammatory and antioxidant activities	Gupta et al., 2013
Allium sativum L.	Amaryllidaceae	Bulbs	Reduces oxidative stress and improves renal function	Banerjee et al., 2003
Trigonella foenum-graecum L.	Fabaceae	Seeds	Improves glucose metabolism and reduces renal oxidative stress	Sharma et al., 1990
Momordica charantia L.	Cucurbitaceae	Fruits, leaves	Enhances insulin sensitivity and reduces renal inflammation	Singh et al., 2011
Camellia sinensis	Theaceae	Leaves	Antioxidant and	Khan et al.,

L.			anti-inflammatory properties	2013
Salvia miltiorrhiza Bunge	Lamiaceae	Roots	Improves renal function and reduces inflammation	Zhang et al., 2012
Vaccinium myrtillus L.	Ericaceae	Fruits	Antioxidant properties and reduces renal damage	Martineau et al., 2006
Aloe vera (L.) Burm.f.	Asphodelaceae	Leaves	Antioxidant and anti-inflammatory effects	Rajasekaran et al., 2006
Panax ginseng C.A.Mey.	Araliaceae	Roots	Improves insulin sensitivity and reduces oxidative stress	Kim et al., 2005
Pterocarpus marsupium Roxb.	Fabaceae	Bark	Regenerates beta cells and reduces renal oxidative stress	Grover et al., 2002
Coccinia indica (L.) Voigt	Cucurbitaceae	Leaves	Reduces blood glucose and oxidative stress in kidneys	Vasudevan et al., 2007
Withania somnifera (L.) Dunal	Solanaceae	Roots	Anti-inflammatory and antioxidant properties	Kulkarni et al., 1993
Ocimum sanctum L.	Lamiaceae	Leaves	Reduces oxidative stress and improves renal function	Chattopadhyay et al., 1993
Syzygium cumini L.	Myrtaceae	Seeds	Antioxidant properties and reduces renal	Ayyanar et al., 2011

			damage	
Zingiber officinale Rosc.	Zingiberaceae	Rhizomes	Antioxidant and anti-inflammatory effects	Afshari et al., 2007
Cinnamomum verum J.Presl	Lauraceae	Bark	Improves glucose metabolism and reduces oxidative stress	Qin et al., 2010
Nigella sativa L.	Ranunculaceae	Seeds	Antioxidant and anti-inflammatory properties	Al-Logmani et al., 2011
Eugenia jambolana L.	Myrtaceae	Seeds	Reduces blood glucose and renal oxidative stress	Srivastava et al., 2012

While these medicinal plants show promise in managing diabetic nephropathy, it's important to consult with a healthcare professional before incorporating them into your treatment regimen, especially if you're already taking medications for diabetes or other medical conditions. Additionally, more research is needed to determine the optimal dosage, safety profile, and long-term effects of these medicinal plants in diabetic nephropathy management.

1.13 Mangroves and their biological significance

Mangroves are critically important ecosystems found in tropical and subtropical coastal regions. They provide numerous ecological, economic, and social benefits. The biological importance of mangroves can be understood through their roles in biodiversity support, coastal protection, carbon sequestration, and as nursery habitats for marine life.

- **Biodiversity Support:** Mangroves are rich in biodiversity, supporting a wide variety of plant and animal species. They provide habitat and breeding grounds for numerous terrestrial and marine species, including fish, crustaceans, mollusks, birds, and insects (Nagelkerken et al., 2008). The complex root systems of mangroves offer shelter and protection, contributing to high levels of species diversity and ecosystem productivity (Dahdouh-Guebas et al., 2002).

- **Coastal Protection:** Mangroves act as natural barriers against coastal erosion and storm surges. Their dense root systems stabilize the shoreline by trapping sediments and reducing the impact of wave action. This function is critical in protecting coastal communities from the devastating effects of storms and tsunamis (Alongi, 2008). Additionally, mangroves mitigate the impacts of rising sea levels and help maintain coastal landforms (Kathiresan & Bingham, 2001).

- **Carbon Sequestration:** Mangroves are highly effective at sequestering carbon, storing it in their biomass and the surrounding soil. They sequester carbon at rates significantly higher than most terrestrial forests, making them vital in the fight against climate change (Donato et al., 2011). The carbon stored in mangrove ecosystems, often referred to as "blue carbon," plays a crucial role in mitigating global carbon dioxide levels (Alongi et al., 2012).

- **Nursery Habitats:** Mangroves serve as critical nursery habitats for many marine species, providing a safe environment for juvenile fish and invertebrates. The complex root structures offer protection from predators and abundant food resources, enhancing the survival and growth rates of young marine organisms (Mumby et al., 2004). This nursery function supports local fisheries and contributes to the overall health of adjacent coral reefs and seagrass beds (Lee et al., 2014).

  These studies underscore the critical role of mangroves in maintaining ecological balance, protecting coastal areas, sequestering carbon, and supporting marine life. The preservation and restoration of mangrove ecosystems are essential for sustaining these valuable functions.

1.14 Medicinal importance of the Sundarbans region

The Sundarbans, a vast mangrove forest in the coastal region of the Bay of Bengal, spanning India and Bangladesh, is renowned not only for its unique biodiversity but also for its rich repository of medicinal plants. These plants have been traditionally used by local communities for treating various ailments, and modern research has begun to uncover their potential pharmaceutical benefits. Below are some notable plants from the Sundarbans region, along with their medicinal importance.

- ***Avicennia alba Blume*** **-** *Avicennia alba*, commonly known as white mangrove, has been studied for its antimicrobial properties. Extracts from this plant have shown significant activity against various bacterial and fungal pathogens.

- ***Acanthus ilicifolius*** **Linn. -** *Acanthus ilicifolius*, known as sea holly, is used traditionally for its anti-inflammatory and hepatoprotective effects. Research has demonstrated its potential in treating liver disorders and inflammation.

- ***Excoecaria agallocha*** **Linn. -** *Excoecaria agallocha*, often referred to as the blind-your-eye mangrove due to its toxic latex, possesses analgesic and anti-inflammatory properties. Studies have highlighted its efficacy in pain management and reducing inflammation.

- ***Heritiera fomes* Buch.- Ham.-** *Heritiera fomes*, also known as sundari, is used in traditional medicine for its astringent and anti-diarrheal properties. It has been investigated for its antioxidant and anti-diabetic activities.

- ***Nypa fruticans* Linn.-** *Nypa fruticans*, commonly known as the nipa palm, has applications in traditional medicine for treating headaches and gastrointestinal issues. Research indicates its potential in managing diabetes and hypertension.

- ***Sonneratia apetala* Buch.- Ham.-** *Sonneratia apetala* is used in folk medicine for its antimicrobial and wound-healing properties. Scientific studies have validated its use in promoting wound healing and treating infections.

1.15 Role of mangrove plants in diabetic nephropathy

Mangrove plants have been explored for their potential role in the treatment of diabetic nephropathy due to their rich content of bioactive compounds. Diabetic nephropathy, a serious complication of diabetes, is characterized by damage to the kidneys' filtering units. Mangrove species, particularly *Avicennia marina, Rhizophora mucronata,* and *Sonneratia apetala,* have shown promise in mitigating this condition.

Mechanism of Action:

- **Antioxidant Properties:** Mangrove plants possess significant antioxidant activities, which help in reducing oxidative stress, a key factor in the progression of diabetic nephropathy. Compounds such as flavonoids, phenolics, and tannins found in mangroves scavenge free radicals and protect renal cells from oxidative damage (Rathod et al., 2021).

- **Anti-inflammatory Effects:** Chronic inflammation is a hallmark of diabetic nephropathy. Extracts from mangrove plants have demonstrated anti-inflammatory properties by inhibiting pro-inflammatory cytokines like TNF-α and IL-6. This helps in reducing inflammation and protecting kidney function (Gupta et al., 2020).

- **Antiglycation Activity:** Advanced glycation end-products (AGEs) contribute to the pathology of diabetic nephropathy. Mangrove extracts have been shown to inhibit the formation of AGEs, thus preventing the glycation of proteins and maintaining renal structure and function (Kumar et al., 2019).

- **Renoprotective Effects:** Specific compounds in mangroves, such as alkaloids and terpenoids, have direct protective effects on kidney cells. These compounds enhance cellular repair mechanisms and improve renal function markers, thereby offering protection against diabetic nephropathy (Verma et al., 2022).

These studies indicate that mangrove plants could serve as a natural therapeutic option for managing diabetic nephropathy through their multifaceted biological activities. Further clinical research is necessary to validate these findings and develop standardized treatments based on mangrove-derived compounds.

References

Afshari, A. T., Shirpoor, A., Farshid, A., Saadatian, R., Rasmi, Y., Sadeghi, H., ... & Allameh, A. (2007). The effect of ginger on diabetic nephropathy, plasma antioxidant capacity and lipid peroxidation in rats. *Food Chemistry*, 101(1), 148-153.

Alicic RZ, Rooney MT, Tuttle KR. Diabetic Kidney Disease: Challenges, Progress, and Possibilities. Clin J Am Soc Nephrol. 2017 Dec 7;12(12):2032-2045. doi: 10.2215/CJN.11491116. Epub 2017 May 18. PMID: 28522654; PMCID: PMC5718284.

Alison B. Evert, Michelle Dennison, Christopher D. Gardner, W. Timothy Garvey, Ka Hei Karen Lau, Janice MacLeod, Joanna Mitri, Raquel F. Pereira, Kelly Rawlings, Shamera Robinson, Laura Saslow, Sacha Uelmen, Patricia B. Urbanski, William S. Yancy; Nutrition Therapy for Adults With Diabetes or Prediabetes: A Consensus Report. Diabetes Care 1 May 2019; 42 (5): 731–754. https://doi.org/10.2337/dci19-0014

Al-Logmani, A., & Zari, T. (2011). Long-term effects of Nigella sativa L. oil on some physiological parameters in normal and streptozotocin-induced diabetic rats. *Journal of Diabetes Mellitus*, 1(3), 46-53.

Alongi, D. M. (2008). Mangrove forests: Resilience, protection from tsunamis, and responses to global climate change. *Estuarine, Coastal and Shelf Science, 76*(1), 1-13. https://doi.org/10.1016/j.ecss.2007.08.024

Al-Waili, N., Al-Waili, H., Al-Waili, T., & Salom, K. (2017). Natural antioxidants in the treatment and prevention of diabetic nephropathy; a potential approach that warrants clinical trials. Redox Report, 22(3), 99-118.

Ayyanar, M., & Subash-Babu, P. (2011). Syzygium cumini (L.) Skeels: A review of its phytochemical constituents and traditional uses. *Asian Pacific Journal of Tropical Biomedicine*, 1(3), 240-246.

Banday, Mujeeb Z., Aga S. Sameer, and Saniya Nissar. "Pathophysiology of diabetes: An overview." Avicenna journal of medicine 10.04 (2020): 174-188.

Banerjee, S. K., & Maulik, S. K. (2003). Effect of garlic on cardiovascular disorders: A review. *Nutrition Journal*, 2, 1-14.

Barnes, J. L., & Gorin, Y. (2011). Myofibroblast differentiation during fibrosis: role of NAD(P)H oxidases. Kidney International, 79(9), 944-956.

Berridge, M. J. (2015). Vitamin D deficiency accelerates diabetic nephropathy in mice. Nature Reviews Nephrology, 11(12), 705-715.

Buchanan TA, Xiang AH, Kjos SL, Watanabe RM. What is gestational diabetes? Diabetes Care. 2007;30(suppl 2):S105–S111.

Chacko, B. K., & Zhi, D. (2011). Mitochondria-targeted antioxidant MitoQ ameliorates oxidative damage and inflammation in diabetic nephropathy. Journal of Biological Chemistry, 286(18), 16436-16446.

Chattopadhyay, R. R. (1993). Hypoglycemic effect of Ocimum sanctum leaf extract in normal and streptozotocin diabetic rats. *Indian Journal of Experimental Biology*, 31(11), 891-893.

Dahdouh-Guebas, F., Jayatissa, L. P., Di Nitto, D., Bosire, J. O., Lo Seen, D., & Koedam, N. (2002). How effective were mangroves as a defence against the recent tsunami? *Current Biology*, *15*(12), R443-R447. https://doi.org/10.1016/j.cub.2005.06.008

Dandona, P., Ghanim, H., Bandyopadhyay, A., Korzeniewski, K., Dhindsa, S., Chaudhuri, A., & Makdissi, A. (2013). Anti-inflammatory effects of insulin and the pro-inflammatory effects of glucose: Relevance to the management of diabetes and its complications. *Journal of Clinical Endocrinology & Metabolism, 98*(11), 4525-4534.

de Zeeuw, D., & Akizawa, T. (2013). Bardoxolone methyl in type 2 diabetes and stage 4 chronic kidney disease. New England Journal of Medicine, 369(26), 2492-2503.

Donato, D. C., Kauffman, J. B., Murdiyarso, D., Kurnianto, S., Stidham, M., & Kanninen, M. (2011). Mangroves among the most carbon-rich forests in the tropics. *Nature Geoscience, 4*(5), 293-297. https://doi.org/10.1038/ngeo1123

Edelstein, Charles L. "Biomarkers in acute kidney injury." Biomarkers of Kidney Disease (2017): 241-315.

Fajans, S. S., Bell, G. I., & Polonsky, K. S. (2001). Molecular mechanisms and clinical pathophysiology of maturity-onset diabetes of the young. *New England Journal of Medicine, 345*(13), 971-980.

Galicia-Garcia, Unai, et al. "Pathophysiology of type 2 diabetes mellitus." International journal of molecular sciences 21.17 (2020): 6275.

Grover, J. K., Vats, V., Rathi, S. S., & Dawar, R. (2001). Traditional Indian anti-diabetic plants attenuate progression of renal damage in streptozotocin induced diabetic mice. Journal of Ethnopharmacology, 76(3), 233-238.

Grover, J. K., Yadav, S., & Vats, V. (2002). Medicinal plants of India with anti-diabetic potential. *Journal of Ethnopharmacology*, 81(1), 81-100.

Gündoğdu Y, Anaforoğlu İ. Effects of Smoking on Diabetic Nephropathy. Front Clin Diabetes Healthc. 2022 Feb 23;3:826383. doi: 10.3389/fcdhc.2022.826383.

Gupta, S. C., Patchva, S., & Aggarwal, B. B. (2013). Therapeutic roles of curcumin: Lessons learned from clinical trials. *The AAPS Journal*, 15(1), 195-218.

Gupta, S., Singh, A., & Chaturvedi, R. (2020). Anti-inflammatory and nephroprotective activities of Rhizophora mucronata in experimental diabetic nephropathy. *Phytomedicine, 70*, 153210.

Hahr, A.J., Molitch, M.E. Management of diabetes mellitus in patients with chronic kidney disease. Clin Diabetes Endocrinol 1, 2 (2015).

Hattersley, A. T., Greeley, S. A. W., Polak, M., Rubio-Cabezas, O., Njølstad, P. R., Mlynarski, W., ... & Ellard, S. (2018). ISPAD Clinical Practice Consensus Guidelines 2018: The diagnosis and management of monogenic diabetes in children and adolescents. *Pediatric Diabetes, 19*(S27), 47-63.

Himmelfarb, J., & Hakim, R. M. (2003). Oxidative stress in uremia. Journal of the American Society of Nephrology, 14(11), 2717-2727.

Hoogeveen, E. K. (2022). The epidemiology of diabetic kidney disease. Kidney and Dialysis, 2(3), 433-442.

Imenshahidi, M., & Hosseinzadeh, H. (2018). Berberis vulgaris and berberine: An update review. *Phytotherapy Research*, 32(9), 1741-1750.

Kathiresan, K., & Bingham, B. L. (2001). Biology of mangroves and mangrove ecosystems. *Advances in Marine Biology, 40*, 81-251. https://doi.org/10.1016/S0065-2881(01)40003-4

Khan, N., & Mukhtar, H. (2013). Tea and health: Studies in humans. *Current Pharmaceutical Design*, 19(34), 6141-6147.

Kim, S. H., & Park, K. S. (2005). Effects of Panax ginseng extract on lipid metabolism in humans. *Pharmacological Research*, 51(2), 159-163.

Kitada, M., & Koya, D. (2013). Renal protective effects of resveratrol in diabetic nephropathy. Biomedicine & Pharmacotherapy, 67(3), 228-234.

Koye DN, Magliano DJ, Nelson RG, Pavkov ME. The Global Epidemiology of Diabetes and Kidney Disease. Adv Chronic Kidney Dis. 2018 Mar;25(2):121-132. doi: 10.1053/j.ackd.2017.10.011.

Kulkarni, S. K., & Dhir, A. (2008). Withania somnifera: An Indian ginseng. *Progress in Neuro-Psychopharmacology and Biological Psychiatry*, 32(5), 1093-1105.

Kumar, M., Dev, S., Khalid, M. U., Siddenthi, S. M., Noman, M., John, C., ... & AKUBUIRO, C. (2023). The bidirectional link between diabetes and kidney disease: mechanisms and management. Cureus, 15(9).

Kumar, V., Bhatia, S., & Bishnoi, R. (2019). Inhibition of advanced glycation end-products by mangrove extracts: A novel therapeutic approach for diabetic nephropathy. *Plant Foods for Human Nutrition, 74*, 273-280.

Lee, S. Y., Primavera, J. H., Dahdouh-Guebas, F., McKee, K., Bosire, J. O., Cannicci, S., ... & Record, S. (2014). Ecological role and services of tropical mangrove ecosystems: A reassessment. *Global Ecology and Biogeography, 23*(7), 726-743.

Lim, A. K., Tesch, G. H., Nikolic-Paterson, D. J., & Atkins, R. C. (2014). Inflammation and progressive renal injury in diabetic nephropathy: Effects of an 11β-hydroxysteroid dehydrogenase type 1 inhibitor in a mouse model. *Nephrology Dialysis Transplantation, 29*(12), 2343-2351.

Lucier, J., & Weinstock, R. S. (2023). Type 1 diabetes. In StatPearls [Internet]. StatPearls Publishing.

MacIsaac, Richard J., Elif I. Ekinci, and George Jerums. "Markers of and risk factors for the development and progression of diabetic kidney disease." American journal of kidney diseases 63.2 (2014): S39-S62.

Martineau, L. C., Couture, A., Spoor, D., Benhaddou-Andaloussi, A., Harris, C., Meddah, B., ... & Haddad, P. S. (2006). Anti-diabetic properties of the Canadian lowbush blueberry Vaccinium angustifolium Ait. *Phytomedicine*, 13(9-10), 612-623.

Mumby, P. J., Edwards, A. J., Arias-González, J. E., Lindeman, K. C., Blackwell, P. G., Gall, A., ... & Wabnitz, C. C. (2004). Mangroves enhance the biomass of coral reef fish communities in the Caribbean. *Nature, 427*(6974), 533-536. https://doi.org/10.1038/nature02286

Murphy, R., Ellard, S., & Hattersley, A. T. (2008). Clinical implications of a molecular genetic classification of monogenic β-cell diabetes. *Nature Clinical Practice Endocrinology & Metabolism, 4*(4), 200-213.

Nagelkerken, I., Blaber, S. J. M., Bouillon, S., Green, P., Haywood, M., Kirton, L. G., ... & Somerfield, P. J. (2008). The habitat function of mangroves for terrestrial and marine fauna: A review. *Aquatic Botany, 89*(2), 155-185. https://doi.org/10.1016/j.aquabot.2007.12.007

Narva AS, Bilous RW. Laboratory Assessment of Diabetic Kidney Disease. Diabetes Spectr. 2015 Aug;28(3):162-6. doi: 10.2337/diaspect.28.3.162.

Navarro-González, J. F., Mora-Fernández, C., Muros de Fuentes, M., & García-Pérez, J. (2011). Inflammatory molecules and pathways in the pathogenesis of diabetic nephropathy. *Nature Reviews Nephrology, 7*(6), 327-340.

Navarro-González, J. F., Mora-Fernández, C., Muros de Fuentes, M., & García-Pérez, J. (2011). Inflammatory molecules and pathways in the pathogenesis of diabetic nephropathy. *Nature Reviews Nephrology*, 7(6), 327-340.

Onyenwenyi, Chijoke, and Ana C. Ricardo. "Impact of lifestyle modification on diabetic kidney disease." Current diabetes reports 15 (2015): 1-8.

Patel, D. K., Prasad, S. K., Kumar, R., & Hemalatha, S. (2012). An overview on antidiabetic medicinal plants having insulin mimetic property. *Asian Pacific Journal of Tropical Biomedicine*, 2(4), 320-330.

Qin, B., Panickar, K. S., & Anderson, R. A. (2010). Cinnamon: Potential role in the prevention of insulin resistance, metabolic syndrome, and type 2 diabetes. *Journal of Diabetes Science and Technology*, 4(3), 685-693.

Rabbani, N., & Thornalley, P. J. (2014). Methylglyoxal, oxidant stress and diabetic nephropathy. Annals of the New York Academy of Sciences, 1043(1), 240-247.

Rajasekaran, S., Ravi, K., Sivagnanam, K., & Subramanian, S. (2006). Beneficial effects of Aloe vera leaf gel extract on lipid profile status in rats with streptozotocin diabetes. *Clinical and Experimental Pharmacology and Physiology*, 33(3), 232-237.

Rapa SF, Di Iorio BR, Campiglia P, Heidland A, Marzocco S. Inflammation and Oxidative Stress in Chronic Kidney Disease-Potential Therapeutic Role of Minerals, Vitamins and Plant-Derived Metabolites. Int J Mol Sci. 2019 Dec 30;21(1):263. doi: 10.3390/ijms21010263.

Rathod, S., Nandgude, T., & Deshmukh, P. (2021). Antioxidant and nephroprotective potential of mangrove plant extracts in diabetic nephropathy. *Journal of Ethnopharmacology, 274*, 113989.

Remuzzi, Giuseppe, et al. "The role of renin-angiotensin-aldosterone system in the progression of chronic kidney disease." Kidney International 68 (2005): S57-S65.

Rivera, L., & Morón, R. (2009). Antioxidant mechanisms of curcumin in kidney and liver. Journal of Physiology and Biochemistry, 65(2), 147-156.

Ruggenenti P, Cravedi P, Remuzzi G. The RAAS in the pathogenesis and treatment of diabetic nephropathy. Nat Rev Nephrol. 2010 Jun;6(6):319-30. doi: 10.1038/nrneph.2010.58. Epub 2010 May 4. PMID: 20440277.

Sagoo, M. K., & Gnudi, L. (2020). Diabetic nephropathy: an overview. Diabetic nephropathy: methods and protocols, 3-7.

Sano, M., & Ueda, M. (2007). Evaluation of the effect of vitamin E on renal function improvement in patients with type 2 diabetic nephropathy. Journal of Nephrology, 20(2), 119-124.

Selby NM, Taal MW. An updated overview of diabetic nephropathy: Diagnosis, prognosis, treatment goals and latest guidelines. Diabetes Obes Metab. 2020; 22(Suppl. 1): 3–15.

Sharma, R. D., Raghuram, T. C., & Rao, N. S. (1990). Effect of fenugreek seeds on blood glucose and serum lipids in type I diabetes. *European Journal of Clinical Nutrition*, 44(4), 301-306.

Singh, N., Gupta, M., & Sirohi, P. (2011). Effects of Momordica charantia fruit juice on glucose and lipid profile of normal and alloxan-diabetic rabbits. *Journal of Ethnopharmacology*, 134(2), 407-412.

Srivastava, S., Kapoor, R., & Thathola, A. (2012). Nutritional quality of leaves of some genotypes of mulberry (Morus alba). *International Journal of Food Sciences and Nutrition*, 53(5), 627-634.

Tanaka, Y., & Aleksunes, L. M. (2020). Role of Nrf2 in mitochondrial function and toxicity: focus on antioxidants and anti-inflammatory functions. International Journal of Molecular Sciences, 21(7), 2463.

Vasudevan, K., Vembar, S., & Devaraj, S. N. (2007). In vivo and in vitro anti-diabetic activity of Coccinia indica. *Journal of Ethnopharmacology*, 118(2), 159-165

Verma, R., Sharma, S., & Kharbanda, S. (2022). Protective role of Sonneratia apetala extracts on renal function markers in diabetic nephropathy. *Journal of Traditional and Complementary Medicine, 12*, 116-124. https://doi.org/10.1016/j.jtcme.2022.02.004

Wang, G., Ouyang, J., Li, S., Wang, H., Lian, B., Liu, Z., & Xie, L. (2019). The analysis of risk factors for diabetic nephropathy progression and the construction of a prognostic database for chronic kidney diseases. Journal of Translational Medicine, 17, 1-12.

Zsom, Lajos, et al. "Estimated glomerular filtration rate in chronic kidney disease: a critical review of estimate-based predictions of individual outcomes in kidney disease." Toxins 14.2 (2022): 127.

CHAPTER 2:

AIM, OBJECTIVE & PLAN OF WORK

2. Plan of work

2.1 Aim

To investigate the potential of *Acrostichum aureum* Linn. to prevent or treat diabetes and diabetic nephropathy, and to determine the mechanisms by which they exert their effects using *in-vitro* and *in-vivo* models, with the ultimate goal of developing new and effective therapeutic options for managing diabetic nephropathy.

2.2 Rationale for plant selection

Many natural remedies have been proposed to treat diabetes. Plant-based medications have been used traditionally to treat a range of diabetic symptoms (Jugran et al., 2021). *Acrostichum aureum* (L.) is a member of the Pteridaceae family and is found primarily along the southern and eastern shores of Bangladesh, SriLanka, Indonesia as well as in the Sundarbans in India. It plays an important role in the coastal ecosystem, although little attention has been paid to its medicinal qualities and phytochemical screening (Akinwumi et al., 2022). The most prevalent pharmacological activity tested was antioxidant, which revealed some potential antioxidant positive aspects (Badhsheeba et al., 2018). *A. aureum* is widely used in traditional medicine, in India, Bangladesh, Indonesia for its capacity to treat boils, rheumatism, myelitis wounds, diabetes and haemorrhages (Wu et al., 2018; Akinwumi et al., 2022). Thus, *A. aureum* is ethnomedicinally claimed to treat diabetes, has anti-inflammatory potential and possesses high phenolic and flavonoid compounds. These properties can be beneficial in treating diabetic nephropathy and have been taken into consideration in selection of this plant (Rapa et al., 2019). As a result, the plant must be reintroduced and its pharmacological activity validated, as well as the phytoconstituents present isolated. This thesis covers the botanical description, distribution, ethnobotanical and traditional uses, as well as antidiabetic effectiveness.

2.3 Objectives

- Extraction of mangrove medicinal plant based on ethnopharmacological relevance.
- Photochemical screening of selected medicinal plant.
- Evaluation of therapeutic potential as antidiabetic agent through in vitro α-amylase, α-glucosidase inhibition assay.
- Validation of therapeutic and ethnopharmacological claim by *in-vivo* screening against diabetic nephropathy.

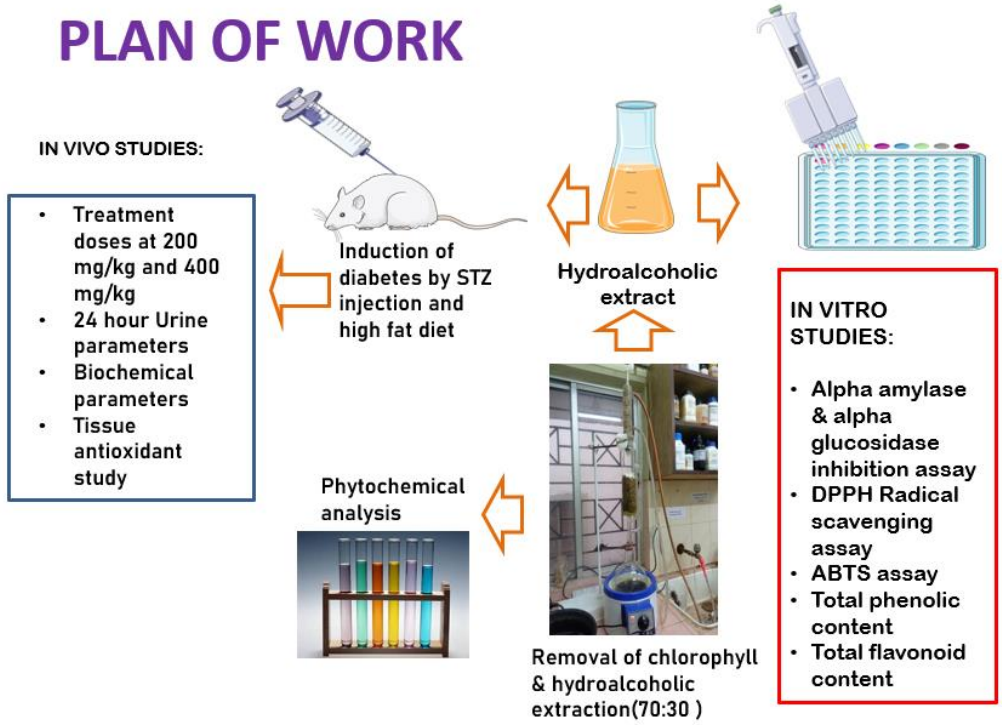


Figure 2.1: Schematic representation of the overall plan of work

2.4 Plan of Work

- Literature Review and selection of plant
- Collection and identification of aerial parts of *Acrostichum aureum*
- Hydro-alcoholic extraction of aerial parts of *Acrostichum aureum*
- 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
  - Oral glucose tolerance test (OGTT)
  - Blood glucose level
  - Glycosylated haemoglobin (HbA1c)
  - Urine parameters:
    - 24 hours urine volume
    - Urine albumin excretion
    - Creatinine clearance
    - Urea clearance
  - Tissue antioxidant parameters
    - Lipid Peroxidation
    - Superoxide dismutase
    - Reduced glutathione

- Serum biochemical parameters
  - SGOT (Serum glutamate oxaloacetate transaminase)
  - SGPT (Serum glutamate pyruvate transaminase)
  - ALP (Alkaline phosphatase)
  - Total Protein
  - Total Cholesterol
  - Triglyceride
  - HDL Cholesterol
- Histopathology

References

Akinwumi, K. A., Abam, E. O., Oloyede, S. T., Adeduro, M. N., Adeogun, Y. A., & Uwagboe, J. E. (2022). Acrostichium aureum Linn: traditional use, phytochemistry and biological activity. *Clinical Phytoscience*, *8*(1), 18.

Badhsheeba, R. A., & Vadivel, V. (2018). Evaluation of in vitro antioxidant activity of Acrostichum aureum Linn. Rachis. *Journal of Pharmacognosy and Phytochemistry*, *7*(6), 1146-1151.

Jugran, A. K., Rawat, S., Devkota, H. P., Bhatt, I. D., & Rawal, R. S. (2021). Diabetes and plant-derived natural products: From ethnopharmacological approaches to their potential for modern drug discovery and development. *Phytotherapy Research*, *35*(1), 223-245.

Rapa, S. F., Di Iorio, B. R., Campiglia, P., Heidland, A., & Marzocco, S. (2019). Inflammation and oxidative stress in chronic kidney disease—potential therapeutic role of minerals, vitamins and plant-derived metabolites. *International journal of molecular sciences*, *21*(1), 263.

Wu, X., Huang, Q., Xu, N., Cai, J., Luo, D., Zhang, Q., ... & Liu, Y. (2018). Antioxidative and anti-inflammatory effects of water extract of Acrostichum aureum Linn. Against ethanol-induced gastric ulcer in rats. *Evidence-Based Complementary and Alternative Medicine*, *2018*(1), 3585394.

CHAPTER 3: LITERATURE REVIEW

3.1 Botanical description

Acrostichum aureum Linn. is a fern belonging to the Pteridaceae family.

A: Leaves

B: Whole plant

Figure 3.1: *Acrostichum aureum* Linn.

3.2 Plant Taxonomy

Kingdom: Plantae
Phylum: Tracheophyta
Class: Polypodiopsida
Order: Polypodiales
Family: Pteridaceae
Genus: *Acrostichum*
Species: *aureum*
Kingdom: Plantae

3.3 Synonymns

Chrysodium aureum (L.) Mett., *Acrostichum cayennense Presl*, *Chrysodium hirsutum* Fée, *Polystichum dissimulans Maxon*, *Chrysodium vulgare* Fée, *Parahemionitis arifolia (Burm. fil.)* Panigrahi

3.4 Local name

Bengali: Hudo
English: Mangrove fern, Tiger fern, Golden leather fern
Chinese: Jin jue
Srilankan: Karen koku

3.5 Parts used

Leaves, rachis, roots, rhizome

3.6 Ethnomedicinal claim

In many regions of Asia, it is a common cure for boils, rheumatism, myelitis wounds, and haemorrhages In Sri Lanka, Malaysia, and Indonesia, young fronds of *A. aureum* are sold as vegetables and can be eaten raw, well-cooked, or blanched. Diabetes, pharyngitis, and syphilitic ulcers are all treated with the fertile fronds and roots. Additionally, the leaves are utilised to cure inguinal hernias, gastritis, diarrhoea, and haemorrhoids. Malaysians use the young fronds of the plants that are under 14 days old in treating hypotension, worms and digestive problems. The Ahanev people of Badagry, South-West Nigeria, use concoctions from the roots of *A. aureum* as newborn baby ointment, while the plant is used for the treatment of severe stomachache and skin infection (Akinwumi et al., 2022). In traditional Sri Lankan medicine, the leaves are used to treat diabetes, while the buds are used to treat digestive and cardiovascular issues (Nijamdeen et al., 2023). *A. aureum* is utilised as a diabetic treatment in Tamil Nadu (Badhsheeba et al., 2018). The Marma tribe in Bangladesh uses the cooked leaf as a vegetable to boost physical strength, alleviate murky urine in women, and stimulate sexual desire (Rahmatullah et al., 2009).

3.7 Geographical distribution

The only fern species that thrives in the marine intertidal zone is "*Acrostichum*." Around the world, *A. aureum* is primarily found growing along tropical and subtropical coasts. Fresh water, however, is where the spores germinate most readily. It usually grows in parts of the mangrove swamp that are sometimes flooded by the sea on small heights. It may grow in freshwater environments as well. The plant is one of the main species in many mangrove environments worldwide and is invasive. In India, it is found in the coasts of inter tidal regions of the Sundarbans and Tamil Nadu (Ganguly et al., 2017).

3.8 Morphological features

The large fronds of the golden leather fern may reach a length of 1.8 metres, or six feet. The pinnae are alternating, dark green, leathery, and widely spread, while the leaves are glossy, broad, and pinnate. The inner fronds are almost straight, while the outside ones arch sideways. Sporangia, or reproductive organs, are present on the upper five to eight pairs of pinnae of some of the larger fronds. They are brick red and give the impression of felt on the pinnae. The shrub grows up to 4m tall and has adventitious roots that are visible at the bottom of the leaves and knots. The leaf's venation is reticulate, with uniformly long, elongated areoles that diverge from the thicker midrib without having free vein ends. The woody stipes grow from a glabrous woody rhizome.

3.9 Phytochemistry

Because phytochemicals offer a number of advantageous biological actions, such as anti-inflammatory, antioxidant, and antibacterial capabilities, they have important medical and

pharmacological benefits. Secondary metabolites, including flavonoids, gum, sterols, glycosides, saponins, alkaloids, gums, tannins, terpenoids, and triterpenoids, are abundant in different regions of *A. aureum*. The ethanol extracts of the fronds were subjected to quantitative analysis, which showed the presence of alkaloids (160 µg/ml), flavonoids (81.5 µg/ml), tannins (12.1 µg/ml), and phenol (64.0 µg/ml). The whole plant contains 0.11, 28.29, 19.90, 3.02, 0.83, 1.11 g/100 g dry weight of proline, phenol, alkaloid, saponin, tannins, and cardiac glycosides, according to another quantitative investigation. Tannin concentrations for n-hexane, ethyl acetate, methanol, ethanol, and 70% were found to be 0.52, 1.39, 9.45, 10.90, and 28.8 TAE/g extract (Thomas et al., 2016; Uddin et al., 2013).

3.11 Pharmacological activities

- **Anti-ulcer and anti-inflammatory activity:** Initial treatment with *A. aureum* mitigated the pathological damage in the gastric tissue caused by alcohol and decreased the incidence of gastric ulcers in a dose-dependent manner. Rats co-exposed to ethanol and an aqueous extract of *A. aureum* showed dose-dependent increases in glutathione, catalase, and superoxide dismutase, while a dose-dependent decrease in ROS produced by ethanol was also observed in the extract's stomach. Moreover, the extract also inhibited the release of proinflammatory cytokines such as interleukin-1 β (IL-1 β), interleukin-6 (IL-6) and tumor necrosis factor-α (TNF-α). Furthermore, the water extract reduced the expressions of phosphorylation of p65 and IkBa. Therefore, the extract's ability to treat stomach ulcers was associated with its ability to decrease oxidative stress and the inflammatory response (Wu et al., 2018).

- **Antioxidant activity:** Using various antioxidant tests, numerous writers have reported on the antioxidant capabilities of various *A. aureum* extracts. An EC50 of 103.0 µg/ml was observed in the plant's methanol twig extract for 2, 2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity, but an IC50 of 28.99 µg/ml was reported for the inhibition of lipid peroxidant. More than 20 µg/ml was reported to be the IC50 for quinone reductase induction. Strong 42 µg/ml in vitro antioxidant activity against DPPH radicals was also reported for the ethanol extract of *A. aureum* (Bunyapraphatsara et al., 2003).

- **Analgesic activity:** Mice were used to test the analgesic effects of *A. aureum*'s ethanol leaf extract against acetic-induced writhing. The findings demonstrated that *A. aureum* exhibited writhing inhibition of 28.86 and 46.77% at doses of 250 and 500 mg/kg body weight, respectively. Although less potent, the analgesic effect was equivalent to the 69.15% achieved with the usual medication of 25 mg/kg body weight diclofenac sodium (Raja et al., 2014).

- **Cytotoxic and anticancer activity:** Compounds isolated from the methanol extract of *A. aureum* were screened for in vitro cytotoxicity against Hep-G2, SKLU-1, and MCF-7cells by Minh et al. using sulforhodamine B assay. anticancer properties of two novel sesquiterpenes, (2R,3S)-sulfated pterosin C and (2S,3S)-sulfated pterosin C, as well as their derivatives, (2S,3S)-pterosin C and (2R)-pterosin P isolated from the methanol

extract of the aerial part of *A. aureum* were evaluated against AGS, HT29, MDA-MB-231, and MCF-7 human cancer cell lines using MTT assay (Minh et al., 2022).

- **Wound healing property:** In a rabbit excision wound model, the plant's ethanol extract was assessed. Comparing the topical application of 10% *A. aureum* rhizome to the control, better wound contraction and epithelization period were observed. The authors explained that *A. aureum*'s ability to heal wounds was due to both its phytochemical makeup and antibacterial activity (Herman et al.,2013).

- **Tyrosinase inhibiting activity:** Lai et al. used Dopachrome assays to examine the tyrosinase-inhibiting potential of *A. aureum* methanol extract. With a tyrosinase inhibitory activity of 33%, the extract was comparable to 91.3 mg of quercetin and 9.8 mg of kojic acid per gram of extract. The herb *A. aureum* may have a role in the management of cutaneous hyperpigmentation due to its potent tyrosinase inhibitory activity.

- **Anti-diarrhoeal activity:** The anti-diarrhea characteristic of *A. aureum* ethanol root extract in a castor oil model of mouse diarrhea development has been investigated. It was discovered that by lowering the rate of defecation and feces inconsistency, the ethanol extract of *A. aureum* root had an anti-diarrheal effect on treated mice. Comparing the extract at 400 mg/kg to loperamide, the conventional medication, which reduced diarrhea by 66%, the extract reduced diarrhea by 55%. Hence, the plant's ethnobotanical application in the treatment of diarrhea is justified (Hossain et al., 2012).

References

Akinwumi, K. A., Abam, E. O., Oloyede, S. T., Adeduro, M. N., Adeogun, Y. A., & Uwagboe, J. E. (2022). Acrostichium aureum Linn: traditional use, phytochemistry and biological activity. Clinical Phytoscience, 8(1), 1-18.

Badhsheeba, R. A., & Vadivel, V. (2018). Evaluation of in vitro antioxidant activity of Acrostichum aureum Linn. Rachis. Journal of Pharmacognosy and Phytochemistry, 7(6), 1146-1151.

Bunyapraphatsara N, Jutiviboonsuk A, Sornlek P, Therathanathorn W, Aksornkaew S, Fong HH, et al. Pharmacological studies of plants in the mangrove forest. Thai J of Phytopharm. 2003;10(2):1–12.

Ganguli S, Rahaman S, Bera AR, Vishal V, Malik S, Roopalakshmi K, et al. Rhizospheric metagenome of the terrestrial mangrove fern Acrostichum from Indian Sunderbans. Genom Data. 2017;5(14):53–5.

Herman HP, Susant D, Saad S, Tahir M, Ramli N. Wound healing properties of ethanolic extract of Acrostichum aureum and Acrostichum speciosum rhizome in rats. J. Trop. Resourc. Sustain Sci. 2013;1(2):42–8.

Minh TT, Thu NH, Toan HK, Quang TT, Thanh NT, Huong TT, et al. Three new phenolic sulfates from Acrostichum aureum collected from coastal area of Thai Binh Province, Vietnam and their cytotoxic activity. Rec Nat Prod. 2022;16(1):66–73.

Nijamdeen, T. M., Ephrem, N., Hugé, J., Kodikara, K. A. S., & Dahdouh-Guebas, F. (2023). Understanding the ethnobiological importance of mangroves to coastal communities: A case study from Southern and North-western Sri Lanka. Marine Policy, 147, 105391.https://doi.org/10.1016/j.marpol.2022.105391

Rahmatullah, M., Hossan, M. S., Hanif, A., Roy, P., Jahan, R., Khan, M., ... & Rahman, T. (2009). Ethnomedicinal applications of plants by the traditional healers of the Marma tribe of Naikhongchhari, Bandarban district, Bangladesh. Adv Nat Appl Sci, 3(78), 392-401.

Raja S, Ravindranadh K. Preliminary phytochemical screening of different solvent extracts of whole plant of Acrostichum aureum. World J Pharm Sci. 2014;1:1753–9.

Thomas A, Peter PK, Chandramohanakumar N. A profiling of anti-tumour potential of sterols in the mangrove fern Acrostichum aureum. Int J Pharmacogn Phytochem Res. 2016;8(11):1828–32.

Uddin SJ, Bettadapura J, Guillon P, Darren Grice I, Mahalingam S, Tiralongo E. In-vitro antiviral activity of a novel phthalic acid ester derivative isolated from the Bangladeshi mangrove fern Acrostichum aureum. J Antivir Antiretrovir. 2013;5(6):139–44.

Wu X, Huang Q, Xu N, Cai J, Luo D, Zhang Q, et al. Antioxidative and anti-inflammatory effects of water extract of Acrostichum aureum Linn. Against ethanol-induced gastric ulcer in rats. ECAM. 2018:3585394.

Wu, X., Huang, Q., Xu, N., Cai, J., Luo, D., Zhang, Q., ... & Liu, Y. (2018). Antioxidative and anti-inflammatory effects of water extract of Acrostichum aureum Linn. against ethanol-induced gastric ulcer in rats. Evidence-based complementary and alternative medicine, 2018.

CHAPTER 4:

COLLECTION IDENTIFICATION AND EXTRACTION

4.1 Collection identification and extraction

The leaves of *Acrostichum aureum* plant was collected from Sajnekhali area of the Sundarbans, West Bengal, India in the month of August, 2023. The plant species was identified and authenticated by the scientist in charge of Botanical Survey of India, Kolkata, West Bengal. Air dried leaves and rachis (aerial parts) of *A. aureum* (500 g) were extracted by ultrasonication assisted cold maceration technique using 2000 mL of hydroalcohol (70:30) for 5 days. to make hydroalcoholic extract of *A. aureum* (HEAA). The solvent was completely removed under reduced pressure in a rotary evaporator. The concentrated extract was obtained by lyophilization and stored in vacuum desiccators (20°C) for further use. The yield of the methanol fraction was about 20.14 %.

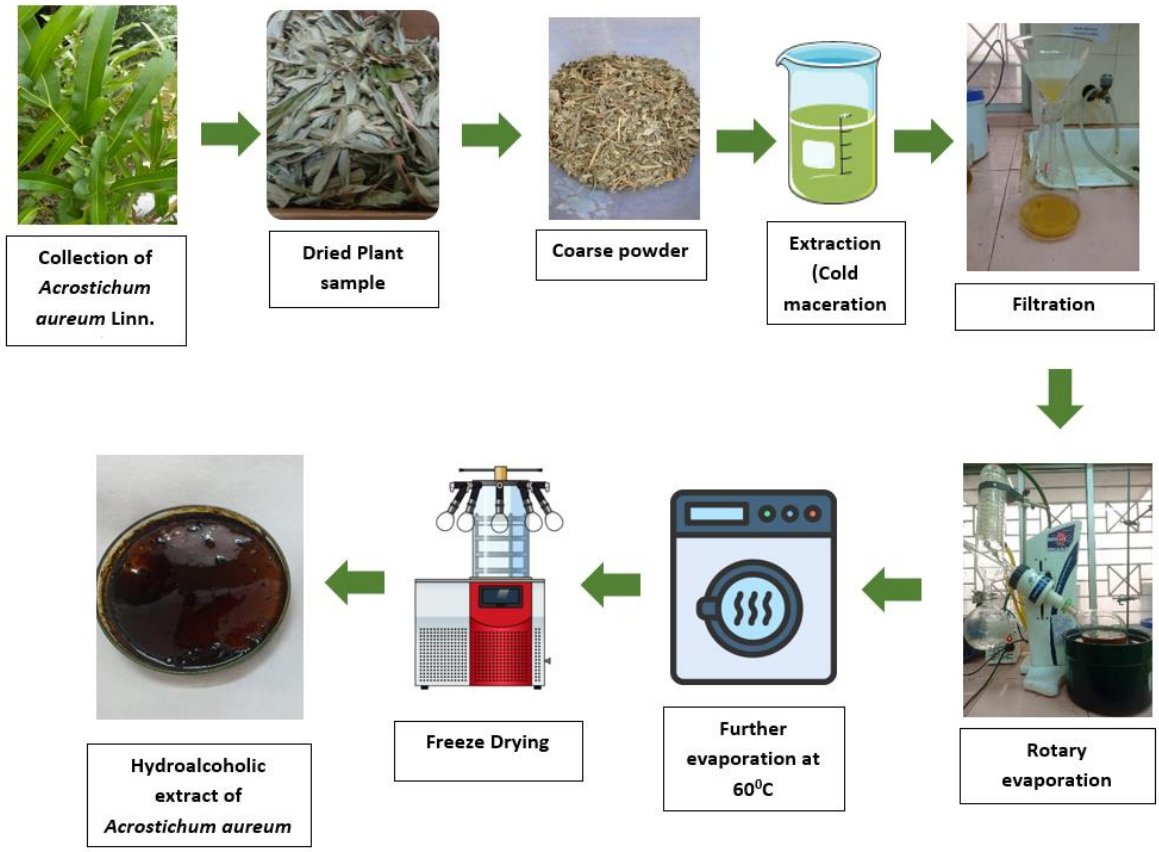


Figure 4: Schematic representation of collection to extraction processes

4.2 Qualitative Analysis

The presence of phytochemical elements in medicinal plants makes them effective for both healing and curing human ailments (A. Nostro et al., 2000). Plant compounds with protective or illness-preventive qualities are known as phytochemicals. Although these compounds are produced by plants as self-defense, new studies show that they can also serve as disease defense. (M. G. Ajuru et al, 2017). Therefore, determination of the phytoconstituents in a plant material or its extract is of utmost importance. To identify the kind of compounds included in the extract, a preliminary qualitative analysis was carried

out. Alkaloids, flavonoids, saponins, tannins, steroids, glycosides, and carbohydrates were all subjected to chemical group tests.

4.3 Chemical Tests

Test for Steroids:

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

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

Salkowski Test (Bosila et al., 2005)

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

Test for Flavonoids:

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

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

Shinoda test (Palanisamy P et al., 2012)

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

Test for Saponins (Shinha et al., 2015)

1 ml solution of the extract was diluted with distilled water to 20 ml and shake in a graduated cylinder for 15 mins. Development of stable foam suggested the presence of saponins.

1 ml extract was treated with 1% lead acetate solution. Formation of white precipitate indicated the presence of saponins.

Test for Tannins (Segelman et al., 2016)

5 ml of extract solution was allowed to react with 1 ml 5% ferric chloride solution black coloration indicated the presence of tannins.

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

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

Test for Glycoside (Salwaan et al. 2012):

Legal's test

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

Bortanger's test

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

Test for Carbohydrate:

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

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

Molish test (Salwaan et al., 2012)

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

Test for Alkaloids (Raffauf et al., 2019)

Mayer's test

1.2 ml of extract was taken in a test tube. To it 0.2 ml of dilute hydrochloric acid and 0.1 ml of Mayer's reagent were added. Formation of yellowish buff coloured precipitate gives positive test for alkaloids.

Dragendroff's test

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

Wagner's test

2 ml of extract solution was treated with dilute hydrochloric acid and 0.1 ml Wagner's reagent formation of reddish brown indicated the positive response for alkaloids.

Hager's test

2ml of the extract was allowed to react with 0.2 ml of dilute hydrochloric acid and 0.1 ml of Hager's reagent. A yellowish precipitate suggested the presence of alkaloids.

Test for Phenols

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

Test for Triterpenoid:

Salkowski test (Nayak et al., 2011)

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

4.4 Results

Phytochemicals	Presence/absence
Flavonoid	+
Phenols	+
Terpenoid	+
Saponin	+
Tannin	+
Glycosides	+
Proteins	+
Steroid	+
Carbohydrates	+
Volatile oils	+
Fixed oils and fats	+
Alkaloids	-

[(+) Presence of the class of compounds; (-) Absence of the class of compounds]

4.5 Conclusion

By conducting the above mentioned phytochemical tests, the hydroalcoholic extract of *Acrostichum aureum* has been found to be phytochemically rich. It has been found to be comprised of flavonoids, phenols, terpenoids, saponins, tannins, glycosides, proteins, steroid, carbohydrates, volatile oils, fixed oils and fats. The test for alkaloids showed negative result.

Reference

Ajuru MG, Williams LF, Ajuru G. Qualititive and Quantitive Phytochemical Screening of Some Plants Used in Ethnomedicine in the Niger Delta Region of Nigeria. 2017; 5(5): 198 205.

Bhandary Sk, Kumari SN, Bhat VS. Sharmila KP and Bekal MP. Preliminary phytochemical screening of various extracts of granatumpeel, whole fruit and seeds Nitte University Journal of Health Science, 2012: 2(4); 34-38,

Nayak Sarojini, Sahoo Anjulata Manjari, Chakraborti Chandra Kanti, Correlation between phytochemical screening and some biological activity using plant extracts, URAP. 2011, 2(4): 1343-1348.

Nostro A, Germano MP, Danelo V, Marino A and Cannatelli MA. Extraction method and bio autography for evaluation of medicinal plant antimicrobial activity, Lett. App Microbial. 2000; 30: 379-384.

Palanisamy P, Jayakar B, Kumuthavalli MV, Kumar Y and Srinath KR. Preliminary phytochemical evaluation of whole plant extract of Dipteracanthus prostrates nees. International Research journal of Pharmacy. 2012; 3(1): 150-153.

Salwaan C. Singh A, Mittal A and Singh P. Investigation of the Pharmacognostical, Phytochemical and antioxidant studies of plant Withania coagulans Dunal. Journal of Phamacognosy and Phytochemistry. 2012; 1(3): 32-39.

Salwaan C. Singh A, Mittal A and Singh P. Investigation of the Pharmacognostical, Phytochemical and antioxidant studies of plant Withania coagulans Dunal. Journal of Pharmacognosy and Phytochemistry. 2012, 1(3): 32-39.

Raffauf RF. A simple field test for alkaloid-containing plants. Economic Botany. 2019: 16: 171-172.

Segelman AB, Farnsworth NR, Quimby MD. False-Negative Saponins test results induced the presence of tannins. The Journal of Biological Chemistry. 2012;32(2): 52- 58.

Sinha SKP, Dogra JVV. A Survey of the Plants of Bhagalpur and Santhal Pargana for Saponin; Flavonoids and Alkaloids. Pharmaceutical Biology. 2018; 23:77-86.

Ugochukwu SC, Arukwe Ul and Ifeanyi O. Preliminary phytochemical screening of solvent extracts of stem bark and roots of Dennetia tripetala G. Baker. Asian Journal of Plant Science and Research. 2013; 3(3): 10-13.

Zhou H, Li YS, Tong XT, Liu HQ, Jiang SH, Zhu DY. Serratane-type Triterpenoids from Huperzia Serrata. Natural Product Research. 2004; 18:453-9. Bosila HA, Mohamed SM, EI SF, IbrahimSIA. Phytochemical screening of some in vivo and in vitro date palm tissues: 2005:23-25.

CHAPTER 5: ACUTE TOXICITY STUDY

5.1 Introduction

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

The term toxicology derived from the word "toxion" means poison and "logos" means science. Toxicology is the science which deals with the harmful effects of chemicals and drugs on living systems. It helps us to determine the quality and quantity of chemical which will turn it into poison (Gupta et al., 2018).

The potential uses of toxicity testing data include:

- Establishing the therapeutic dose.
- Acquiring information about the harmful effects specific organs.
- Establishment of the mode of toxic action.
- Establishment of the toxic as a future reference. (Krewski et al., 2020).

5.2 METHODOLOGY:

5.2.1 Animals

Healthy and nulliparous Swiss Albino female mice weighing 22-25g were taken for this study. Each group included five mice with a total of ten. They were kept in polyacrylic cages (38cm x10cm) with not more than five animals in each cage. They were maintained under standard laboratory conditions (temperature 25ºC, 12 hours light and12 hours dark cycle) with sufficient food and water.

5.2.2 Procedure

According to the Organization for Economic Co-operation and Development 420 Guidelines for Acute oral toxicity test, fasted animals of single sex were dosed in a stepwise procedure using the fixed dose of 5, 50, 300 and 2000 mg/kg orally (OECD 2000). All the animals were

observed for any signs of toxicity or mortality at least for 24 hours. The initial dose level was selected on the basis of a sighting study as the dose expected to produce some signs of toxicity without causing severe toxic effects or mortality. Further groups of animals were dosed at higher or lower fixed doses, depending on the presence or absence of signs of toxicity or mortality. This procedure continued until the dose causing evident toxicity or death is identified, or when no effects are seen at the highest dose or when deaths occur at the lowest dose. All the animals were subjected for sharp observation for a period of 14 days, HEAA showed no mortality or toxic effect up to 25 mg/kg body weight in mice. No deaths were observed when the animals were given a dose of 2000 mg/kg body.

5.3 RESULT

No death of the animals were observed upto the dose of 2000 mg/kg body.

5.4 DISCUSSION

Oral administration of HEAA did not show any significant change in behaviour, breathing, itching sensation, sensory nervous system responses or gastrointestinal effects. During the experiment period no deaths were observed. So from the results concluded that Hydro-alcoholic extract of *Acrostichum aureum* is safe up to dose of 2000 mg/kg.

REFERENCES

Gupta, P. K. (2018). Illustrated toxicology: with study questions. Academic Press.

Krewski, D., Andersen, M. E., Tyshenko, M. G., Krishnan, K., Hartung, T., Boekelheide, K., ... & Cote, I. (2020). Toxicity testing in the 21st century: progress in the past decade and future perspectives. *Archives of toxicology*, *94*, 1-58.

Arora Ps, Mishra Ck. Mavani P. asha R. Shrivastava B and Nema RL. Effective attomane authods of LD50 help to save number of experimental animals. Journal of chemical and Pharmaceutical research, 2010, 2(6), 450-453,

Lachfield JT, Wilcoxon, F. A shuplified method of evaluating those effect experiments. Journal of Pharmacology and Experimental therapeutics, 1949, (96).90-113

Looke D.A new approach to practical acute toxicity testing Arch Toxicology. 1983,54275-87

OECD (2000) Guidance Document on Acute Oral Toxicity. Environmental Health and Sality

Turner R. Screening Methods in Pharmacology. New York: Academic Press Acute toxicity. The determination of LD, 1965 61-61

CHAPTER 6:
IN VITRO ASSAYS

6.1 Introduction

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

α-glucosidase activity was measured by determining the colour developed by the release of p-nitrophenol arising from the hydrolysis of substrate PNPG by α-glucosidase using spectrophotometric method. Glucose transport across the yeast cell membrane is based on the principle of Facilitated Diffusion down the concentration gradient. Glucose transport occurs only after intracellular glucose is effectively utilized (reduced). Therefore the aim of our desired plant extract should be enhancement of effective glucose utilization thereby controlling blood glucose level.

The generation of highly reactive oxygen species (ROS) with a lone unpaired electron induce oxidative stress and plays a key role in the pathogenesis of numerous physiological conditions, including cellular injury, aging, cancer, and hepatic, neurodegenerative, cardiovascular and renal disorders.

Phenolic compounds are important plant constituents with redox properties responsible for antioxidant activity. Natural phenolic and flavonoid compounds are plant secondary metabolites that hold an aromatic ring bearing at least one hydroxyl group. Phenolic compounds are good electron donors because their hydroxyl groups can directly contribute to antioxidant action. Furthermore, some of them stimulate the synthesis of endogenous

antioxidant molecules in the cell. According to multiple reports in the literature, phenolic compounds exhibit free radical inhibition, peroxide decomposition, metal inactivation or oxygen scavenging in biological systems and prevent oxidative disease burden. Many epidemiological studies have shown that the consumption of leafy plant vegetables containing phenolic and flavonoid compounds with potent antioxidant activity are associated with a lower incidence of cardiovascular diseases, cancer, diabetes and neurodegenerative diseases (Aryal et al., 2019). Scientific studies have revealed that polyphenols play an important role as an antidiabetic agent. The potential of these secondary compounds as antidiabetic agents may be due to its inhibitory action in the gut for glucose absorption or by its peripheral tissue uptake. Inhibition of αglucosidase found in gut mucosa is responsible for such type of effect. Along with this, polyphenols have been investigated for their glucose transporter and intestinal glycosidase inhibitory activity (Mutha et al., 2021).

6.2 Materials and Methods

6.2.1 Materials required

α-amylase, α-glucosidase, Acarbose (≥95% (HPLC),2-diphenyl-1-picrylhydrazyl (DPPH), Ascorbic acid (analytical standard) were used which were procured from Merck (Mumbai, Maharashtra, India).

6.2.2 Evaluation of Total Phenolic and Flavonoid Content

The total phenolic content (TPC) and total flavonoid content (TFC) of *A. aureum* hydroalcoholic extract was estimated by a previously published literature (Gupta et al., 2023). The sample (HEAA) or standard (gallic acid) 30 µL/well in the concentration range of 15.6–500 µg/mL were used to estimate the TPC by microtiter plate method. The gallic acid equivalent (GAE) per gram of material was determined by taking the sample and standard absorbance at 725 nm. Triplicates of this study have been done for statistical analysis. For TFC analysis 80 µL/well of standard (Quercetin) and sample (HEAA) were mixed with $NaNO_2$, NaOH, and $AlCl_3$ and the absorbance was measured at 415 nm after to compute quercetin equivalent per gram of the sample (QE).

6.2.3 Free Radical Scavenging Activity

Different concentrations (made by serial dilution) of HEAA was prepared in distilled water and reference (2-diphenyl-1-picrylhydrazyl) was prepared in Methanol.

The principle of this assay is based on reduction of alcoholic DPPH solution in the presence of hydrogen donating antioxidant. Antioxidant activity was measured spectrophotometrically on the basis of decrease the intensity of the purple-coloured methanolic solution of 2,2-diphenyl-1- picrylhydrazyl (DPPH). DPPH radical scavenging assay has been used to assess the antioxidant potential of HEAA. HEAA (100 µL) and 100 µL Ascorbic acid (standard) was combined with 100 µL of 0.1 mM DPPH (2,2-diphenyl-1-picrylhydrazyl) at various concentrations (200, 100, 50, 25, 12.50 µg/mL). After 30 minutes of storage (in dark) at room temperature, the absorbance of the mixture was

measured at 517 nm against a blank (Chanu et al., 2023). The percentage of DPPH free radical scavenging capacity was calculated using the following equation:

% inhibition = (Absorbance of control − Absorbance of sample)/Absorbance of control) ×100

6.2.4 α -amylase inhibitory activity

Different concentrations (made by serial dilution) of HEAA was prepared in distilled water and reference (Acarbose) was prepared in phosphate buffer (100 mM, pH: 6.8)

Various concentrations of the sample HEAA and standard acarbose (20, 40, 60, 80, 100, and 120 µg/mL) were produced. 10 µL of α-amylase (2U/mL) soluble starch (1%), was combined with 50 µL of sodium phosphate buffer (100 mM, pH 6.8). Once the substrate had been incubated for 30 minutes at 37°C, 20 µL of 1% soluble starch made in phosphate buffer 100 mM (pH: 6.8) was added, and the mixture was once again incubated for 30 minutes at 37°C. After adding 100 µL of dinitrosalicylic acid reagent solution and boiling for 10 minutes, the reaction was stopped. The enzyme activity was evaluated by measuring absorbance at 540 nm using a microplate reader (Gayen et al., 2024).

The following formula was used to compute the % inhibition, which was used to express the results.

Inhibitory activity (%) = (1 − As/Ac) ×100 where As is the absorbance in the presence of test substance and Ac is that of control

6.2.5 α -glucosidase inhibition assay

Different concentrations (made by serial dilution) of HEAA was prepared in distilled water and reference (Acarbose) was prepared in phosphate buffer (100 mM, pH: 6.8)

The α-glucosidase inhibitory activity of HEAA was carried out according to the standard method previously done in the laboratory with slight modifications. Acarbose, the standard inhibitor, and the sample HEAA extract (20, 40, 60, 80, 100, and 120 µg/mL) were produced in different concentrations. Subsequently, 10 µL of alpha-glucosidase (1 U/mL) and 50 µL of 0.1 M potassium phosphate buffer (pH: 6.8) were combined and allowed to incubate. 20 µL of p-nitrophenyl glucopyranoside (pNPG, 5 mM) was added, thoroughly mixed, and then incubated again for 30 minutes at 37°C after the initial 20 minutes. A solution of 0.1 M Na2 CO3 in 40 µL was added to halt the reaction. Enzyme activity was determined by measuring the absorbance of the end product p-nitrophenol at 410 nm with a microplate reader). The results were expressed as percentage inhibition, which was calculated using the following formula;

Inhibitory activity (%) = (1 − As/Ac) ×100

where As is the absorbance in the presence of test substance and Ac is the absorbance of control (Patra et al., 2020).

6.3 Statistical Analysis

All the results are shown as mean SEM. The results were analyzed for statistically significance by one-sway analysis of variance (ANOVA) using Graph Pad Prism 8.2.1 software (Graph Pad Software, USA). p values of < 0.05 were considered as statistically significant.

6.4 Result

6.4.1 Evaluation of Total phenolic and total flavonoid content

Gallic acid equivalents (GAE) per gram of dry extract were used to assess the amount of total phenols present in the dry extracts. The dry extract's total phenolic content was 4.06±0.010 mg GAE/gm of dry extract. The equation for the gallic acid standard curve was Y= 0.002781x+ 0.01359, and the correlation coefficient was $R^2 = 0.9916$. Quercetin equivalents (QE) per gram of dry extracts were used to assess the amount of total flavonoids present in the samples. Total flavonoids content of HEAA was 0.78±0.0277 mg QE/g of dry extract. The standard curve equation of quercetin was $y = 0.001516x + 0.4766$ with a correlation coefficient of $R^2 = 0.9719$.

6.4.2 Antioxidant potential

The anti-oxidant potential of HEAA was evaluated in the concentration range of 15.65- 500 µg/ml and the percentage inhibition of the respective concentrations of HEAA are given in the table 3 below, compared to that of the reference, Ascorbic acid.

Table 1: DPPH radical scavenging activity of HEAA and Ascorbic acid

Concentration (µg/ml)	HEAA	Ascorbic acid
15.625	18.16 ± 1.03	39.65 ± 1.45
31.25	24.81 ± 2.09	49.15 ± 1.98
62.5	28.91 ± 1.66	59.0 ± 1.88
125	47.2325 ± 1.43	70.40 ± 2.45
250	68.52 ± 1.05	75.5 ± 1.78
500	75.43 ± 1.87	90.64 ± 1.49

Each value is expressed as Mean ± SEM

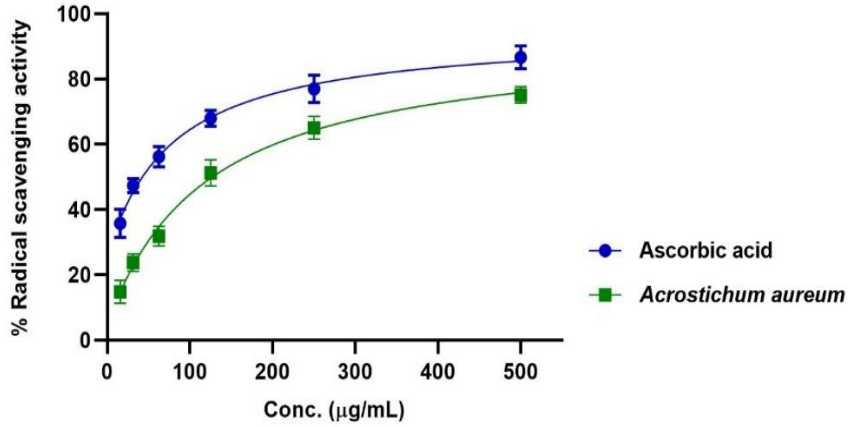


Figure 6.1: DPPH radical scavenging activity of different concentrations of HEAA and Ascorbic acid

6.4.3 *In vitro* anti diabetic activity

The in vitro anti-diabetic potential of HEAA was evaluated in the concentration range of 15.65- 500 µg/ml by alpha amylase and alpha glucosidase inhibition assays and the percentage inhibition of the respective concentrations of HEAA are given in the tables 2 and 3 respectively below, compared to that of the reference compound, Acarbose.

Table 2: Percentage inhibition of alpha amylase by HEAA and Acarbose

Concentration (µg/ml)	HEAA	Acarbose
7.81	9.97 ± 2.06	28.3 ± 1.12
15.62	18.27± 1.19	38.42 ± 1.09
31.25	30.96 ± 2.61	57.46 ± 1.98
62.5	49.86 ± 3.04	67.49 ± 1.11
125	52.69 ± 1.11	80.65 ± 1.04
250	69.42 ± 2.78	84.94 ± 2.21

Each value is expressed as Mean ± SEM

Table 3: Percentage inhibition of alpha glucosidase by HEAA and Acarbose

Concentration (µg/ml)	HEAA	Acarbose

7.81	8.97 ± 1.45	25.02 ± 1.07
15.62	20.27 ± 2.08	40.29 ± 1.79
31.25	39.56 ± 1.88	59.04 ± 1.56
62.5	47.15 ± 2.03	72.62 ± 0.09
125	55.24 ± 1.98	82.26 ± 1.78
250	67.22 ± 1.06	89.13 ± 2.56
500	71.95 ± 2.51	94.6 ± 2.01

Each value is expressed as Mean ± SEM

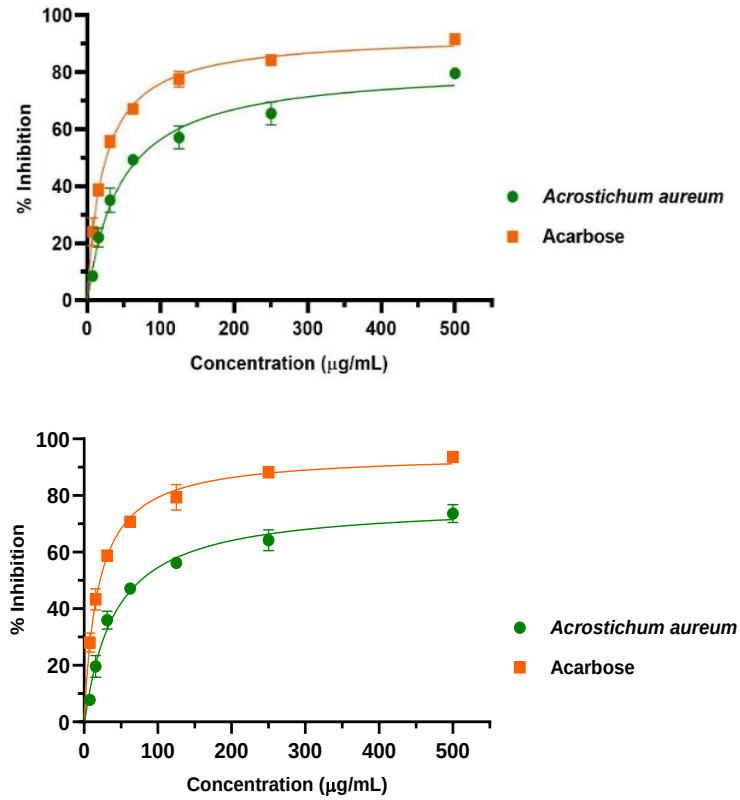


Figure 6.2 & 6.3: Percentage inhibition of alpha amylase and alpha glucosidase respectively by different concentrations of HEAA and Acarbose

6.5 Discussion

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

6.6 Conclusion

In the current study, HEAA has shown significant inhibition of alpha-amylase and alpha glucosidase suggesting its anti-diabetic potential. It has also shown significant anti-oxidant potential by DPPH radical scavenging activity.

References

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

Elya Berna, Basah Katrin, Mun'im Abdul, Yuliastuti Wulan, Bangun nastasia, Septiana Eva Kurnia. Screening of a-Glucosidase Inhibitory Activity from Some Plants of Apocynaceae Clusiaceae, Euphorbiaceae, and Rubiaceae. Journal of Biomedicine and Biotechnology Volume 2012.

GL Miller, Use of dinitrosalicylic acid reagent for determination of reducing sugar. Anal Chem. 1959:31:426-8

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

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

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

Mutha, R. E., Tatiya, A. U., & Surana, S. J. (2021). Flavonoids as natural phenolic compounds and their role in therapeutics: An overview. Future journal of pharmaceutical sciences, 7, 1-13.

Nair SS, Kavrekar V and Mishra A. Evaluation of In Vitro Anti diabetic Activity of Selected plant Extracts. International Journal of Pharmaceutical Science Invention. 2013;2(4):12-19.

R. T. Dewi, Y. M. Iskandar, M. Hanafi et al., "Inhibitory effect of Koji Aspergillus terreus on a glucosidase activity and postprandial hyperglycemia," Pakistan Journal of Biological Sciences, vol. 10, no. 18, pp. 3131-3135, 2007.

Wickramaratne M. Nirmali, Punchihewa J. C., Wickramaratne D. B. M. In-vitro alpha amylase inhibitory activity of the leaf extracts of Adenanthera pavonina. BMC Complementary and Alternative Medicine (2016) 16:466.

CHAPTER 7:
IN VIVO STUDIES

7.1 Introduction

Increased basal insulin production and hyperproinsulinemia are two symptoms of an active β-cell compensatory mechanism that accompany obesity and kidney impairment associated with impaired insulin sensitivity in patients with type 2 diabetes (Kahn SE et al., 2005). In the late stages of diabetes mellitus, these pathological problems develop early and before the β-cells drastically fail (Skovs S et al., 2014). Streptozotocin enters the pancreatic cells via a glucose transporter GLUT2 and causes alkylation of deoxyribonucleic acid (DNA), furthermore STZ induces activation of poly adenosine-diphosphate ribosylation and nitric oxide release causing destruction of pancreatic cells by necrosis (Zhang Met al., 2008). DN has been linked to dyslipidemia, which is caused by an increase in extracellular matrix synthesis and macrophage infiltration in the glomeruli of diabetes patients (Kawanami et al., 2016). Excessive ROS build up in hyperglycemia through a variety of processes, including the oxidative phosphorylation of glucose, the polyol pathway, advanced glycosylation, the mitochondrial respiratory process, and the uncoupling of NADPH oxidase (Forbes et al., 2008). Nutritional overload in long term can also lead to obesity transitioning to diabetes (Wang J et al., 2001). Problems like hyperinsulinemia, insulin resistance, dyslipidemia, inflammatory and dysfunctional adipose tissues characterizes the prediabetic state (Srinivasan K et al., 2005). DN can be mitigated by ameliorating oxidative stress, hyperglycaemia, dyslipidemia by following any of these pathways. The present chapter deals with the *in-vivo* activity of HEAA against high fat diet and low dose streptozotocin induced diabetic rats in treating DN. The following study deals with various parameters which should be checked as a consequence of diabetics like tissue antioxidant, urine parameters, serum parameters, blood glucose level, glycosylated haemoglobin and a histopathology of the affected organ.

7.2 Animals

Male Wistar Albino rats weighing 180–200 g and age between 8 to 12 weeks, obtained from State Centre for Laboratory Animal Breeding (SCLAB, Buddha Park, Kalyani, West Bengal) were used for the present study. They were given unrestricted access to food and water for seven days throughout their acclimatisation phase (07 days). They were housed in a 25°C environment with a 12-hour light/dark cycle, and they were fed a high-fat diet for 28 days. The experiment protocol was approved by Institutional Animal Ethics Committee, Department of Pharmaceutical Technology, Jadavpur University (Approval Number JU/IAEC-22/15).

7.3 Acute oral toxicity study

Acute oral toxicity study of HEAA was performed on Swiss Albino mice according to the Organization for Economic Cooperation and Development guidelines 425. It was found that the HEAA extract was safe up to a dose of 2 g/kg b.w. p.o. Based on the findings of Oral Acute Toxicity study, two dosages of HEAA were chosen for the study: 200 (1/10th) and 400 (1/5th) mg/kg of maximum tolerance dose (2000 mg/kg b.w. p.o.) (Dolai et al., 2012).

7.4 Test for oral glucose tolerance

Rats who were fasted overnight and had a normal glycemic level of 85–90 mg/dL were used for the oral glucose tolerance test (OGTT). Three groups (n = 6) of rats were used: Group I received distilled water (5 mL/kg b.w., p.o.); Groups II and III were given HEAA at dosages of 200 and 400 mg/kg b.w., p.o., respectively based on the acute toxicity study. Following these treatments, each group was given an oral dose of glucose (2 g/kg b.w.). Blood samples from tail vein was taken at 0, 30, 60, and 120 minutes intervals after oral glucose treatment. A single-touch glucometer was used to measure the blood glucose levels (Jana et al., 2024).

7.5 Diabetes induction

Rats were fed with high fat diet (HFD) for 28 days. On the 29th day, low dose of Streptozotocin (STZ) (35 mg/kg b.w.; dissolved in 0.1 M citrate buffer of pH 4.5) was injected via intraperitoneal route. Prior to it, the rats were denied water for eighteen hours in order to develop diabetes. Long term administration of HFD daily for 28 days promoted oxidative stress and mitochondrial dysfunction induced renal damage (Sun et al., 2020). The rats with fasting blood glucose levels more than 250 mg/dL were taken as diabetic rats in the study (Gayen et al., 2024).

High Fat Diet composition: The high fat diet was fed in accordance with the previously used ingredients as per exhaustive literature survey (Sun et al., 2020)

Ingredients	**Diet (g/kg)**
Powdered NPD	365
Lard	310
Casein	250
Cholesterol	10
Vitamin and mineral mix	60
dl- Methionine	03
Yeast Powder	01

7.6 Experimental design

The 28-day experimental procedure was planned. There were six rats included in each group (n = 6) after the rodents were split up into five groups.

- **Normal control group:** For 28 days, the rats were given normal saline (0.5 mL/kg, p.o.).
- **Diabetic control group:** Rats fed with High fat diet for 28 days and injected with low dose STZ (35 mg/kg, b.w.) comprised the diabetic group.
- **Test Group I (HEAA 200 mg/kg):** For 28 days, the diabetic rats received oral treatment with HEAA (200 mg/kg b.w.).
- **Test Group II (HEAA 400 mg/kg**): HEAA 400 mg/kg b.w. was given orally for 28 days.

- **Metformin treated group:** For 28 days, the diabetic rats received metformin as the reference drug (250 mg/kg, p.o.).

7.7 Serum biochemical parameters

7.7.1 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.

7.7.2 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 (ALP), Total protein, Total Bilirubin, Direct Bilirubin, Indirect Bilirubin. All the analyses were performed by using commercially available kits from Span Diagnostics Ltd. India.

SGOT and SGPT are the enzymes found mainly in heart muscle, skeletal muscle, liver cells, Kidneys. Injury to these tissues causes releases of these enzymes in blood. Elevated levels are found in myocardial infraction, cardiac operations, hepatitis, acute renal diseases and lower level found in pregnancy, diabetic keto acidosis. All the reagents are used in this assay was already prepared in kit which was ready to be used. The reagents are named as enzyme reagent and starter reagent. Clean and dry test tubes with proper label were used for this assay. Enzyme reagent (0.8 ml) mixed with 0.2 ml samples (serum collected from each animal groups free from haemolysis), and serum was incubated at room temperature for 1 minute. After that initiator reagent (0.2 ml) was added to the mixture. All the reaction mixture was mix properly and read the initial absorbance was taken at 340 nm Ao and repeated measure of the absorbance was taken in every 1, 2 & 3 minutes. Mean was calculated for absorbance change per minute, value was estimated by following calculations.

SGOT activity in U/L 25°C/ 30°C = A A/min x 952

Serum ALP is an important determinant of inflammatory condition of body. Pathological conditions like liver disease, parathyroidism, increased blood sugar level causes increase level of ALP in serum. In this study serum ALP is estimated by commercially available kit. Buffer and Substrate liquid reagents were supplied in kit, ready-to-use. Working Reagent was prepared in the ratio of 5 parts Buffer (R1) to 1 part Substrate (R2) (l.e., 25 ml. Buffer and 5 mL Substrate). Serum was separated from each animal group, where tube and warm to 37°C for 3 minutes. In sample tube 20 µl. (0.020 mL) of serum was added and mixed gently. After that absorbance was taken at 405nm against distill water. At first absorbance was recorded for 1 minute and incubated at 37°C and repeated absorbance was measured up to 3 minutes. Reaction rate remain constant.

Values are derived based on the "absorptivity micromolar extinction coefficient" of 4-nitrophenol at 405 nm (0.01845). Units per liter (U/L) of Alkaline Phosphatase activity is that amount of enzyme which products one mmol/L, of 4-nitrophenol per minute.

U/L=AA/Min/ Absorptivity x Total Volume/Sample volume

U/L=AA/Min/0.01845 x 1.020/0.020

U/LA/Min x 2764

Proteins are the constitute of muscle, enzymes, hormones and other structural and functional entities of the body. Main plasma proteins like albumin and globulin fractions vary widely depend upon various disease condition. In this study, plasma protein level was estimated by using kit. Biuret reagent, which was provided in kit was used for the detection of serum protein. Serum was collected from each group, where n= 6. Three test tubes were labelled as blank, test and standard, where blank contains only biuret reagent and distill water, standard tube contains biuret reagent and protein standard and samples tube contains serum sample and biuret reagent. Samples of each tube were mixed properly and followed by incubation at 37°C for 10min. Absorbance was measured for test and standard tubes at 550 nm against blank within 60 min.

Total proteins (g / d * l) = Abs Test/Abs. Sample x 8

7.7.3 Serum lipid profiles

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

Total cholesterol, triglycerides, high density lipoproteins are the main lipids found in serum. Certain pathophysiological conditions, such as hyperlipidaemia, hypothyroidism, uncontrolled hypercholesterolaemia, diabetes, nephrotic syndrome and cirrhosis result increased levels of serum lipid. Malabsorption, malnutrition, hyperthyroidism, anemias and serum diseases are the causes of lower level of serum lipid. Cholesterol kit uses CHOD / PAP method to determine cholesterol activity in serum or plasma All the reagents were ready to use.

For HDL cholesterol determination three tubes were prepared and labelled as blank, sample and test. Blank tubes constituted with working reagent and distilled water, standard tubes constituted with working reagent and HDL standard and test tubes constituted with serum sample and working reagent. All the contents were mixed property and incubated at 37 deg. C for 5 min, followed by measuring the absorbance at 505nm against blank.

HDL Cholesterol in (mg /d * l) = Abs Of test Abs. Of standard * 25 * 2 Cholesterol in mg / d * l = Abs Of test / Abs. Of standard x 200

For estimation of triglycerides in serum sample, procedure was same, only standard tubes contained standard triglyceride with working reagent,

Triglycerides in (mg /d* l) = Abs Of test/ Abs. Of Standard x 200

7.7.4 Urine parameters test

The urine of each rat was collected after placing them in their respective metabolic cages. Using commercial diagnostic kits, the following parameters were measured: urine output, urine microalbumin, urine creatinine clearance.

7.8 Tissue antioxidant study

7.8.1 Lipid peroxidation level (TBARS)

Degree of lipid peroxidation in tissue homogenate was determined in terms of thiobarbituric active reactive substances (TBARS) formation (Borgohain et al., 2017). 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 on a water bath until a stable pink colour 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 malondialdehyde (MDA), TBARS concentrations of the samples were calculated using the extinction coefficient of MDA ($1.56 \times 105\ M^{21}cm^{21}$).

7.8.2 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 minutes. 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.

7.8.3 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 tris buffer (pl 8.9) followed by DTNB (0.01 M) were added and the absorbance was measured at 412 mm 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.

7.9 Histopathological investigation

The isolated rat kidneys, pancreas and liver was cleaned in regular saline water and preserved in 10% formalin solution for histology. Hematoxylin-Eosin was used to make

and stain kidney, pancreas and liver tissues were 5 µm thick. The stained samples were then seen under a light microscope at a 20× magnification.

7.10 Statistical analysis

All values were expressed as mean ± standard error of mean (SEM). One-way analysis of variance (ANOVA) was used for analyzing the data, $p<0.05$ was considered as statistically significant.

7.11 Results

7.11.1 Test for oral glucose tolerance

The OGTT was conducted on rats with normal blood sugar levels (85-90 mg/dl). Blood glucose levels in normal rats surged for the first 30 minutes after glucose administration, then steadily declined for 60 minutes until returning to normal

HEAA-treated groups showed substantial renal, pancreatic and hepatic cell protection, according to the histopathology studies. When compared to diabetic rats, HEAA treated groups showed a dose dependent progressive improvement in pancreatic cell density.

Table 1: Effect of HEAA on Oral Glucose Tolerance test

Groups	**0 min**	**30 mins**	**60 mins**	**120 mins**
Normal control group	84 ±1.590	135.33 ±1.786	121 ±1.447	110.21±1.998
Test Group I (HEAA 200 mg/kg)	90 ±1.789	139 ±1.908	117 ±1.585	112.6±2.887
Test Group II (HEAA 400 mg/kg)	86.33 ±1.987	133.33 ±2.776	126 ±1.879	110 ±1.543
Metformin treated group	81.66 ±2.098	140 ±3.112	115.34 ± 2.778	108.33 ± 1.994

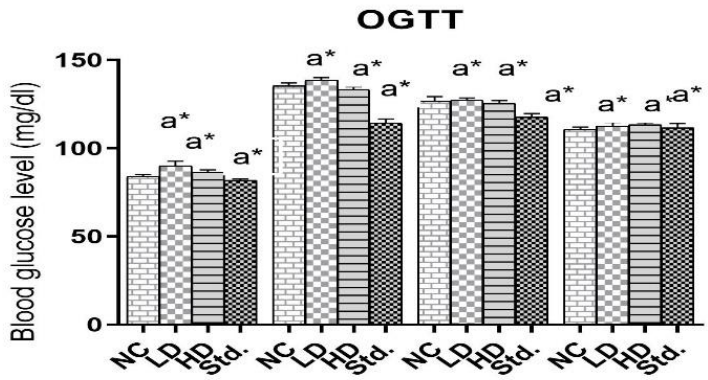


Figure 7.1 : Effect of HEAA on OGTT. Each value is expressed as Mean ± SEM where n=6. a* p<0.05 when compared to Normal Control .NC= Normal Control, DC= Diabetic Control, LD= Test Group I (HEAA 200 mg/kg), HD = Test Group II (HEAA 400 mg/kg), STD.= Metformin treated group

7.11.2 Effect of HEAA on fasting blood glucose (FBG) levels

The level of Fasting Blood Glucose (FBG) in HFD-STZ induced diabetic rats was found to be considerably (P< 0.05) higher than in the normal control group. When diabetic rats were given HEAA at doses of 200 and 400 mg/kg for 28 days, the level was found to be significantly lower (P<0.05) than in the diabetic control group [Table 2].

Table 2: Effect of HEAA on fasting blood glucose (FBG) levels of rats

Groups	Day 0	Day 7	Day 14	Day 21	Day 28
Normal control group	92.66 ± 2.09	95.33± 1.56	86± 2.98	86.66± 1.67	87± 2.45
Diabetic control group	327.33± 3.21	374.33± 2.34	438± 3.67	430.66± 3.21	459.33±1.98
Test Group I (HEAA 200 mg/kg)	358.66± 3.16	340±4.12	272.33± 2.87	215±2.77	119.33± 2.87
Test Group II (HEAA 400 mg/kg)	237.66± 2.78	195.66± 2.16	152.33± 3.01	113± 3.99	104± 1.22
Metformin	300.12±	158.66±	133± 3.21	110.34±4.09	87.33± 3.09

treated group	3.77	4.02			

*Each value are expressed as mean ±SEM (n=6), a*Diabetic control group versus normal control group (P<0.05), b*All treated group versus diabetic control group on corresponding day (P<0.05), *Values significantly differ from each other where P<0.05. SEM: Standard error of mean, HEAA: Hydroalcoholic extract of *Acrostichum aureum* ,

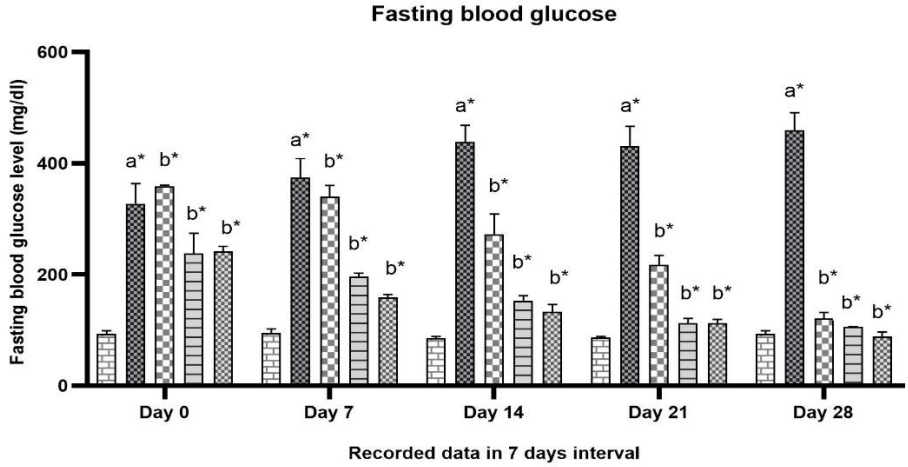


Figure 7.2 : Effect of HEAA on Fasting Blood Glucose. Each value is expressed as Mean ± SEM where n=6. A* p<0.05 when compared to Normal Control and b* p<0.05 when compared to disease control. NC= Normal Control, DC= Diabetic Control, LD= Test Group I (HEAA 200 mg/kg), HD = Test Group II (HEAA 400 mg/kg), STD.= Metformin treated group

7.11.3 Estimation of HEAA on the body weights

The body weights of the diabetes control group were statistically substantially (P<0.05) lower than that of the normal control group. When compared to the diabetic control group, HEAA administration at doses of 200 and 400 mg/kg significantly improved body weight (P<0.05). which has been shown in Table 3.

Table 3: Effect of HEAA on Body weight

Groups	Day 0	Day 7	Day 14	Day 21	Day 28
Normal control group	195 ± 3.60	206 ± 3.17	209.66± 4.50	221 ± 2.84	236.12±4.80
Diabetic control group	314.66 ±3.05	308.66± 4.66	301 ± 3.45	294 ± 2.84	256 ± 3.21
Test Group I (HEAA 200 mg/kg)	295 ± 1.85	291.33± 4.35	289.33± 4.81	291.33± 1.86	291 ± 4.38

Test Group II (HEAA 400 mg/kg)	304 ± 2.72	299.33± 3.12	297.33± 4.17	300 ± 1.73	302 ± 3.98
Metformin treated group	308.33± 4.09	306 ± 2.01	309 ± 5.17	311 ± 1.76	313.32±4.80

*Each value is expressed as mean ±SEM (n=6), a*Diabetic control group versus normal control group (P<0.05), b*All treated group versus diabetic control group on corresponding day (P<0.05), *Values significantly differ from each other where P<0.05. SEM: Standard error of mean, HEAA: Hydroalcoholic extract of *Acrostichum aureum*,

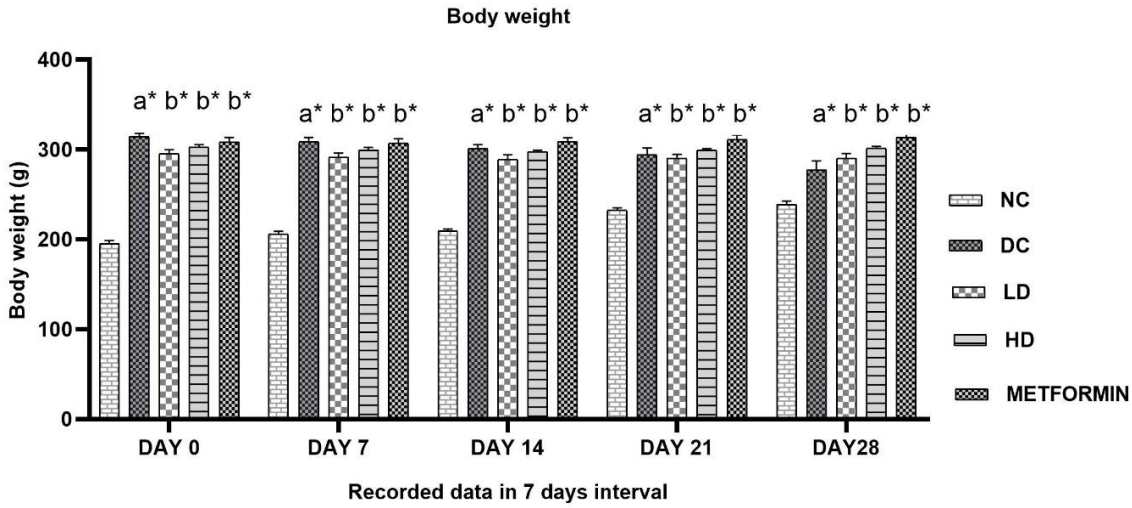


Fig 7.3: Effect of HEAA on body weight. Each value is expressed as Mean ± SEM where n=6. A* p<0.05 when compared to Normal Control and b* p<0.05 when compared to disease control. NC= Normal Control, DC= Diabetic Control, LD= Test Group I (HEAA 200 mg/kg), HD = Test Group II (HEAA 400 mg/kg), STD.= Metformin treated group

7.11.4 Estimation of Glycosylated Haemoglobin

Glycosylated haemoglobin level in HFD and STZ-induced diabetic rats Was significantly ($p < 0.001$) increased compared to normal control group. Treatment with HEAA at the doses of 200 and 400 mg/kg significantly ($p < 0.05$) reduced the HbA1c level when compared to the diabetic control group (Table 4).

Table 4: Effect of HEAA on HbA1c

Groups	HbA1c (%)
Normal control group	5.56 ± 0.052
Diabetic control group	9.54 ± 0.031
Test Group I (HEAA 200 mg/kg)	7.12 ± 0.025

Test Group II (HEAA 400 mg/kg)	6.31 ± 0.055
Metformin treated group	5.82 ± 0.041

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

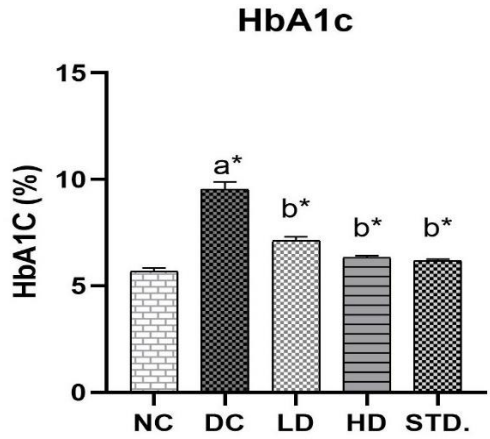


Fig 7.4: Effect of HEAA on Glycated Haemoglobin level. Each value is expressed as Mean ± SEM where n=6. A* p<0.05 when compared to Normal Control and b* p<0.05 when compared to disease control. NC= Normal Control, DC= Diabetic Control, LD= Test Group I (HEAA 200 mg/kg), HD = Test Group II (HEAA 400 mg/kg), STD.= Metformin treated group

7.11.5 Estimation of serum liver function test parameters

Biochemical parameters like SGOT, SGPT, ALP, Total Protein, Total Bilirubin in HFD and 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 HEAA at the doses of 200 and 400 mg/kg significantly ($p < 0.05$) reduced the SGOT, SGPT, ALP, Total Protein, Total Bilirubin levels and significantly ($p < 0.05$) increased the total protein level as compared to the diabetic control group (Table 5).

Groups	**SGOT (IU/L)**	**SGPT (IU/L)**	**ALP (IU/L)**	**Total Protein (g/dl)**	**Total Bilirubin (mg/dl)**
Normal control group	84.62 ± 1.66	49.92 ± 1.59	38.19 ± 0.94	9.6 ± 0.30	0.64 ± 0.07

Diabetic control group	245.45± 1.22	126.28 ±2.87	88.54 ± 1.68	4.47 ± 0.25	3.51 ± 0.27
Test Group I (HEAA 200 mg/kg)	141.18 ±3.01	90.37 ± 1.29	66.13 ± 1.69	6.5 ± 0.23	2.10 ± 0.07
Test Group II (HEAA 400 mg/kg)	107.12 ±4.32	58.36 ± 1.99	47.06 ± 2.10	7.05 ± 0.10	1.28 ± 0.04
Metformin treated group	90.96 ± 1.59	52.21± 1.68	40.75 ± 1.04	8.12 ± 0.09	1.04 ± 0.03

*Each value are expressed as mean ± SEM (n=6), a*Diabetic control group versus normal control group (P<0.05), b*All treated group versus diabetic control group on corresponding day (P<0.05), *Values significantly differ from each other where P<0.05. SEM: Standard error of mean, HEAA: Hydroalcoholic extract of *Acrostichum aureum*

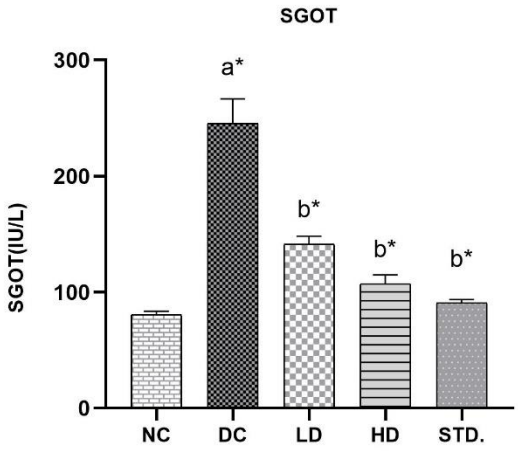


Fig 7.5: Effect of HEAA on SGOT. Each value is expressed as Mean ± SEM where n=6. A* p<0.05 when compared to Normal Control and b* p<0.05 when compared to disease control. NC= Normal Control, DC= Diabetic Control, LD= Test Group I (HEAA 200 mg/kg), HD = Test Group II (HEAA 400 mg/kg), STD.= Metformin treated

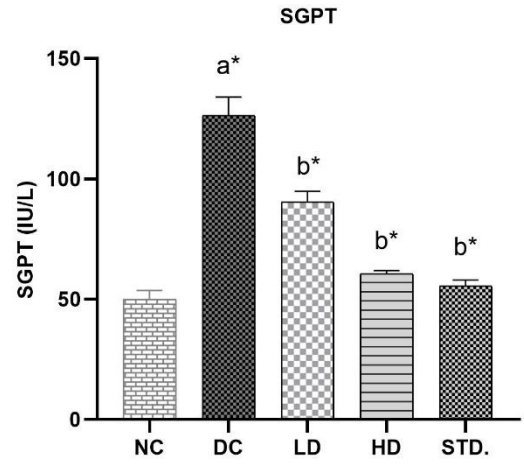


Fig 7.6: Effect of HEAA on SGPT. Each value is expressed as Mean ± SEM where n=6. A* p<0.05 when compared to Normal Control and b* p<0.05 when compared to disease control. NC= Normal Control, DC= Diabetic Control, LD= Test Group I (HEAA 200 mg/kg), HD = Test Group II (HEAA 400 mg/kg), STD.= Metformin treated group

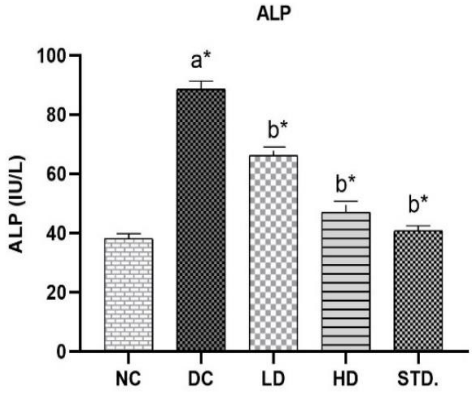


Fig 7.7: Effect of HEAA on ALP (Alkaline Phosphatase). Each value is expressed as Mean ± SEM where n=6. A* p<0.05 when compared to Normal Control and b* p<0.05 when compared to disease control. NC= Normal Control, DC= Diabetic Control, LD= Test Group I (HEAA 200 mg/kg), HD = Test Group II (HEAA 400 mg/kg), STD.= Metformin treated group

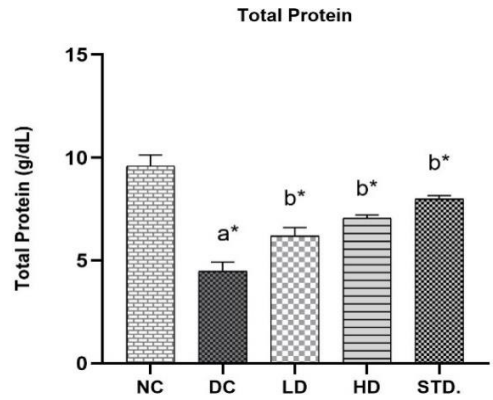


Fig 7.8: Effect of HEAA on Total Protein. Each value is expressed as Mean ± SEM where n=6. A* p<0.05 when compared to Normal Control and b* p<0.05 when compared to disease control. NC= Normal Control, DC= Diabetic Control, LD= Test Group I (HEAA 200 mg/kg), HD = Test Group II (HEAA 400 mg/kg), STD.= Metformin treated group

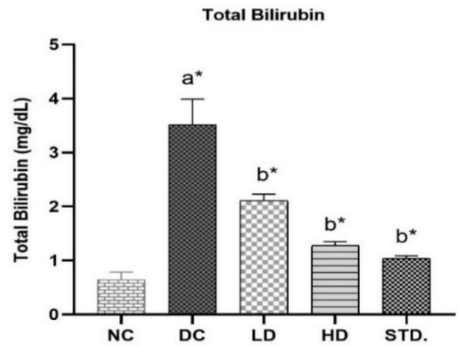


Fig 7.9 : Effect of HEAA on Total Bilirubin. Each value is expressed as Mean ± SEM where n=6. A* p<0.05 when compared to Normal Control and b* p<0.05 when compared to disease control. NC= Normal Control, DC= Diabetic Control, LD= Test Group I (HEAA 200 mg/kg), HD = Test Group II (HEAA 400 mg/kg), STD.= Metformin treated group

7.11.6 Estimation of serum lipid profiles

Serum lipid profiles like total cholesterol, triglyceride & HDL cholesterol 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 HEAA at the doses of 200 and 400 mg/kg significantly ($p < 0.05$) reduced the total cholesterol, triglyceride level and significantly ($p<0.05$) increased the HDL level when compared to the diabetic control group (Table 6)

Groups	Total Cholesterol (mg/dl)	HDL Cholesterol (mg/dl)	Triglycerides (mg/dl)
Normal control group	35.71± 0.84	75.23 ± 2.02	55.68 ± 0.87
Diabetic control group	114.28 ± 0.57	31.31 ± 2.33	314.28 ± 0.58
Test Group I (HEAA 200 mg/kg)	53.52 ± 1.37	42.99 ± 1.62	103.53 ± 1.37
Test Group II (HEAA 400 mg/kg)	46.66 ± 0.78	53.30 ± 2.02	84.66 ± 0.78
Metformin treated group	36.03 ± 2.57	63.96 ± 2.31	70.03 ± 0.64

*Each value are expressed as mean±SEM (n=6), a*Diabetic control group versus normal control group (P<0.05), b*All treated group versus diabetic control group on corresponding day (P<0.05), *Values significantly differ from each other where P<0.05. SEM: Standard error of mean, HEAA: Hydroalcoholic extract of *Acrostichum aureum*

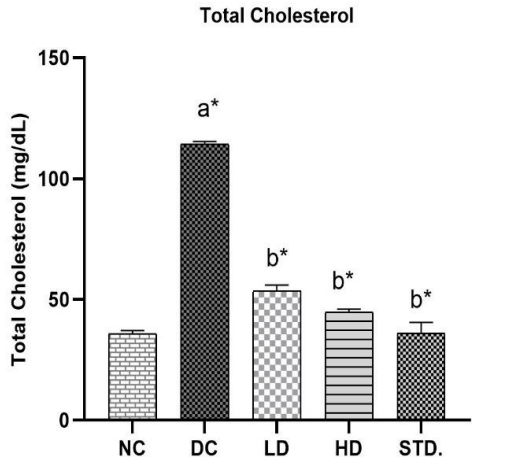


Fig 7.10: Effect of HEAA on Total Cholesterol. Each value is expressed as Mean ± SEM where n=6. A* p<0.05 when compared to Normal Control and b* p<0.05 when compared to disease control. NC= Normal Control, DC= Diabetic Control, LD= Test Group I (HEAA 200 mg/kg), HD = Test Group II (HEAA 400 mg/kg), STD.= Metfomin treated group

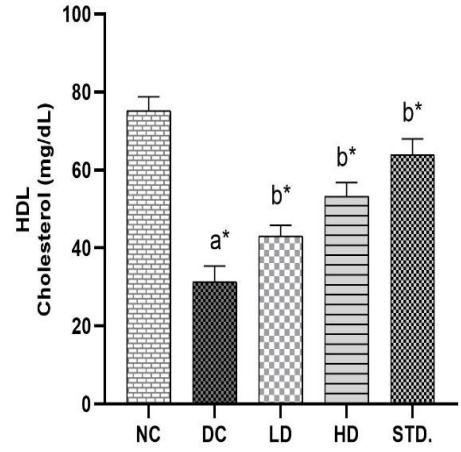


Fig 7.11: Effect of HEAA on HDL Cholesterol. Each value is expressed as Mean ± SEM where n=6. A* p<0.05 when compared to Normal Control and b* p<0.05 when compared to disease control. NC= Normal Control, DC= Diabetic Control, LD= Test Group I (HEAA 200 mg/kg), HD = Test Group II (HEAA 400 mg/kg), STD.= Metformin treated group

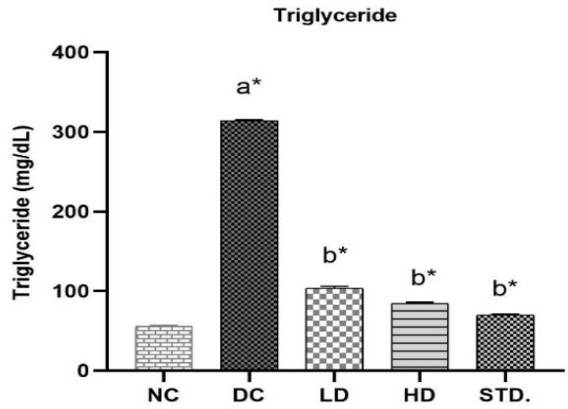


Fig 7.12: Effect of HEAA on Serum Triglyceride. Each value is expressed as Mean ± SEM where n=6. A* p<0.05 when compared to Normal Control and b* p<0.05 when compared to disease control. NC= Normal Control, DC= Diabetic Control, LD= Test Group I (HEAA 200 mg/kg), HD = Test Group II (HEAA 400 mg/kg), STD.= Metformin treated group

7.11.7 Estimation of serum kidney function test parameters

Biochemical parameters like Creatinine, Urea, Uric acid in HFD-STZ induced diabetic rats were significantly ($p < 0.05$) elevated as compared to the normal control group. Treatment with HEAA at the doses of 200 and 400 mg/kg significantly ($p < 0.05$) reduced the Creatinine, Urea, Uric acid levels (Table 7)

Groups	**Serum Creatinine**	**Blood Urea Nitrogen (BUN)**	**Uric acid**
Normal control group	0.68 ± 0.017	19.46 ± 0.73	1.73 ± 0.08
Diabetic control group	1.91 ± 0.03	50.01 ± 1.30	6.15 ± 0.10
Test Group I (HEAA 200 mg/kg)	1.68 ± 0.02	35.84 ± 1.56	3.2 ± 0.09
Test Group II (HEAA 400 mg/kg)	1.01 ± 0.01	26.65 ± 1.15	1.89 ± 0.04

Metformin treated group	0.93 ± 0.03	21 ± 1.15	1.69 ± 0.09

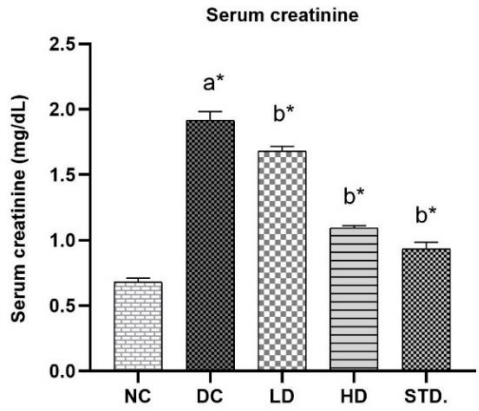


Fig 7.13: Effect of HEAA on Serum Creatinine. Each value is expressed as Mean ± SEM where n=6. A* p<0.05 when compared to Normal Control and b* p<0.05 when compared to disease control. NC= Normal Control, DC= Diabetic Control, LD= Test Group I (HEAA 200 mg/kg), HD = Test Group II (HEAA 400 mg/kg), STD.= Metformin treated group

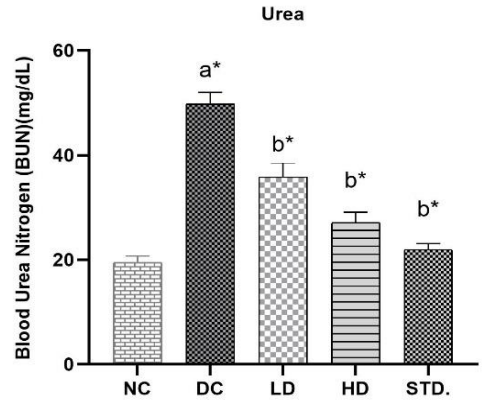


Fig 7.14: Effect of HEAA on Blood Urea Nitrogen. Each value is expressed as Mean ± SEM where n=6. A* p<0.05 when compared to Normal Control and b* p<0.05 when compared to disease control. NC= Normal Control, DC= Diabetic Control, LD= Test Group I (HEAA 200 mg/kg), HD = Test Group II (HEAA 400 mg/kg), STD.= Metformin

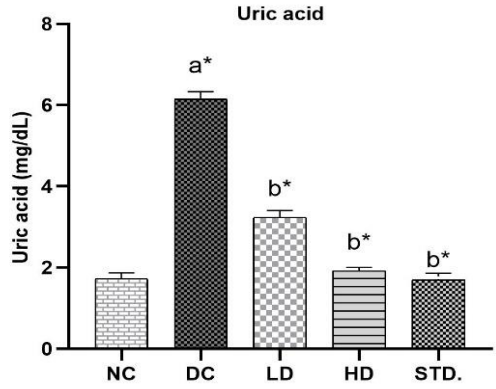


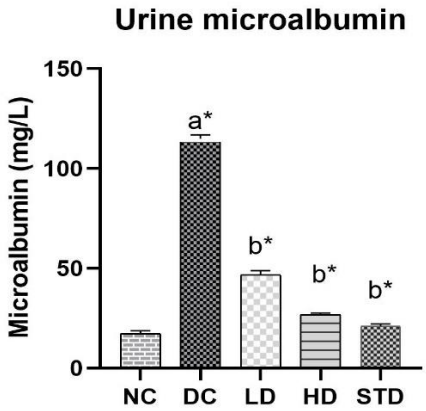
Fig 7.15: Effect of HEAA on Serum Uric Acid. Each value is expressed as Mean ± SEM where n=6. A* p<0.05 when compared to Normal Control and b* p<0.05 when compared to disease control. NC= Normal Control, DC= Diabetic Control, LD= Test Group I (HEAA 200 mg/kg), HD = Test Group II (HEAA 400 mg/kg), STD.= Metformin treated group

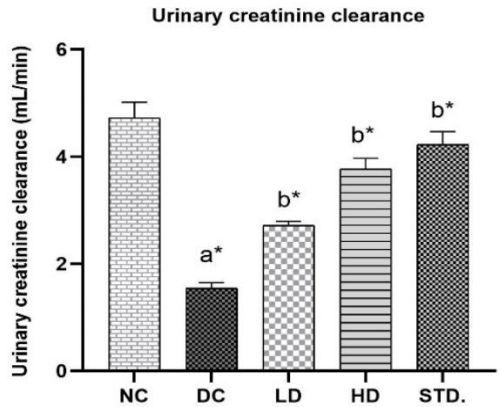
7.11.8 Estimation of urine function test parameters

Urine parameters like Microalbumin, Urine volume, Creatinine clearance in HFD-STZ-induced diabetic rats were significantly ($p < 0.05$) elevated as compared to the normal control group. Treatment with HEAA at the doses of 200 a nd 400 mg/kg significantly ($p < 0.05$) reduced the Microalbumin, Urine volume, creatinine clearance levels (Table 8).

Table 8: Effect of HEAA on Urine microalbumin and Urinary creatinine clearance

Groups	Urine Microalbumin	Urinary Creatinine Clearance
Normal control group	19.36 ± 2.16	4.8 ± 0.01
Diabetic control group	112.41 ± 2.02	1.50 ± 1.02
Test Group I (HEAA 200 mg/kg)	46.32 ± 0.02	2.60 ± 2.31
Test Group II (HEAA 400 mg/kg)	31.34 ± 3.02	3.70 ± 3.12
Metformin treated group	24.01 ± 2.11	4.21 ± 0.016





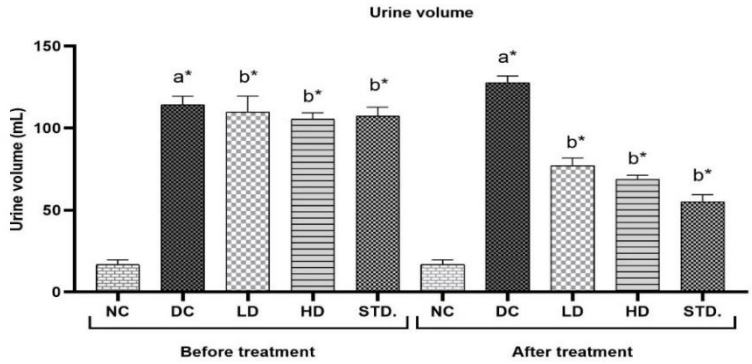


Fig 7.16: Effects of different concentrations of HEAA on Urine Microalbumin, Urinary Creatinine clearance, Urine volume levels of kidney and liver. Values are represented as Mean ± SEM, where n=b6. A*p<0.05 when compared to normal control, b* when compared to diabetic control p<0.05.

7.11.9 Estimation of tissue antioxidant parameter

Lipid peroxidation results in the formation of ROS species and subsequently elevates the level of malondialdehyde (MDA) in liver tissue of STZ-induced diabetic rats. In the present study the MDA level was significantly ($p <$ increased in HFD/STZ-induced diabetic rats compared to normal control group. Interestingly, treatment with HEAA at the doses of 200 and 400 mg/kg significantly ($p < 0.05$) reduced the MDA levels compared to diabetic control group. The levels of reduced GSH and SOD were significantly ($p <0.05$) decreased in HFD/STZ-induced diabetic rats compared to normal control group. Administration of HEAA at the doses of 200 and 400 mg/kg significantly ($p < 0.05$) increased GSH, SOD antioxidant enzyme levels in the liver of HFD/STZ- induced diabetic rats compared to the diabetic control

Table 9: Effect of HEAA on LPO

Groups	**LPO (µM/100gm Tissue Homogenate) Kidney**	**LPO (µM/100gm Tissue Homogenate) Liver**
Normal control group	1.34 ± 0.17	1.27 ± 0.14
Diabetic control group	3.85 ± 0.09	3.30 ± 0.13

Test Group I (HEAA 200 mg/kg)	2.1 ± 0.06	2.23 ± 0.10
Test Group II (HEAA 400 mg/kg)	1.73 ± 0.10	1.64 ± 0.12
Metformin treated group	1.49 ± 0.13	1.5 ± 0.05

Values are represented as Mean ± SEM, where n=b6. A*p<0.05 when compared to normal control, b* when compared to diabetic control p<0.05.

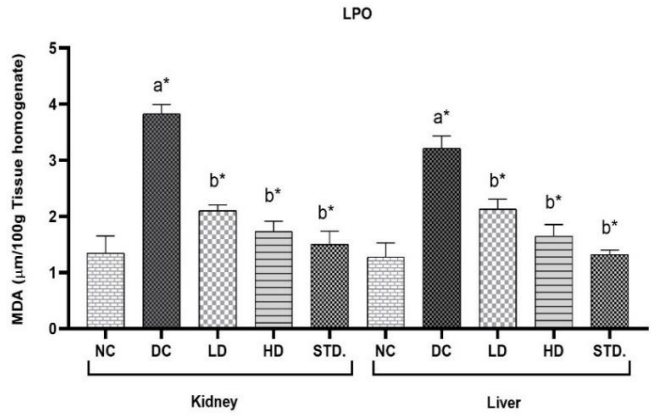


Fig 7.17: Effects of different concentrations of HEAA on LPO levels of kidney and liver. Values are represented as Mean ± SEM, where n=b6. A*p<0.05 when compared to normal control, b* when compared to diabetic control p<0.05.

Table 10: Effect of HEAA on SOD

Groups	**SOD Kidney (U/mg)**	**SOD Liver (U/mg)**
Normal control group	3.73 ± 0.08	2.82 ± 0.09
Diabetic control group	1.01 ± 0.02	0.86 ± 0.06
Test Group I (HEAA 200 mg/kg)	1.67 ± 0.07	1.88 ± 0.07

Test Group II (HEAA 400 mg/kg)	2.65 ± 0.11	2.23 ± 0.06
Metformin treated group	3.06 ± 0.07	3.00 ± 0.05

Values are represented as Mean ± SEM, where n=b6. A*p<0.05 when compared to normal control, b* when compared to diabetic control p<0.05.

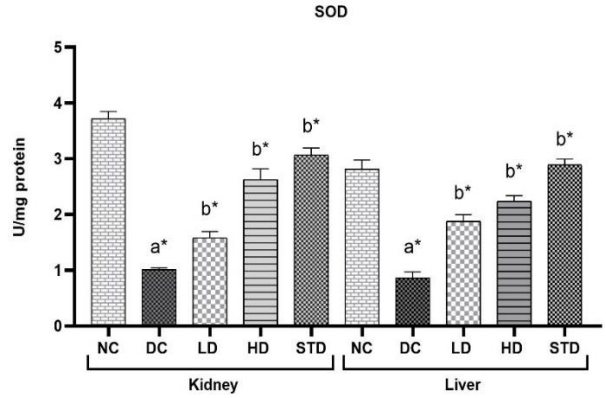


Fig 7.18; Effects of different concentrations of HEAA on SOD of kidney and liver. Values are represented as Mean ± SEM, where n=b6. A*p<0.05 when compared to normal control, b* when compared to diabetic control p<0.05.

Table 11: Effect of HEAA on GSH

Groups	GSH Kidney (µg/mg tissue)	GSH Liver (µg/mg tissue)
Normal control group	3.45 ± 0.17	3.34 ± 0.19
Diabetic control group	1.47 ± 0.16	1.39 ± 0.11
Test Group I (HEAA 200 mg/kg)	2.33 ± 0.19	2.2 ± 0.20
Test Group II (HEAA 400 mg/kg)	3.00 ± 0.12	2.90 ± 0.15
Metformin treated group	3.35 ± 0.21	3.25 ± 0.14

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

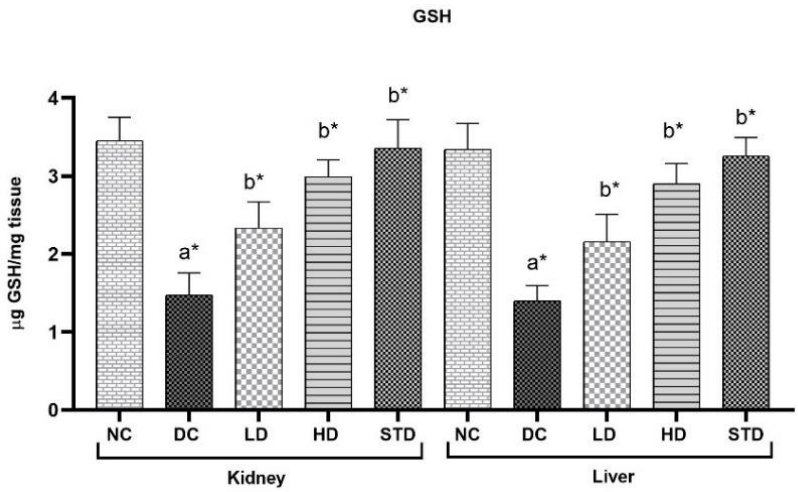
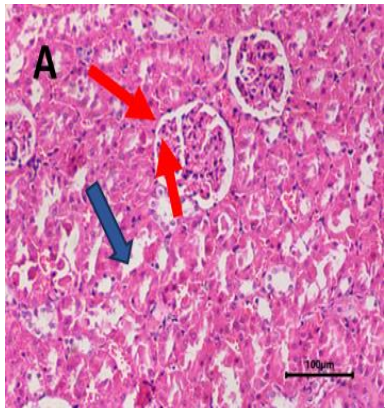
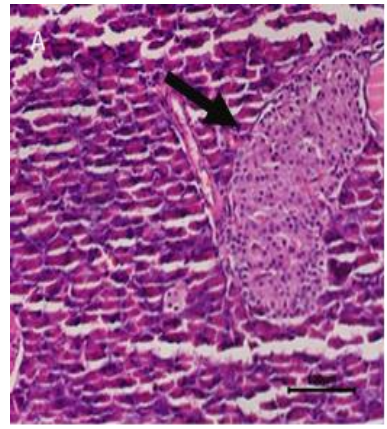


Fig 7.19; Effects of different concentrations of HEAA on GSH levels of kidney and liver. Values are represented as Mean ± SEM, where n=b6. A*p<0.05 when compared to normal control, b* when compared to diabetic control p<0.05

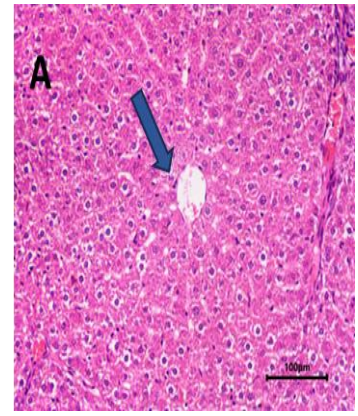
7.11.10 Histopathology of kidney, pancreas and liver tissues



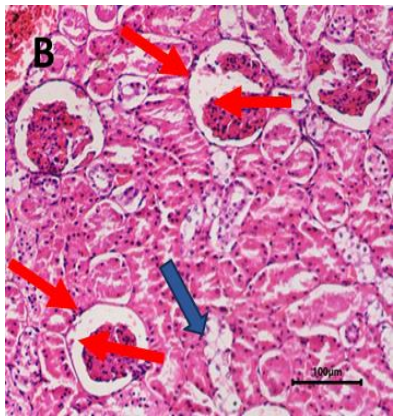
A- Normal Control Kidney



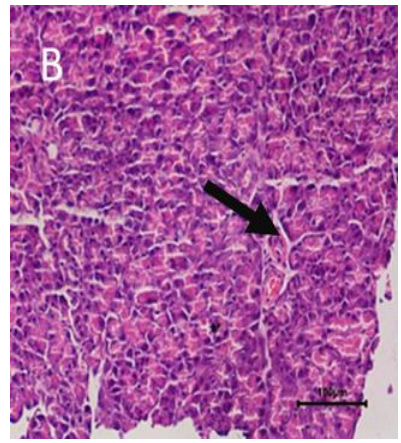
A - Normal Control Pancreas



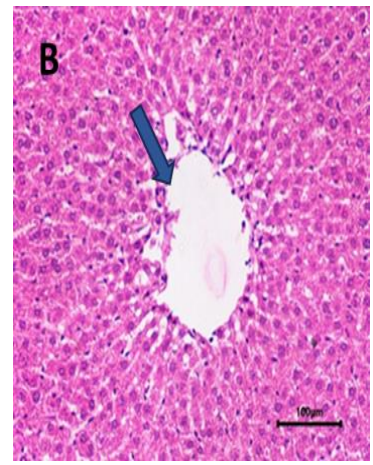
C- Normal Control Liver



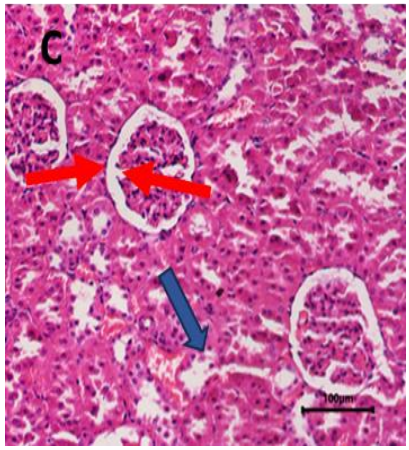
B- Disease Control Kidney



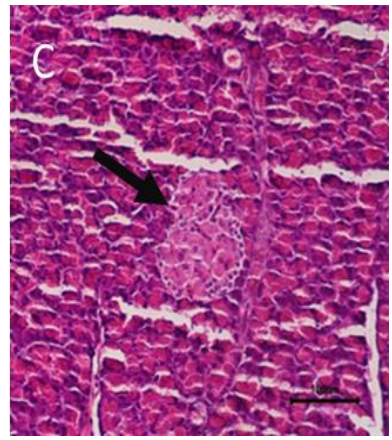
B- Disease Control Pancreas



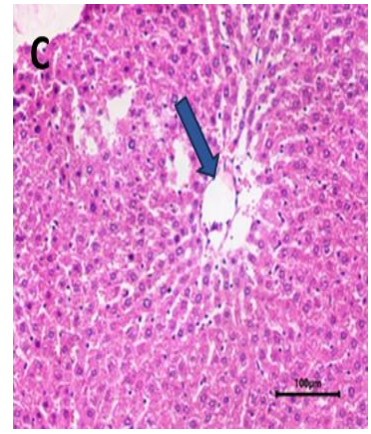
B- Diabetic Control liver



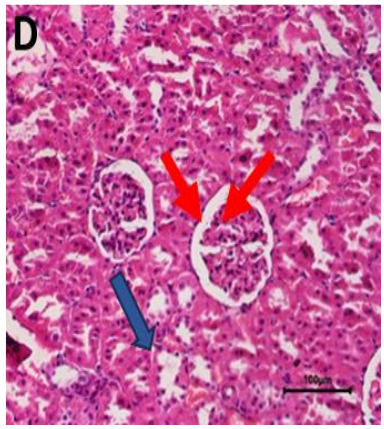
C - HEAA (200 mg/kg) treated Kidney



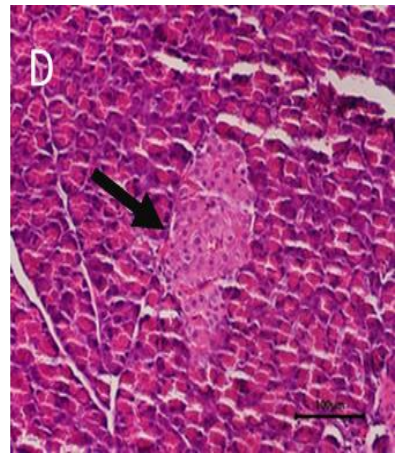
C - HEAA (200 mg/kg) treated Pancreas



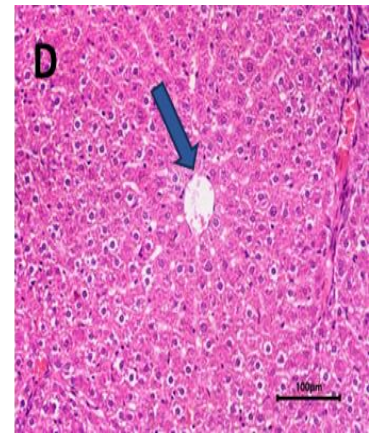
C- HEAA (200 mg/kg) treated liver



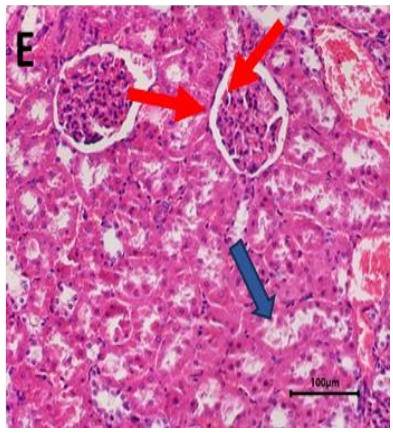
D - HEAA (400 mg/kg) treated Kidney



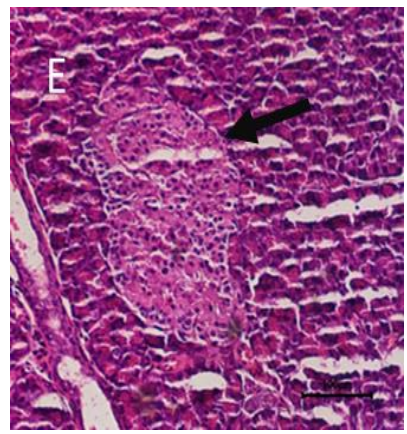
D - HEAA (400 mg/kg) treated Pancreas



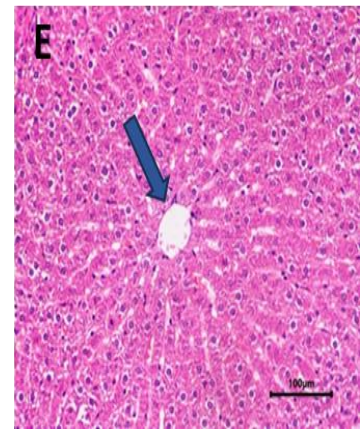
D -HEAA (400 mg/kg) treated Liver



E - Metformin treated Kidney



E- Metformin treated Pancreas



E-Metformin treated liver

Effect of HEAA on kidney histopathology (HE stain). A: normal control group, B: untreated HFD- STZ, C: Test group I (HEAA 200 mg/kg), D: Test group II (400 mg/kg HEAA) E: Standard treated group (treated with metformin). Mesangial matrix expansion in the glomerulus (red arrow) and renal tubule dilatation (Blue arrow) have been marked in the images. HFD: High fat diet; STZ: Streptozotocin; HE stain: Hematoxylin Eosin stain. *

Effect of HEAA on histopathology of pancreas (HE stain) A: Normal control, B: Disease Control (HFD+ STZ); there is hydropic degeneration and necrosis (arrows) of cells of islets of Langerhans., C: Test group I (200 mg/kg HEAA), slight hydropic degeneration and a few necrotic cells of islets of Langerhans, D: Test group II (400 mg/kg HEAA); Almost recovered degenerated cells of islets of Langerhans E: Standard group (250 mg/kg Metformin treated); Almost normal histopathology of the islets of Langerhans. HFD: High fat diet; STZ: Streptozotocin; HE stain: Hematoxylin Eosin stain

Hematoxylin and eosin-stained sections of liver. A: Normal Control group: Normal histological appearance. B: Diabetic group: Disseminated vacuolization (arrows) in the hepatocytes and dilation of sinusoids C: HEAA (200 mg/kg) treated Liver: Slight hydropic

degeneration in some hepatocytes (arrows) and dilation of sinusoids (star). D: HEAA (400 mg/kg) treated Liver: Almost normal histological appearance of the liver. E: Metformin treated group: rat administered with acarbose showing slight hydropic degeneration (arrows) and dilation of sinusoids.

7.11.11 Discussion

The present study has potrayed the antidiabetic, and antioxidant activities of methanol extract of *Acrostichum aureum* aerial parts (HEAA) in STZ and HFD induced diabetic rats.

Type 2 diabetes oral hypoglycemic treated with a combination of diet restriction, exercise, and oral hypoglycemics. These drugs can control hyperglycaemia and glycosuria, but they also raise cardiovascular difficulties and cannot avoid the macrovascular and microvascular complications that come with them in the long term (Chakraborty M et al., 2018). Medicinal plants and their natural compounds have always been important in drug development, serving as a pivot for early treatments. (Biswas M et al. 2018). This has become a landmark in the search for alternate treatments to conventional or folk medicine for this ailment. HEAA was proven to be safe orally at a dose of 2000 mg/kg in an acute toxicity investigation. The digestive endoenzymes alpha amylase and alpha glucosidase are responsible for the of starch and disaccharides to glucose and the breakdown of long-chain carbohydrates. These enzyme inhibitors are being investigated as potential targets in the treatment of diabetes mellitus. (Tiwari N et al., 2014). HEAA has in vitro alpha amylase and alpha glucosidase inhibitory activity, and the results imply that HEAA can limit monosaccharide absorption, which helps manage blood sugar levels. The OGTT was used to assess the rate of glucose elimination from the blood after consuming glucose. It's a crucial metric for determining diabetes and insulin resistance (Kumar A et al., 2011). When compared to the normal control group, HEAA was found to have a considerable glucose tolerance impact. When rats were given HEAA at oral doses of 200 mg/kg and 400 mg/kg b.w. each day for 28 days, the FBG level was considerably lower than in diabetic control rats, indicating antidiabetic efficacy. The concentration of HbA1c in the blood is an important diagnostic measure for determining diabetic status and glycemia. A HbA1c level of 6.5 percent is considered a high-risk diabetic state, and it is proportional to FBG content. In diabetic rats, HEAA dramatically lowered HbA1c levels. The level of free fatty acids in the systemic circulation is higher in diabetes patients. The circulating free fatty acids have deleterious effect on the endothelial functions by various pathways and mechanisms which include free radical generation and protein kinase C activation, and thus aggravate dyslipidemia (Goldberg U et al., 2001). Dyslipidemia is defined as a high plasma level of triglycerides, low plasma level of HDL cholesterol, and a low plasma level of cholesterol Due to little or no scavenging of these extra free radicals in diabetes patients, the generation of free radicals (reactive oxygen and nitrogen species) increases, resulting in increased oxidative stress (Matough FA et al., 2012). Several micro and macrovascular problems of diabetes are caused by oxidative stress. When compared to diabetes management, HEAA has a considerable potential to lower lipids (triglycerides and total

cholesterol). HDL cholesterol, which is considered good cholesterol, increased considerably in HEAA-treated groups.

Endogenous antioxidant mechanisms become less effective in diabetics to scavenge overproduced free radicals, resulting in a variety of pathophysiological alterations in various organs. Because the liver and kidney are key organs for metabolite storage, detoxification, metabolism, and excretion, they are particularly prone to oxidative injury. Oxidative stress, which involves the secretion of cytokines such as tumour necrosis factor, interleukin 1, and interferon c, is the cause of liver and kidney tissue deterioration (Aboonabi A et al., 2014). Hepatotoxicity causes a rise in liver function enzymes such as SGOT, SGPT, and SALP. The serum hepatic marker levels in the diabetic group were considerably higher than in the normal control group. When compared to diabetic control, SGOT, SGPT, and SALP levels significantly decreased toward normal values following 28 days of HEAA treatment.

The beginning of tissue LPO is caused by free radicals such as superoxide (02) and hydroxyl (OH). The development of TBA reactive material, i.e. MDA, is commonly used to test LPO (Biswas M et al., 2011). Increased hepatic and renal MDA levels in diabetic rats suggested that endogenous antioxidant mechanisms were impaired. MDA levels in HEAA-treated groups were considerably lower than in diabetic control groups, indicating that HEAA has a protective effect against oxidative damage at Indicating level. SOD is an endogenous enzymatic antioxidant that provide organs from free radical oxidative damage. GSH is a nonenzymatic endogenous antioxidant that works with glutathione peroxidase to detoxify hydrogen peroxide radicals and prevent LPO (Forman HJ et al., 2016). Due to oxidative damage and decreased GSH synthesis in diabetics, SOD and GSH were reduced in the diabetic control group. Hepatic and renal SOD activity, as well as GSH levels, were considerably higher in HEAA-treated diabetic rats than in diabetic control rats, indicating a strong presence of endogenous antioxidative defence at the tissue level.

In comparison to diabetic control rats, histopathological analysis of the pancreas of HEAA-treated diabetic rats revealed a dose-dependent increase in cell density, indicating that HEAA treatment promotes cellular development, HEAA revealed significant antidiabetic effect in albino rats in a dose-dependent manner in the current investigation. HEAA contains flavonoids and alkaloids, according to preliminary phytochemical investigations. Flavonoids are natural antioxidants that have been studied extensively (Bhattacharya S et al., 2018).

References

Aboonabi, A., Rahmat, A., & Othman, F. (2014). Effect of pomegranate on histopathology of liver and kidney on generated oxidative stress diabetic induced rats. *J Cytol Histol*, *6*(1), 2-5.

Bhattacharjee, S., Chakraborty, S., Chorell, E., Sengupta, P. K., & Bhowmik, S. (2018). Importance of the hydroxyl substituents in the B–ring of plant flavonols on their preferential binding interactions with VEGF G–quadruplex DNA: Multi-spectroscopic and molecular modeling studies. *International journal of biological macromolecules*, *118*, 629-639.

Biswas, M., Kar, B., Bhattacharya, S., Kumar, R. S., Ghosh, A. K., & Haldar, P. K. (2011). Antihyperglycemic activity and antioxidant role of Terminalia arjuna leaf in streptozotocin-induced diabetic rats. *Pharmaceutical biology*, *49*(4), 335-340.

Chakraborty, A., Hegde, S., Praharaj, S. K., Prabhu, K., Patole, C., Shetty, A. K., ... & Upadhya, D. (2021). Age related prevalence of mild cognitive impairment in type 2 diabetes mellitus patients in the indian population and association of serum lipids with cognitive dysfunction. *Frontiers in endocrinology*, *12*, 798652.

Dolai, N., Karmakar, I., Kumar, R. S., Kar, B., Bala, A., & Haldar, P. K. (2012). Evaluation of antitumor activity and in vivo antioxidant status of Anthocephalus cadamba on Ehrlich ascites carcinoma treated mice. *Journal of ethnopharmacology*, *142*(3), 865-870.

Forbes, J. M., & Cooper, M. E. (2013). Mechanisms of diabetic complications. *Physiological reviews*, *93*(1), 137-188.

Gayen, S., Jana, S., Das Gupta, B., Ghosh, A., Kar, A., Bala, A., ... & Haldar, P. K. (2024). Exploration of anti-diabetic activity and metabolite profiling of Bruguiera cylindrica (l.) Bl.—in vivo anti-diabetic activity, exploration of molecular mechanism, and network pharmacological analysis. *Journal of Pharmacy and Pharmacology*, rgae030.

Gayen, S., Jana, S., Das Gupta, B., Ghosh, A., Kar, A., Bala, A., ... & Haldar, P. K. (2024). Exploration of anti-diabetic activity and metabolite profiling of Bruguiera cylindrica (l.) Bl.—in vivo anti-diabetic activity, exploration of molecular mechanism, and network pharmacological analysis. *Journal of Pharmacy and Pharmacology*, rgae030.

Kawanami, D., Matoba, K., & Utsunomiya, K. (2016). Dyslipidemia in diabetic nephropathy. *Renal Replacement Therapy*, *2*, 1-9.

Kumar, A. Y., Nandakumar, K., Handral, M., Talwar, S., & Dhayabaran, D. (2011). Hypoglycaemic and anti-diabetic activity of stem bark extracts Erythrina indica in normal and alloxan-induced diabetic rats. *Saudi Pharmaceutical Journal*, *19*(1), 35-42.

Li, R., Zhang, P., Barker, L. E., Chowdhury, F. M., & Zhang, X. (2010). Cost-effectiveness of interventions to prevent and control diabetes mellitus: a systematic review. *Diabetes care*, *33*(8), 1872-1894.

Skov, V., Cangemi, C., Gram, J., Christensen, M. M., Grodum, E., Sørensen, D., ... & Rasmussen, L. M. (2014). Metformin, but not rosiglitazone, attenuates the increasing plasma levels of a new cardiovascular marker, fibulin-1, in patients with type 2 diabetes. *Diabetes Care*, *37*(3), 760-766.

Srinivasan, K., Viswanad, B., Asrat, L., Kaul, C. L., & Ramarao, P. J. P. R. (2005). Combination of high-fat diet-fed and low-dose streptozotocin-treated rat: a model for type 2 diabetes and pharmacological screening. *Pharmacological research*, *52*(4), 313-320.

Wang, T. J., Larson, M. G., Vasan, R. S., Cheng, S., Rhee, E. P., McCabe, E., ... & Gerszten, R. E. (2011). Metabolite profiles and the risk of developing diabetes. *Nature medicine*, *17*(4), 448-453.

CHAPTER 8:

SUMMARY & CONCLUSION

8.1 Summary and conclusion

Even if there are well-known anti-diabetic medications available on the market, diabetes and its aftereffects can be successfully treated using herbal therapies. The present study has been conducted to evaluate the anti-diabetic as well as nephroprotective effect of HEAA in High fat diet and STZ induced diabetic rats. The rats treated with HFD and STZ showed signs of significant weight loss, polydipsia, polyuria, and glycosuria—all classic signs of diabetes mellitus. (OKON et al., 2012) The current findings imply that in HFD fed STZ induced diabetic rats, HEAA has notable nephroprotective effect in rats through modulation of hyperglycaemia, oxidative stress and hyperlipidaemia. Research conducted on diabetic animal models reveals that oxidant species play a significant role in the pathogenesis of diabetic kidney damage (Sagoo et al., 2018)

The existence of flavonoids and phenolic substances was shown by preliminary phytochemical investigations in HEAA. One well-reviewed class of potential natural antioxidants are phenolic chemicals. (Lao et al., 2017). According to a recent study, *A. aureum* aerial parts showed antioxidant andanti-inflammatory properties. (Wu et al., 2018) The presence of phenolics and flavonoids may be the cause of the observed antioxidative protection in vivo.

In the digestive tract, α-amylase is a crucial enzyme that converts dietary carbohydrates like starch into simple monosaccharides (Kaur et al., 2021). α-glucosidases proceed to break them down into glucose, which is absorbed and goes into the circulation. Thus, blocking the actions of α-amylase and α-glucosidase enzymes might hinder the breakdown of carbohydrates, postpone the absorption of glucose, and ultimately lower blood sugar levels (Kashtoh et al., 2022). The α-amylase and α-glucosidase inhibitory assay demonstrated the enzyme inhibitory activity of HEAA, and the results indicate that HEAA may lower blood sugar levels by preventing the absorption of monosaccharides.

The current investigation used higher levels of urine microalbumin, urine volume, serum creatinine, Uric acid and impaired creatinine clearance as in vivo indicators of nephropathy in HFD STZ induced diabetic rats, indicating that HFD and STZ had induced diabetic nephropathy (Kumari et al., 2020). High levels of Microalbumin and serum creatinine are the prime biomarkers of diabetic nephropathy (Ide et al., 2022). An increase in BUN levels suggests that hyperglycemia may cause the glomerular filtration barrier to be destroyed, which can then result in albumin release and worsen the course of diabetic nephropathy (Abdou et al., 2022). Elevation of liver function test parameters like SGOT, SGPT, ALP also indicated progression towards T2DM. It has been reported that dyslipidemia in diabetes mellitus (DM) is a significant factor in the hepatic lipid and free fatty acid accumulations that cause excessive ROS generation and lipid peroxidation. These procedures intensify the damage and oxidative stress in the liver, leading to a rise in the blood levels of liver enzymes in individuals with diabetes (Singh et al., 2020). HEAA has shown considerable decrease in the serum liver function parameters. Research has demonstrated that in individuals with diabetes mellitus, insufficient insulin causes a range of disruptions in metabolic and regulatory functions, ultimately resulting in the build-up of lipids such as TC and

triglycerides in diabetic patients (Rahmani et al., 2023). DN has been linked to dyslipidemia, which is caused by an increase in extracellular matrix synthesis and macrophage infiltration in the glomeruli of diabetes patients (Kawanami et al., 2016). Lipid-lowering medication has been shown in clinical trials to have a protective impact on renal function (Wong et al., 2016). HEAA, in this present study has shown to normalize the serum lipid profile of the diabetic rats.

Reactive oxygen species (ROS) are produced in greater amounts when blood glucose levels are elevated. ROS have a role in the development of several diabetic complications, such as diabetic nephropathy. Reactive oxygen species degrade the cell's antioxidant defences, increasing its vulnerability to oxidative damage. It also targets proteins, lipids, and DNA, causing their oxidation, which alters cellular structure and function (Cheng et al., 2013). Excessive ROS build up in hyperglycemia through a variety of processes, including the oxidative phosphorylation of glucose, the polyol pathway, advanced glycosylation, the mitochondrial respiratory process, and the uncoupling of NADPH oxidase. These processes ultimately result in the oxidation or glycosylation of antioxidant enzymes, which lowers antioxidant capacity (Forbes et al., 2008). The findings demonstrated that HEAA may successfully raise SOD and GSH activities, lower MDA synthesis in renal tissue thereby reducing Lipid peroxidation, and lessen renal damage and oxidative stress.

A histopathological analysis showed that treated diabetic rats had less apparent glomerulosclerosis than untreated diabetic rats, with the former showing significant glomerulosclerosis. Additionally, glomerular membrane thickening was observed to be less in rats treated with HEAA (particularly at higher test doses) than in rats with diabetes.

Hydroalcoholic extract of *A.aureum*, aerial parts exhibits a dose-dependent, considerable protective effect against HFD-STZ induced diabetic nephropathy and albuminaria in Wistar rats, as well as an anti-diabetic effects. As a result, this plant may be effectively used to control diabetic complications.

References

Abdou, H. M., & Abd Elkader, H. T. A. E. (2022). The potential therapeutic effects of Trifolium alexandrinum extract, hesperetin and quercetin against diabetic nephropathy via attenuation of oxidative stress, inflammation, GSK-3β and apoptosis in male rats. *Chemico-Biological Interactions*, *352*, 109781.

Cheng, D., Liang, B., & Li, Y. (2013). Antihyperglycemic effect of Ginkgo biloba extract in streptozotocin-induced diabetes in rats. BioMed research international, 2013.

Ebaid, H., Bashandy, S. A., Abdel-Mageed, A. M., Al-Tamimi, J., Hassan, I., & Alhazza, I. M. (2020). Folic acid and melatonin mitigate diabetic nephropathy in rats via inhibition of oxidative stress. *Nutrition & metabolism*, *17*, 1-14.

Ide, H., Iwase, M., Ohkuma, T., Fujii, H., Komorita, Y., Oku, Y., ... & Kitazono, T. (2022). Usefulness of urinary tubule injury markers for predicting progression of renal dysfunction in patients with type 2 diabetes and albuminuria: The Fukuoka Diabetes Registry. Diabetes Research and Clinical Practice, 186, 109840.

J. M. Forbes, M. T. Coughlan and M. E. Cooper, Oxidative Stress as a Major Culprit in Kidney Disease in Diabetes, Diabetes, 2008, 57, 1446–1454

Kashtoh, H., & Baek, K. H. (2022). Recent updates on phytoconstituent alpha-glucosidase inhibitors: An approach towards the treatment of type two diabetes. Plants, 11(20), 2722.

Kaur, N., Kumar, V., Nayak, S. K., Wadhwa, P., Kaur, P., & Sahu, S. K. (2021). Alpha-amylase as molecular target for treatment of diabetes mellitus: A comprehensive review. Chemical biology & drug design, 98(4), 539-560.

Kawanami, D., Matoba, K., & Utsunomiya, K. (2016). Dyslipidemia in diabetic nephropathy. Renal Replacement Therapy, 2, 1-9.

Kumari, R., Goldar, W. A., Mondal, S., Patra, S., Bhattacharya, S., & Haldar, P. K. (2021). Protective effect of Basella alba leaf against diabetic nephropathy in rats. Advances in Traditional Medicine, 21, 111-119.

Lao, F., Sigurdson, G. T., & Giusti, M. M. (2017). Health benefits of purple corn (Zea mays L.) phenolic compounds. Comprehensive Reviews in Food Science and Food Safety, 16(2), 234-246.

Rahmani, A. H., Alsahli, M. A., Khan, A. A., & Almatroodi, S. A. (2023). Quercetin, a plant flavonol attenuates diabetic complications, renal tissue damage, renal oxidative stress and inflammation in streptozotocin-induced diabetic rats. Metabolites, 13(1), 130.

Sagoo, M. K., & Gnudi, L. (2018). Diabetic nephropathy: is there a role for oxidative stress?. Free Radical Biology and Medicine, 116, 50-63.

Singh, B., Kumar, A., Singh, H., Kaur, S., Kaur, S., Buttar, H. S., ... & Singh, B. (2020). Zingerone produces antidiabetic effects and attenuates diabetic nephropathy by reducing oxidative stress and overexpression of NF-κB, TNF-α, and COX-2 proteins in rats. Journal of Functional Foods, 74, 104199.

UA O, O. D., Udokang, N., Udobang, J., & Ekpenyong, C. (2012). Oral administration of aqueous leaf extract of Ocimum gratissimum ameliorates polyphagia, polydipsia and weight loss in streptozotocin-induced diabetic rats. American Journal of Medicine and Medical Sciences, 2(3), 45-9.

Wong, M. G., & Perkovic, V. (2016). Knowing what we do not know: statin therapy in advanced chronic kidney disease. The lancet Diabetes & endocrinology, 4(10), 801-803.

Wu, X., Huang, Q., Xu, N., Cai, J., Luo, D., Zhang, Q., ... & Liu, Y. (2018). Antioxidative and anti-inflammatory effects of water extract of Acrostichum aureum Linn. against ethanol-induced gastric ulcer in rats. Evidence-based complementary and alternative medicine, 2018.