CAPITAL UNIVERSITY OF SCIENCE AND TECHNOLOGY, ISLAMABAD

A Study on Antioxidant and Alpha Amylase Inhibitory Attributes of *Adhatoda vasica* Leaf Extract

by

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A thesis submitted in partial fulfillment for the degree of Master of Science

in the

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I dedicate this thesis to my loving and supportive family and friends who have fully helped me in achieving my life goals.

CERTIFICATE OF APPROVAL

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Abstract

Diabetes stands as one of the most significant health challenges of the 21st century and ranks among the top 10 global causes of death. The study explores the hypoglycemic (blood sugar-lowering) potential of phenolic compounds derived from the plant *Adhatoda vasica* in treating Type 2 Diabetes Mellitus (T2DM). T2DM, a major global health challenge, has no cure, leading to ongoing research into alternative treatments such as herbal medicines. Traditional herbal remedies, although commonly used, often lack scientific validation. This research aimed to evaluate *Adhatoda vasica*'s potential through in vitro testing, focusing on its bioactive compounds such as flavonoids, quinolines, and essential oils, which may aid in managing diabetes more effectively and with fewer side effects than conventional drugs.

The study assessed the antioxidant properties of *Adhatoda vasica*'s ethanolic leaf extract using a DPPH free radical scavenging assay. Results showed concentration-dependent antioxidant activity, with increased scavenging activity over time, confirming the plant's effectiveness in neutralizing harmful free radicals. This was further correlated with the extract's phenolic content, measured using the Folin-Ciocalteu (FC) method, which demonstrated a positive relationship between phenolic concentration and antioxidant activity. These findings suggest that higher phenolic concentrations contribute to greater antioxidant efficacy.

Additionally, the study examined the plant extract's inhibitory effects on alpha-amylase, an enzyme crucial for starch digestion. At concentrations ranging from 20 ppm to 5000 ppm, significant enzyme inhibition was observed, indicating that the extract may help regulate blood sugar levels by slowing carbohydrate breakdown. This inhibition is attributed to the presence of bioactive compounds such as phenols, flavonoids, saponins, steroids, and alkaloids.

The in vivo section of the research involved administering *Adhatoda vasica* extracts to diabetic Sprague Dawley rats induced with diabetes through streptozotocin injections. The rats were divided into several groups, including a negative control, a

positive control treated with metformin, and groups treated with varying concentrations of the plant extract (100 mg/kg, 200 mg/kg, and 400 mg/kg). Over 14 days, the extract, especially at higher doses, significantly lowered blood glucose levels, matching the effects of metformin. Additionally, the plant extract led to improved body weight, reduced serum creatinine levels (indicating better kidney function), and improved liver function test (LFT) results, suggesting a protective effect on the liver.

The study concludes that *Adhatoda vasica* has promising antidiabetic properties, effectively reducing blood sugar levels and improving liver and kidney function in diabetic rats. The plant's bioactive compounds, particularly phenolic compounds, contribute to its hypoglycemic, antioxidant, and enzyme-inhibiting properties, supporting its traditional use in managing diabetes.

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Abbreviations

AChE	Acetyl cholinesterase
AKT	Protein Kinase B
ALT	Alanine aminotransferase
AMPK	Adenosine Monophosphate – activated Protein Kinase
AST	Aspartate aminotransferase
BuChE	Butyrylcholinesterase
CCK-8	Cholecystokinin Octapeptide
COX-2	Cyclooxygenase-2
CRD	Complete Randomized Design
D2M	Diabetes- Mellitus Type II Diabetes
DCM	Dichloromethane
DM	Diabetes- Mellitus
DNSA	Deoxyribonucleic Acid Sequence Analysis
DPPH	2, 2-Diphenyl-1-Picrylhydrazyl Radical Scavenging Assay
ELEAV	Eosinophils Lymhocytes Erthrocytes Agranulocytes Vesicles
GDM	Gestational diabetes mellitus
GK	Glucokinase
GLUT	Glucose Transporter
HbA1c	Glycated haemoglobin
HPLC	High – Performance Liquid Chromatography
HRMS	High Resolution Mass Spectrometry
IRS-1	Insulin Receptor Substrate
IR	Insulin Receptor
LADA	Latent Autoimmune diabetes of Adults

MET	Mitochondrial electron transport chain
NMR	Nuclear magnetic resonance
P13k	Phosphoinositide 3- Kinases
PPARs	Peroxisome proliferator – Activated receptors
PTB	Phosphtyrosine Binding
RAAS	Renin Angiotensin System
ROS	Rective Oxygen Species
S.D	Standard Deviation
S.G.O.T	Serum glutamic oxaloacetic transaminase
S.G.P.T	Serum glutamic pyruvic transaminase
TLC	Thin Layer Chromatography
TPC	Total Phenolic Content
iNOS	Inducible Nitric oxide Synthase

Chapter 1

Introduction

Diabetes mellitus is a serious worldwide public health issue that is typified by abnormalities in the metabolism of carbohydrates, which results in blood glucose levels that are persistently raised because of decreased insulin function or production. It happens when blood glucose, sometimes referred to as blood sugar, concentrations rise too high [1]. Diabetes can be classified as follows: An autoimmune attack on β-cells causes type 1 diabetes, which usually results in total lack of insulin production, including latent autoimmune diabetes of adulthood [2]. A non-autoimmune, progressive decrease in β-cell insulin production causes type 2 diabetes, which is frequently associated with insulin resistance and metabolic syndrome [2].

According to a study, approximately 537 million adults between the ages of 20 and 79 are presently affected by diabetes, constituting 10.5% of this demographic globally. Projections suggest that by 2030, this figure will climb to 643 million (11.3%) and further to 783 million (12.2%) by 2045. Additionally, around 240 million individuals worldwide have diabetes but have not been diagnosed, indicating that nearly half of adults with the condition are unaware of their status [3].

The most frequently encountered chronic complications resulting from diabetes pertain to cardiovascular problems, which involve the heart and the network of blood vessels. These complications encompass conditions such as coronary artery

disease, heart attacks, stroke, and atherosclerosis [4]. Moreover, high blood pressure places additional strain on the heart, contributes to vascular damage, and amplifies the risk of experiencing a heart attack [5].

Among diabetics, hypertension is highly prevalent and ranks as one of the most common diseases worldwide. The coexistence of these two conditions significantly heightens the likelihood of complications entail retinopathy and nephropathy [6].

Managing diabetes can also affect mental well-being. Compared to people without diabetes, people with diabetes have a two to three times higher chance of developing depression [4].

These complications largely stem from persistently elevated blood glucose levels. Insulin and glucagon play crucial roles in maintaining glucose and lipid homeostasis through signaling pathways [7].

Attaining lasting metabolic stability in diabetes requires a blend of lifestyle adjustments and pharmaceutical interventions. Achieving glycated hemoglobin levels close to normal substantially diminishes the risks associated with both macrovascular and microvascular complications. Currently, there exists a range of treatments, including oral and injectable options, for managing type 2 diabetes mellitus. Treatment protocols aimed at curbing the onset or advancement of diabetes-related complications underscore the importance of maintaining optimal glycemic control. The primary approach should prioritize lifestyle modifications. While lifestyle changes have demonstrated significant benefits, sustaining them long-term can pose challenges for many patients. Metformin continues to be the preferred initial treatment for the majority of patients. The selection of alternative or second-line treatments should be based on the particular needs of each patient [8].

In earlier times, the primary focus of diabetes medications was to manage and normalize blood glucose levels within the bloodstream. However, many modern drugs were associated with numerous side effects that could lead to significant medical issues during treatment. Consequently, plants had been employed as alternative medicines for an extended period and played a pivotal role in diabetes management. Moreover, in recent years, newly discovered bioactive compounds isolated

from plants exhibited superior antidiabetic properties compared to conventional oral hypoglycemic agents utilized in clinical treatment [9].

Medicinal plants wield considerable influence in addressing diabetes mellitus, a critical metabolic ailment. Traditional botanical remedies are noted for their potent anti-diabetic properties without adverse effects. They harbor valuable anti-diabetic constituents like flavonoids, alkaloids, phenolics, and tannins, enhancing pancreatic tissue function by augmenting insulin secretion or curbing glucose absorption in the intestines [10].

Adhatoda vasica, a renowned medicinal plant, harbors phytoconstituents with promising anti-diabetic properties. Its extracts and isolates exhibit potential in delaying carbohydrate digestion and absorption, as well as demonstrating insulinotropic and insulin-mimetic effects, antioxidant capabilities, and anti-glycation activity.

This exploration aims to shed light on the plant's potential as an anti-diabetic agent, offering insights to address contemporary therapeutic challenges in diabetes management [11].

1.1 Problem Statement

High blood sugar levels are a defining feature of the chronic illness known as diabetes mellitus, affecting millions worldwide. Insulin resistance and impaired insulin secretion lead to hyperglycemia, causing damage to organs and tissues. Current medications have limitations and side effects, highlighting the need for more effective management strategies such as exploring the novel phytotherapy may present an avenue for reducing adverse effects.

1.2 Aim and Objectives

The main aim is to explore the antioxidant and alpha-amylase inhibitory properties of *Adhatoda vasica* leaf extract and studying the its antidiabetic potential on

Sprague Dawley rats.

The objectives of the study are as follows:

- To investigate the antioxidant potential following DPPH assay and total polyphenolic content of *Adhatoda vasica* leaf extract.
- To evaluate the alpha-amylase inhibitory activity of *Adhatoda vasica* leaf extract.
- To determine the anti-diabetic effect of this plant extract in Sprague Dawley rats.

1.3 Scope

The research seeks to delve into its properties for regulating blood sugar, uncover bioactive components, gain insights into its mode of operation, and play a role in the creation of cost effective and secure pharmaceutical choices for diabetes treatment. Furthermore, the investigation extends to examining *Adhatoda Vasica*'s wider health advantages, encompassing its antimicrobial and antioxidative attributes, all with the overarching objective of progressing scientific understanding in the realms of herbal medicine and diabetes care.

1.4 Impact on Society

Diabetes exerts a notable influence on society, encompassing challenges within healthcare, financial ramifications, and an expanding public health concern. Genetic and environmental elements contribute to this impact, while investigations into alternative remedies like *Adhatoda vasica* offer potential solutions for diabetes management

Chapter 2

Literature Review

2.1 Diabetes

A significant lack of insulin is the hallmark of type 1 diabetes, which is caused by the autoimmune destruction of pancreatic β cells. In contrast, insulin resistance which is thought to be a fundamental physiological condition that precedes and contributes to the evolution of the disease and insufficient insulin production from the pancreatic β cells are the hallmarks of type 2 diabetes. Environmental and genetic variables interact intricately to influence the development of type 2 diabetes [12].

2.1.1 Diabetes and its Types

Diabetes falls into a number of categories: An autoimmune attack on β-cells causes type 1 diabetes, which frequently results in total lack of insulin production, including adult-onset latent autoimmune diabetes. A non-autoimmune, progressive decrease in β-cell insulin production is the cause of type 2 diabetes, which is often associated with insulin resistance and metabolic syndrome [3]. Monogenic diabetes syndromes, pancreatic exocrine abnormalities, and variants brought on by drugs or chemicals (such glucocorticoid therapy) are among the other forms of the disease. [12]. Diabetes that was not evident before to pregnancy but was discovered

during the second or third trimester of pregnancy is known as gestational diabetes mellitus [3]. Diabetes is characterised by elevated blood glucose levels; however, the underlying causes of each form of diabetes vary [3, 12].

Precise categorization of diabetes is essential for clinical and epidemiological research, as well as clinical therapy. While diabetes was once categorized by age of onset or treatment type, current classification primarily focuses on the pathogenic processes leading to hyperglycemia [14]. The major types of diabetes include:

- Type 1 diabetes mellitus (T1DM)
- Type 2 diabetes mellitus (T2DM)
- Gestational diabetes mellitus (GDM)

2.2 Type 2 Diabetes Mellitus

The most prevalent kind of diabetes, type 2 diabetes mellitus (T2DM), accounts for around 90% of cases [15]. It is often referred to as adult-onset diabetes or non-insulin-dependent diabetes [16]. In T2DM, hyperglycemia arises from insulin resistance and relative (rather than absolute) insulin deficiency. T2DM differs from type 1 diabetes in that pancreatic β-cells are not destroyed by the immune system. Although the precise origin of type 2 diabetes is unknown, environmental variables (bad food, obesity, and physical inactivity) and genetic predispositions all have a role [16]. An unhealthy diet typically includes high levels of fat, salt, sugar, and cholesterol, with insufficient fiber content. Less than 30 minutes of physical activity on at least five days a week is considered physical inactivity.

Being overweight or obese, having a family history of diabetes, being older than 45, having high blood pressure ($\geq$140/90 mmHg), having gestational diabetes in the past, having a history of heart disease or stroke, and being a member of specific ethnic groups (such as African or African American) are risk factors for developing type 2 diabetes. Due to rising rates of obesity, physical inactivity, and poor diet, T2DM is now more commonly diagnosed in children and adolescents

than in adults, despite the condition's historical prevalence in adults. The usual approach to managing type 2 diabetes is to lose weight, lead a healthy lifestyle, and seek medical attention as needed [16].

2.2.1 Gestational Diabetes Mellitus

Approximately 13% of all infants are affected by gestational diabetes mellitus (GDM), which is characterised by glucose intolerance initially discovered during pregnancy [16]. GDM typically resolves after childbirth, but around 50% of affected women may develop type 2 diabetes mellitus (T2DM) later in life [17]. During pregnancy, the placenta produces hormones that can reduce the effectiveness of insulin, leading to the development of GDM. While many symptoms of T2DM are present in GDM, diagnosis usually relies on prenatal screening such as fasting glucose tests rather than symptoms reported by the patient [16].

Management of gestational diabetes involves strategies such as adopting a healthy diet, engaging in physical exercise, monitoring blood glucose levels, and sometimes using oral medications [15].

2.2.2 Other Causes of Diabetes Mellitus

The causes of diabetes encompass, insulin resistance: the primary origin of Type 2 diabetes lies in insulin resistance, where cells in muscles, fat, and the liver do not react appropriately to insulin. Various factors contribute to differing degrees of insulin resistance, such as obesity, limited physical activity, dietary habits, hormonal imbalances, genetic predisposition, and specific medications. Autoimmune disorder: Both type 1 diabetes and latent autoimmune diabetes of adults (LADA) are caused by the immune system attacking the pancreatic cells that produce insulin. Hormonal imbalances: Pregnancy prompts the placenta to release hormones that induce insulin resistance. If the pancreas fails to generate sufficient insulin to counter this resistance, gestational diabetes may develop. Conditions related to hormonal imbalances, such as acromegaly and Cushing syndrome, can also lead

to Type 2 diabetes. Pancreatic impairment: Physical harm to the pancreas, resulting from conditions, surgeries, or injuries, can impede its capacity to produce insulin, leading to Type 3 diabetes. Genetic mutations: Specific genetic mutations contribute to conditions like Maturity-Onset Diabetes of the Young (MODY) and neonatal diabetes [4]. Prolonged elevation of blood glucose levels can lead to harm in your body's tissues and organs. The primary cause of this harm is attributed to damage occurring in your blood vessels and nerves, which provide essential support to your body's tissues [12].

This category, however less prevalent, was created to include diabetic disorders resulting from distinct, non-classical causes of diabetes mellitus (DM), which may have acquired or hereditary origins [18]. These include pancreatic problems, endocrine abnormalities, drug-induced DM, and monogenic forms of diabetes. Mutations in a single gene that impair β-cell activity or, less frequently, lead to insulin resistance are the cause of monogenic forms of diabetes [18].

Drug-induced DM can result from a variety of commonly used medications that disrupt glucose homeostasis, leading to hyperglycemia or exacerbating glycemic control in individuals with pre-existing DM [19]. Glucocorticoids, diuretics, β-blockers, phenytoin, cyclosporine, diazoxide, and derivatives of nicotinic acid are a few examples of these drugs. For example, the anti-seizure drug phenytoin can cause hyperglycemia by preventing the release of insulin. Immunosuppressive therapy with cyclosporine can impair insulin synthesis and secretion. When used to treat hypoglycemia, diazoxide can cause the body to produce more glucose, less insulin, and decrease the absorption of glucose. Anti-inflammatory medications called glucocorticoids have the potential to worsen glucose intolerance, insulin resistance, and decrease glucose synthesis [20]. When used to treat hypertension, β-blockers can hinder the release of insulin from pancreatic β-cells, while diuretics can cause insulin resistance, obstruct glucose absorption, and reduce insulin release [21].

Acromegaly, Cushing syndrome, pheochromocytoma, glucagonoma, and thyrotoxicosis are among the endocrine disorders that can induce diabetes mellitus (DM) because of high hormone levels that counter regulate insulin and prevent insulin

from being secreted or acting upon the body [22]. An uncommon but important cause of diabetes mellitus is pancreatic diseases. Damage to the pancreas's endocrine and exocrine processes, such as that caused by chronic pancreatitis, can result in diabetes mellitus. [23].

2.3 Statistics

According to current diabetes statistics, the worldwide number of individuals with diabetes has surpassed 500 million, with older individuals being the most heavily impacted. Projections indicate that the prevalence of the condition will surge by 134% in Africa, 68% in South-East Asia, and 13% in Europe. Within the United States, 37 million people, constituting 11.3% of the population, have diabetes. In Australia, the figure stands at one in twenty people, or approximately 1.3 million individuals.

Remarkably, China records the highest number of diabetes cases, totaling 141 million individuals. Alarmingly, there are an estimated 283,000 Americans under the age of 20 who are affected by the disease, and the U.S. sees around 1.4 million new cases annually [13].

Around the globe, approximately 463 million adults are living with diabetes, with 90% of this group facing type 2 diabetes mellitus. According to a report from "The News," Pakistan holds the third position globally in terms of diabetes prevalence, following China and India. The prevalence of diabetes within Pakistan has shown an increase, reaching 11.77% in 2016, 16.98% in 2018, and 17.1% in 2019 as depicted in Figure 2.1.

As per the International Diabetes Federation, the year 2022 witnesses a 26.7% diabetes incidence among adults in Pakistan, accounting for an estimated total of around 33,000,000 cases. This alarming figure is not only significantly high but is also on a rising trajectory each year. It is plausible that a substantial number of patients remain undiagnosed, thereby elevating both the true prevalence of the condition and the associated risk of complications due to untreated diabetes [24].

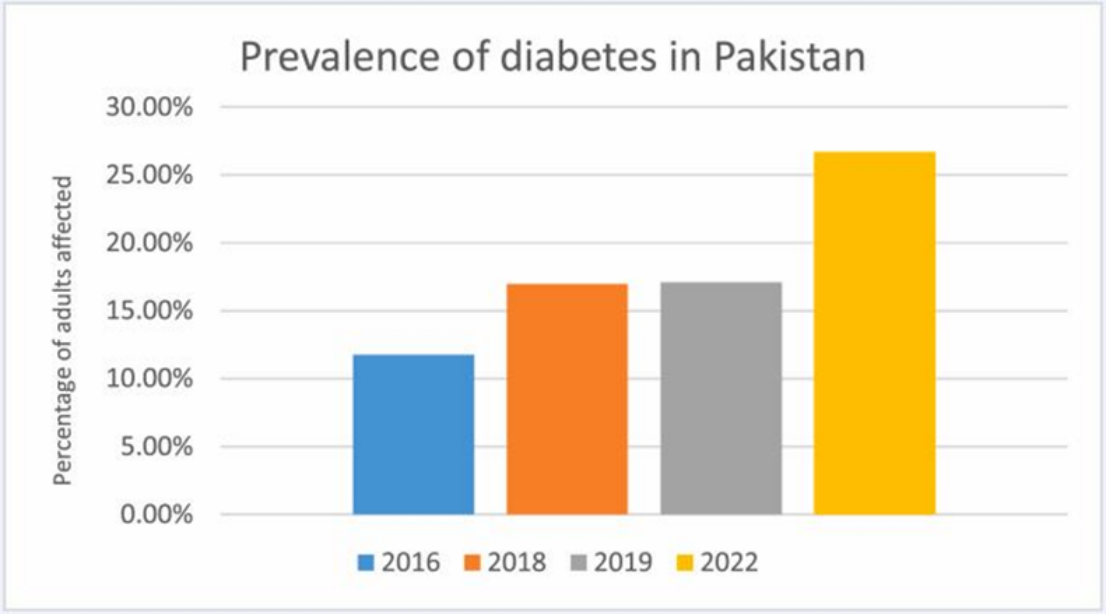


FIGURE 2.1: Prevalence of diabetes in Pakistan. The graph had shown the prevalence of diabetes. The blue area depicted 11.77% of adults who were affected in 2016. The orange area depicted 16.98% of adults who were affected in 2018. The grey area depicted 17.1% of adults who were affected in 2019, and the yellow area showed that in 2022, 26.7% of adults were affected in Pakistan [13]

To put it in perspective, about 537 million people aged 20 to 79 are living with diabetes, equivalent to 10.5% of the global population within this age range. Projections indicate that by the year 2045, the worldwide prevalence of diabetes will exceed 12% [13].

2.4 Diabetes Health Complications

Studies revealed that diabetes can give rise to additional disorders and significant complications. Among diabetics, hypertension is highly prevalent and ranks as one of the most common diseases worldwide. A staggering sixty percent of individuals with type 2 diabetes also contend with hypertension as a comorbidity. The coexistence of these two conditions significantly heightens the likelihood of experiencing complications, encompassing both microvascular and macrovascular issues. Macrovascular complications comprise myocardial infarction and stroke, while microvascular complications entail retinopathy and nephropathy [6].

2.4.1 Cardiovascular Diseases

A study revealed that the most frequently encountered chronic complications resulting from diabetes pertain to cardiovascular problems, which involve the heart and the network of blood vessels.

These complications encompass conditions such as coronary artery disease, heart attacks, stroke, and atherosclerosis [4]. Diabetes-related elevated blood glucose levels can harm blood vessels as well as the neurons controlling heart and vascular function. Over time, this damage can culminate in the development of heart disease. Moreover, high blood pressure places additional strain on the heart, contributes to vascular damage, and amplifies the risk of experiencing a heart attack [5]. In a study conducted in China, additional complications associated with diabetes were presented in Figure 2.2.

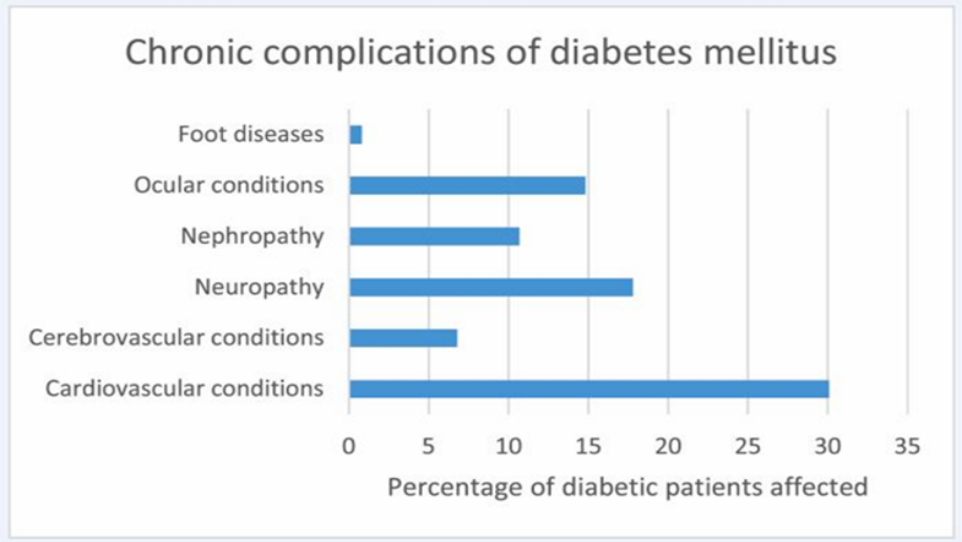


FIGURE 2.2: Chronic complications of diabetes mellitus. The blue area in the graph showed the percentages of complications or diseases that occurred through diabetes. Below 5% showed foot diseases. 15% showed ocular conditions (related to the eye). 10 – 11% showed kidney diseases (nephropathy). More than 15% showed neuropathy (nerve damage). Below 10% showed cerebrovascular conditions. 30% showed cardiovascular conditions [13]

2.4.2 Diabetic Neuropathy

Neuropathy, which could manifest as sensations of numbness, tingling, and pain, was one of these complications. Nephropathy, which had the potential to progress

to kidney failure, necessitating dialysis or transplantation, was another significant concern [4]. The onset of hypertension in individuals with diabetes varied depending on the diabetes type when diabetes type 1 was first diagnosed, hypertension usually appeared several years later and was frequently associated with diabetic nephropathy. On the other hand, hypertension may exist in individuals with type 2 diabetes even in the absence of elevated glucose levels. Diabetic nephropathy is more common in patients with both diabetes and hypertension.

On the other hand, hypertension may exist in individuals with type 2 diabetes even in the absence of elevated glucose levels. Diabetic nephropathy is more common in patients with both diabetes and hypertension. Renal cells were activated by hyperglycemia, which led to the release of growth factors, humoral mediators, and cytokines. This production therefore contributed to increased glomerular basement membrane permeability, increased extracellular collagen deposition, and structural changes in the glomeruli of diabetes patients.

These structural alterations cause the renin-angiotensin system (RAAS) to become chronically activated, which in turn causes micro albuminuria and eventually hypertension [6].

2.4.3 Diabetic Retinopathy

Furthermore, retinopathy is a complication that could lead to vision impairment or even blindness [4]. The main cause of diabetic retinopathy is elevated blood sugar levels carried by the disease. Excessive blood sugar levels have the potential to harm the retina over time. The retina is the portion of the eye that detects light and sends messages to the brain through the optic nerve.

This damage initiated when sugar obstructed blood vessels. Diabetes inflicted damage on blood vessels throughout the body, including the tiny vessels leading to the retina, resulting in fluid leakage or bleeding. In response to these blocked vessels, the eyes would generate new blood vessels, but these newly formed vessels were often inefficient [25].

2.4.4 Periodontal (gum) Disease

Oral health issues, including periodontal (gum) disease, can arise as a consequence of diabetes. Diabetes has the potential to impact oral health by altering the composition of saliva, the essential fluid responsible for maintaining mouth moisture. Through its ability to wash away food particles, stop bacterial development, and neutralise acid produced by bacteria, saliva plays a critical role in preventing tooth decay. Saliva also contains minerals, which protect oral tissues and help to prevent tooth decay. Both diabetes itself and certain medications used for its management can lead to reduced saliva production by the salivary glands in the mouth. A decrease in saliva flow elevates the risk of dental cavities, gum disease, and other oral complications.

Furthermore, diabetes can elevate the glucose levels in saliva. In cases of diabetes, where blood glucose levels are excessively high, this surplus glucose can also accumulate in saliva. The presence of glucose in saliva can serve as a nutrient source for harmful bacteria, which, in combination with food particles, forms a soft, adhesive film known as plaque. The most important component in the formation of cavities is plaque. Failure to remove plaque can lead to its hardening near the gum line, forming a deposit referred to as tartar, which can provoke gum disease [26].

2.4.5 Diabetic Neuropathy

Diabetes can also give rise to foot-related issues. Diabetic neuropathy is a condition that can result from diabetes that affects nerves over time and causes tingling, pain, and diminished feeling in the foot. When sensation is compromised, individuals may not notice foreign objects such as a stone lodged in their sock or a blister on their foot, which might cause wounds and blisters. Diabetes can also reduce blood supply to the feet, which can delay the healing of wounds or infections. These cuts and sores are also more likely to get infected. In severe cases, untreated infections might lead to gangrene. Other complications associated with diabetes

encompass skin infections, gastroparesis (delayed stomach emptying), and hearing impairment [27].

Maintaining a healthy mental state is another effect of managing diabetes. People with diabetes have a higher chance of developing depression—two to three times higher than people without diabetes [4]. The main cause of these complications arises from persistently elevated levels of blood glucose. Insulin and glucagon play crucial roles in regulating the balance of glucose and lipids through signaling pathways [7].

2.5 Available Treatment

According to a study the treatment paradigm for diabetes mellitus was centered on the regulation and reduction of plasma glucose concentrations to within the normal range. During that era, six fundamental categories of contemporary medications, as well as two classes of injectable agents, were globally employed to manage blood glucose levels. These groups included tablet-form biguanides, sulfonylureas, thiazolidinediones, and alpha-glucosidase inhibitors [28].

2.5.1 Oral Drugs

When managing underlying metabolic abnormalities such insulin resistance or insufficient insulin production, oral medications are recommended. These medications should be used in conjunction with a balanced diet and regular exercise [29].

2.5.2 Sulfonylureas

Sulfonylureas (Figure 2.3) are a group of medications that facilitated increased insulin production by the body, although some individuals experienced weight gain as an adverse effect upon initiation of treatment. Additionally, certain individuals were susceptible to allergic reactions associated with sulfonylurea usage [30]. On the other hand, metformin, another drug used in the past, functioned by reducing

endogenous glucose production. However, it was observed that individuals with diabetes who were prescribed metformin could develop a condition known as acidosis, characterized by an abundance of acid in the bloodstream. This illness may present as nausea, circulatory shock, or trouble breathing. As a result, metformin was generally not prescribed to patients with impaired renal function, impaired cardiac function, or a history of alcohol abuse [30].

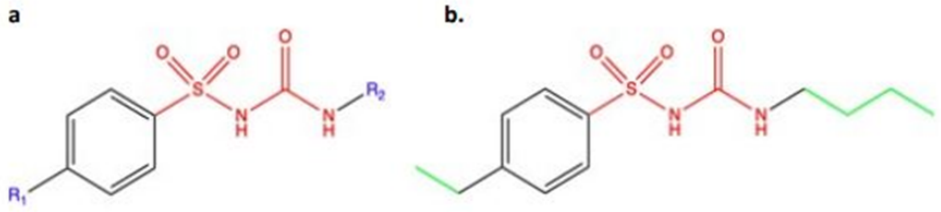


FIGURE 2.3: (a) The side chains that differentiate distinct sulfonylurea compounds are indicated in blue, and the sulfonylurea backbone is highlighted in red in the following figure of the fundamental structure of sulfonylureas. (b) The methyl group (R1) and butane chain (R2) of tolbutamide, a sulfonylurea medication, are both shown in green.

2.5.3 Gliflozins

Furthermore, gliflozins, including drugs such as dapagliflozin, empagliflozin, and canagliflozin, were administered to enhance urinary excretion of glucose, thereby lowering blood sugar levels. It was noted, however, that the use of gliflozins was connected to possible negative effects like vaginal thrush and a possible increased risk of developing acidosis (excessive acidity in the blood) [28, 30].

Alpha-glucosidase inhibitors were used to treat type 2 diabetes, according to a research, though this is no longer the general use for them. These medications operated by slowing down the absorption of glucose (sugar) in the gastrointestinal tract. Among the drugs in this category, acarbose was the most frequently prescribed. However, it was noted that acarbose did not produce as significant a reduction in blood sugar levels when compared to alternative treatment options [30].

2.5.4 Metformin

Metformin (Figure 2.4) is the preferred initial therapy for Type 2 diabetes mellitus (T2DM) due to its superior effectiveness compared to other oral medications. Its mode of action involves altering the microbiota of the gut and stimulating mucosal AMP-activated protein kinase (AMPK), which is essential for maintaining the integrity of the intestinal barrier [31].

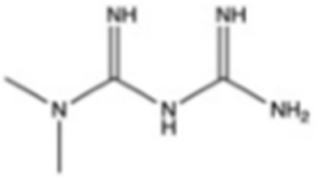


FIGURE 2.4: The structure of metformin [31]

Numerous mechanisms have been identified by research to explain how metformin can inhibit gluconeogenesis. These mechanisms include inhibiting mitochondrial glycerol phosphate dehydrogenase and nicotinamide adenine dinucleotide (NADH) coenzyme Q oxidoreductase in the mitochondrial electron transport chain (MET), which increases the AMP/ATP ratio and activates AMPK. Metformin also uses liver kinase B1 to activate hepatic AMPK. Metformin is generally well tolerated, however adverse effects on the gastrointestinal tract (GIT) such as nausea, upset stomach, and diarrhoea are possible [31].

2.5.5 Thiazolidinediones

Thiazolidinediones (TZD) Figure 2.5 enhance insulin sensitivity. They work as agonists for nuclear peroxisome proliferator-activated receptor-gamma (PPAR-l). Peroxisome proliferator-activated receptors (PPARs), which are involved in the regulation of genes linked to adipocyte growth, insulin signal transduction, and glucose and lipid metabolism, are found in muscle, adipose tissue, and the liver. Thus, TZDs attach to PPARs to regulate fat and carbohydrate metabolism, as well as to enhance glucose uptake and reduce glucose production [31]. Two of its main side effects are fluid retention and weight gain [32].

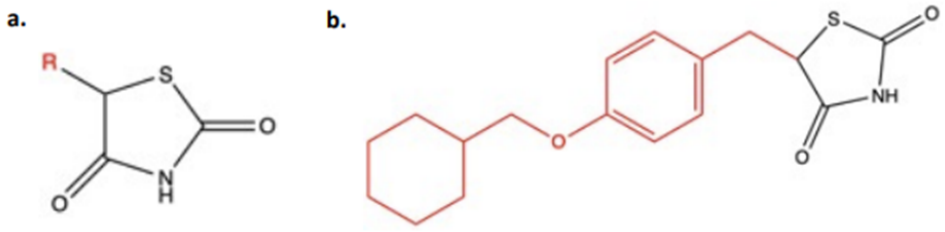


FIGURE 2.5: (a) This diagram shows the general structure of thiazolidinediones, with the backbone shown in black and the side chain highlighted in red. (b) Ciglitazone is an example of a thiazolidinedione medication [32]

2.5.6 Biguanides

Phenformin, buformin, and metformin are the three biguanides that are used to treat diabetes. Because of their link to lactic acidosis, the first two named were no longer used in the United States of America [86]. Metformin, derived from *Galega officinalis* L., also known as French lilac, originates from galegine, a guanidine derivative found in this perennial herb.

Galega officinalis has been recognized for centuries for its ability to alleviate diabetes symptoms. Metformin successfully completed clinical trials in 1995 and was subsequently approved for use in the United States. While the exact mode of action of biguanides, such as metformin, remains unclear, they do not require the existence of functional pancreatic beta cells in order to lower blood glucose levels.

Reduced plasma glucagon levels, hepatic gluconeogenesis, gastrointestinal tract slowdown, enterocyte improvement of glucose to lactate conversion, direct stimulation of glycolysis in tissues, and enhanced elimination of glucose from the bloodstream are some of the hypothesised mechanisms of action [86]. Patients with insulin-resistant hyperglycemia and persistent obesity are frequently given biguanides. Known for its ability to preserve insulin, metformin has an advantage over sulfonylureas and insulin in that it does not cause hypoglycemia or encourage weight gain. Metformin's gastrointestinal side effects are the most prevalent, and lactic acidosis is a possible side effect.

2.5.7 Meglitinides

A new class of insulin secretagogues is represented by meglitinides (figure 2.6). First approved by the FDA for clinical use in 1998, repaglinide is the first drug in this category. By affecting potassium efflux through potassium channels, these drugs regulate the release of insulin from beta cells. Meglitinides share molecular binding sites with sulfonylureas, with two sites in common and one distinct site. However, unlike sulfonylureas, meglitinides do not directly affect insulin exocytosis [86].

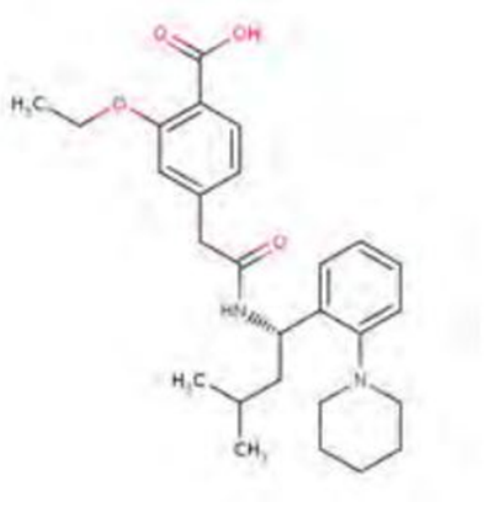


FIGURE 2.6: Chemical structure of the Meglitinide repaglinide [86]

2.5.8 Injectable Drugs

Additionally, there were two types of injectable drugs used for diabetes management in the past: incretin mimetics and insulin [28]. Incretin mimetics, which resembled hormones, were administered by injection in conjunction with metformin and/or sulfonylurea tablets, rather than as a replacement for oral antidiabetic tablets. These injectable drugs were delivered beneath the skin using pre-filled pens and stimulated the pancreas to increase insulin production. Nausea and vomiting are possible side effects of incretin mimetics, including liraglutide, dulaglutide, lixisenatide, exenatide, and albiglutide. Additionally, blood pressure-lowering drugs (antihypertensives), low doses of acetylsalicylic acid (the active ingredient in medications like Aspirin) to prevent blood clot formation, and statins

to lower cholesterol levels were among the medications intended to lower the risk of cardiovascular diseases [20].

2.5.9 Lifestyle Change

While pharmacological treatments provide therapeutic options, lifestyle modifications are crucial for managing diabetes mellitus. Adopting a healthier diet and engaging in regular physical activity are essential to achieving optimal outcomes alongside medical interventions. Blood pressure, weight control, and blood glucose levels are all greatly influenced by diet. There are several advantages to physical activity, including increased tissue sensitivity to insulin, better glycemic management, favourable effects on blood pressure and lipid profiles, encouragement of weight reduction, and cardiovascular benefits [31].

2.5.10 Biological activity of α- and β Glucosidase Inhibitors

Many studies have been conducted recently in an attempt to find alpha glucosidase inhibitors from natural sources, such as plants. Many promising compounds have been identified, particularly secondary metabolites, including phenols, terpenoids, alkaloids, and flavonoids. However, studies on the topic of triglycosides and their importance in numerous metabolic processes, including the processing of glycoproteins and glycolipids and the intestinal digestion of carbohydrates, have been done. Among the many enzymes, glucosidases are considered a promising therapeutic target because they catalyse the breakdown of glycosidic bonds, which releases glucose from the non reducing end of an oligo- or polysaccharide chain involved in the formation of glycoproteins. Glucosidase inhibitors are now being researched for the treatment of lysosomal storage diseases, diabetes, HIV infection, and metastatic cancer due to their fascinating therapeutic potential. Additionally, useful for investigating biochemical pathways and determining the patterns of structure-activity connections required to model an enzyme's transition state are glucosesidase inhibitors. Among the several types of glucosidase inhibitors, disaccharides, amino sugars, carbasugars, thiosugars, and non-sugar derivatives

have attracted a lot of attention. This study's main goal was to demonstrate the main classes of glucosidase inhibitors and how they affect α- and β-glucosidases biologically [33]. (Figure 2.7)

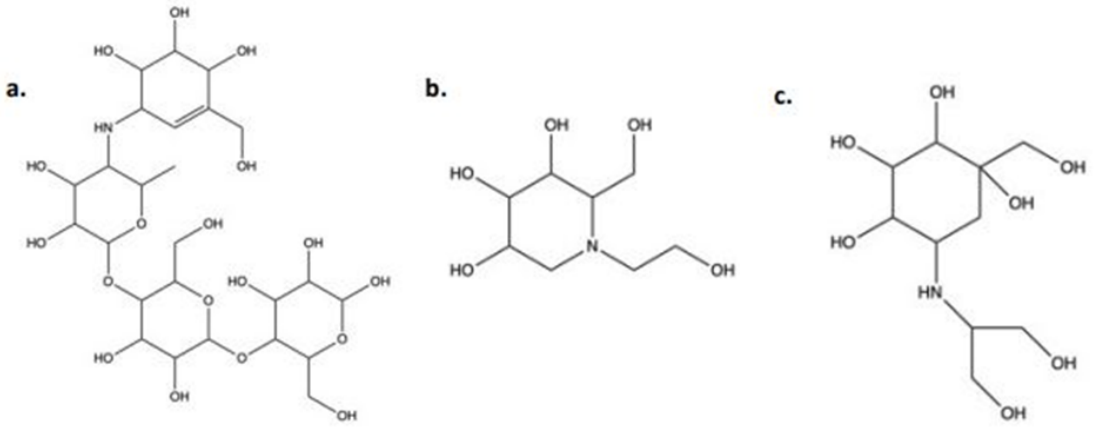


FIGURE 2.7: Structures of anti-diabetic medications: (a) acarbose, (b) miglitol, and (c) voglibose

2.6 T2DM Control & the Metabolism of Glucose

2.6.1 Metabolism of Glucose

The main function of glucose, a monosaccharide, is to serve as the body's major metabolite for the creation of energy. Simple sugars like glucose, fructose, and galactose are produced when complex carbs are broken down [34]. The production, storage, and absorption of glucose are controlled by the pancreas, liver, and small intestine.

The production, storage, and absorption of glucose are controlled by the pancreas, liver, and small intestine. The ratio of glucose entering the bloodstream to glucose leaving it is what determines blood glucose concentration.

Blood glucose levels are regulated by hormones such glucagon, cortisol, and insulin [35]. These hormones control the uptake of glucose into cells and have an impact on glycolysis, gluconeogenesis, and glycogenolysis, among other metabolic activities. The primary catabolic process in the human body is glycolysis. The primary

catabolic process in the human body is glycolysis. It produces ATP (adenosine triphosphate)—energy—by using glucose as its substrate [36].

TABLE 2.1: The metabolic pathways affecting the amount of blood glucoses [34]

Process	**Description**	**Glucose**	**Organ**
Glycolysis	When glucose molecules break, two three-carbon units are produced (pyruvate).	↓glucose	Liver
Gluconeogenesis	From non-carbohydrate and carbohydrate precursors, new glucose molecules are produced.	↑glucose	Liver Kidney
Glycogenolysis	When glucose is broken down from glycogen,	↑glucose	Liver Muscle
Glycogenesis	Glycogen synthesis involves the addition of glucose molecules to chains of glycogen.	↓glucose	Liver

2.6.2 Insulin and the regulation of blood sugar levels

The pancreatic islets of Langerhans' β-cells produce the peptide hormone insulin (Figure 2.8). In order to maintain appropriate blood glucose levels, this hormone is essential for promoting cellular glucose absorption and controlling the metabolism of fats, proteins, and carbohydrates. The principal factor governing the synthesis and release of insulin is the level of glucose. Insulin secretion can also be influenced by other hormones such growth hormone, melatonin, leptin, and estrogen [37].

Pancreatic β-cells are triggered to release insulin in response to rising glucose levels, which in turn facilitates glucose uptake into cells and inhibits the body's synthesis of glucose [38]. Particular receptors on the surface of target cells in skeletal muscle, liver, and adipose tissue are bound by insulin. The translocation of glucose transporters (GLUT) to the cell membranes is facilitated by the phosphorylation events that are set off by this binding. Blood glucose levels are lowered as a result of improved cellular glucose uptake made possible by the increased presence of glucose transporters.

Insulin resistance, or the decreased sensitivity of organs like the liver, adipose tissue, and skeletal muscles to the actions of insulin, is a hallmark of type 2 diabetes mellitus (T2DM). This resistance causes these tissues to absorb less glucose and increases the production of gluconeogenesis in the liver [39], which ultimately causes hyperglycemia.

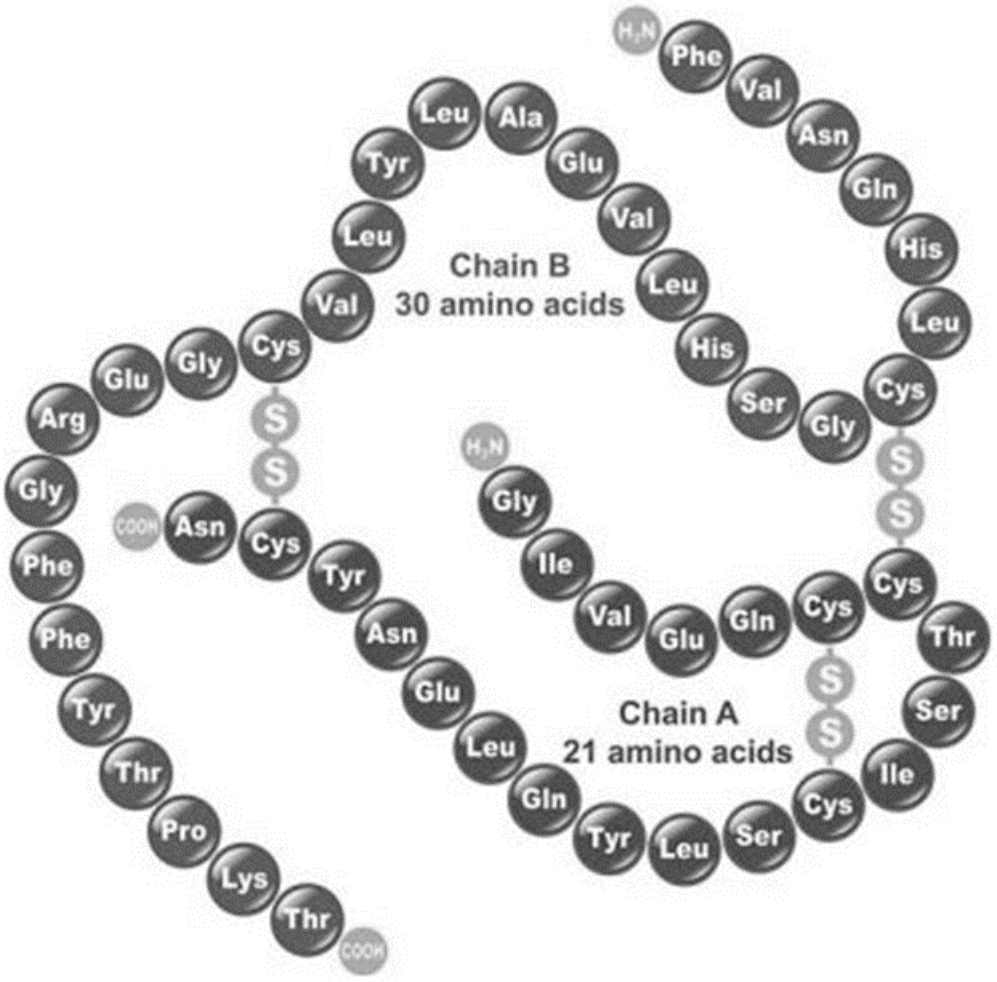


FIGURE 2.8: The Insulin Structure [40]

Insulin controls the glucose in blood using the two important organs in the body that is in the muscles as well as in our liver. It is secreted by the beta cells of pancreas. Following tasks are produced by the insulin; At first, uptake of glucose inside the cell through insulin. Conversion of glucose into glycogen (glycogenesis process) means glucose level is dropping. Preventing any other process which produces glucose inside the cell by breaking glycogen so, the process is called gluconeogenesis [12].

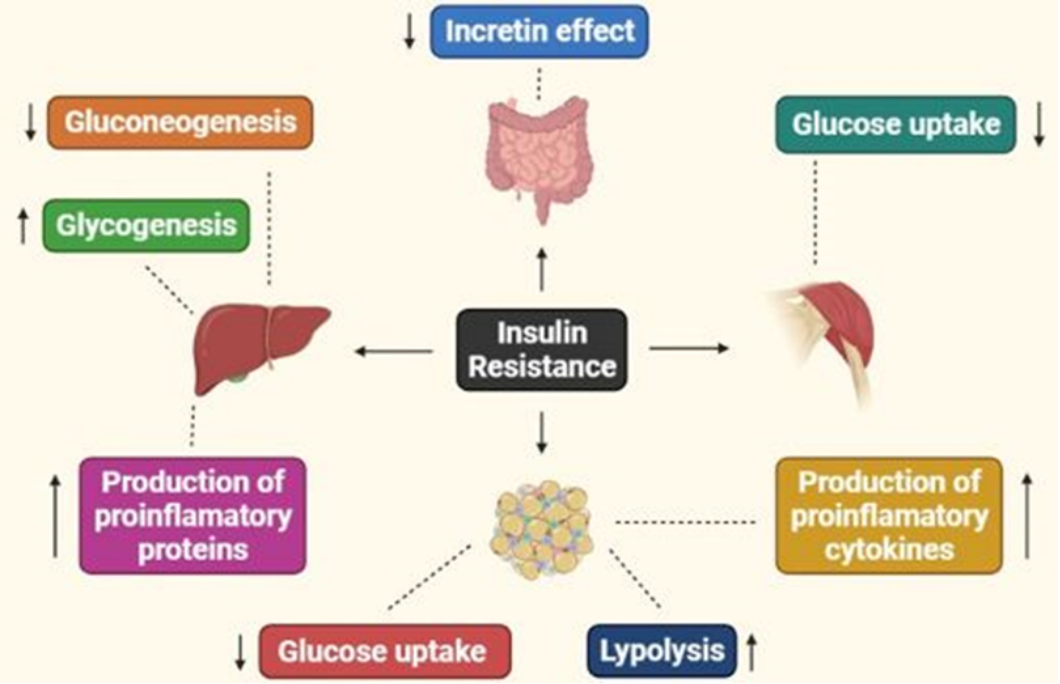


FIGURE 2.9: Showing the way insulin resistance operates in the human body [28]

The function of pancreatic hormones is to initiate the translocation of the glucose transporter isoform 4 (GLUT4) from the interior to the exterior of the cell. This mechanism allows glucose to be rapidly stored in muscle and adipose tissue cells in an insulin-dependent manner (Fig. 2.9). Insulin resistance in people with type 2 diabetes is primarily caused by abnormalities in the processes involved in glucose uptake [7].

2.6.3 Insulin Signaling Pathways

Insulin can be achieved through different signaling processes. There are many phytochemicals derived from plants, including Alkaloids, tannins, glycosides, phenols, anthraquinones, terpenoids, flavonoids, and steroids, activate diverse insulin signaling. Figure 2.10 showing the way insulin resistance operates in the human body pathways [41].

These insulin receptors are made up of two β-subunits that bind insulin and two β-subunits that have tyrosine kinase activity. They are members of the receptor

tyrosine kinase family. Tyrosine residues on the intracellular regions of the β subunits can be phosphorylated by insulin when it interacts to its receptor, causing a conformational shift. This phosphorylation increases the insulin receptor's kinase activity by providing access to ATP and substrate-binding sites. The insulin receptor and intracellular molecules with phosphotyrosine-binding (PTB) domains, including insulin receptor substrate (IRS-1), are connected via specific tyrosine sites that are close to the cell membrane [42].

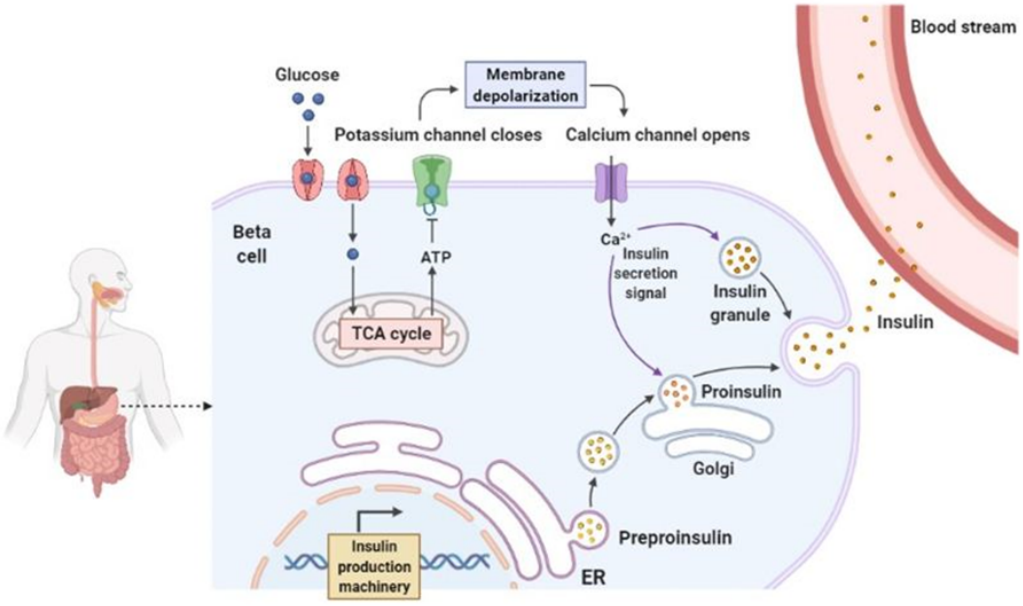


FIGURE 2.10: Illustrating the pathways involved in glucose-induced insulin secretion [28]

In the glucose regulation pathway, the activation of IRS-1 is initiated through its binding with insulin, instigating a cascade of signaling events. This then triggers the activation of other kinases, including phosphoinositide 3-kinase (PI3K) and protein kinase B (PKB-Akt). Akt is a serine/threonine kinase that is involved in insulin signalling and functions through the PI3K pathway, after receptor protein kinases. Akt activation initiates through its translocation to the cellular external membrane, precipitating a structural change that leads to its phosphorylation. This phosphorylation, in turn, serves as a Figure 2.10. Illustrating the pathways involved in glucose-induced insulin secretion, catalyst for subsequent steps within the insulin signaling cascade [42]. A class of transcription factors called peroxisome proliferator-activated receptors (PPARs) is crucial for regulating lipid and glucose

metabolism. These receptors are notably present in pancreatic beta cells, where they exert control over insulin secretion [43].

2.6.4 Insulin Secretion

Insulin is produced by pancreatic β-cells at a baseline rate and at significantly higher rates in response to glucose and other stimuli. Elevated blood glucose levels cause cells' levels of adenosine triphosphate (ATP) to rise, which inhibits ATP-dependent potassium channels. This closure results in a decrease in outward potassium current, depolarizing the β-cell and opening voltage-gated calcium channels. Hormone release is triggered by the subsequent increase in intracellular calcium levels. Insulin, also known as glutathione insulin transhydrogenase, is mainly responsible for removing insulin from the bloodstream through the liver and kidneys. It is thought that hydrolyzation of the disulfide bond between the A and B chains is occurring here. Proteolysis is used to carry out further degradation after this decrease. Because the liver is the primary target for blood flow from the portal vein, it normally removes around 60% of the insulin generated by the pancreas, whereas the kidneys only remove 35–40% of the hormone. This distribution is changed in diabetic individuals getting subcutaneous insulin injections, since the liver removes no more than 30–40% of the exogenous insulin while the kidneys remove 60% of it. Insulin has a circulation half-life of around three to five minutes [86].

2.6.5 The Insulin Receptor

Insulin attaches to specific receptors on different cell membranes as it enters circulation. However, the biological consequences of these insulin-receptor complexes have only been observed in specific target organs, such as muscle, adipose tissue, and liver.

With remarkable selectivity and affinity, the insulin receptors bind insulin at pico molar concentrations. Each complete insulin receptor consists of two heterodimers: the alpha subunit, which is entirely extracellular and serves as the recognition site,

and the beta subunit, which crosses the membrane and comprises a tyrosine kinase domain. When insulin binds to the alpha subunit outside of the cell, it initiates tyrosine kinase activity in the beta subunit. Although the beta subunit's dimeric form can bind insulin, its affinity for doing so is much lower than that of the tetrameric form. The beta subunit's self-phosphorylation promotes the formation of beta heterodimers as well as the maintenance of the receptor tyrosine kinase's activated state. Insulin receptor concentration is lower in clinical situations including obesity and insulinoma that are marked by high circulating insulin levels. Target cells appear to use this natural process of downregulating insulin receptors to limit their responsiveness to high hormone concentrations [86].

2.6.6 Effects of Insulin on Its Targets

Insulin promotes the storage of fat and glucose inside certain target cells and has an impact on cell division and metabolic activities in a variety of organs.

2.6.7 Action of Insulin on Glucose Transporters (GLUT)

Insulin has a substantial impact on a number of transport molecules that help move glucose across cell membranes. Diabetes is linked to these transporters in both its onset and manifestation. Insulin releases GLUT 4, a crucial factor in lowering blood glucose levels, from intracellular storage vesicles and inserts it into the membranes of muscle and fat cells. Deficits in the transport of glucose into pancreatic β-cells by GLUT 2 may also be a factor in the reduced insulin production seen in type II diabetes [86].

2.6.8 Action of Insulin on the Liver

When endogenous insulin reaches the portal circulation, it mostly affects the liver. Here, it plays a role in stimulating the storage of glucose as glycogen and reestablishing a fed state in the liver by inhibiting multiple catabolic processes, including ketogenesis and glycogenolysis.

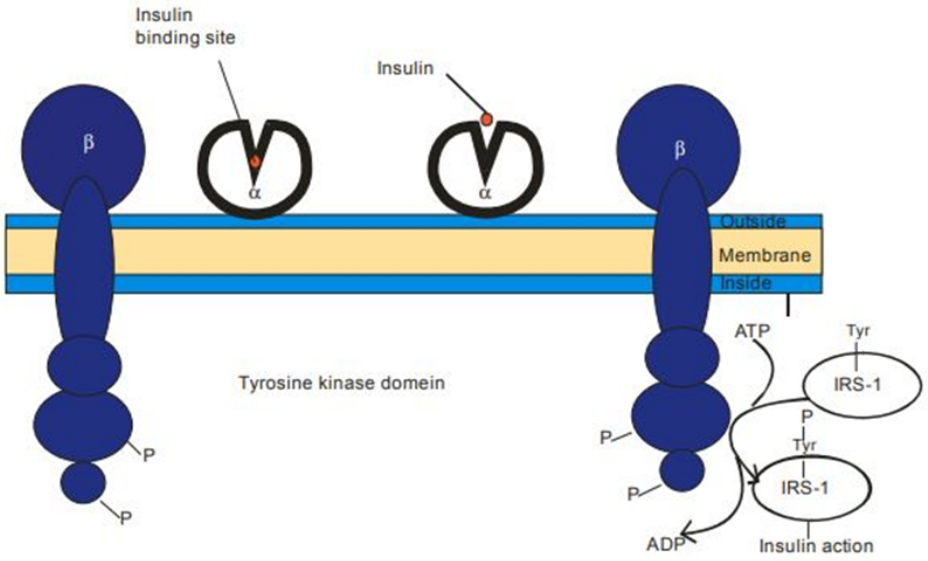


FIGURE 2.11: Schematic diagram of the probable structure of the insulin receptor tetramer in the activated state [86]

Insulin exerts its effects on the liver by directly inducing phosphorylation events. This activation enhances enzymes like pyruvate kinase, phosphofructokinase, and glucokinase, which promote glucose storage and utilization pathways. On the other hand, gluconeogenic enzymes that are normally active during the post-absorptive state—such as fructose bisphosphatase, phosphoenolpyruvate carboxykinase, and pyruvate carboxylase—are inhibited by insulin. Insulin directly induces phosphorylation processes, which impact the liver. This activation increases the activity of enzymes such as phosphofructokinase, glucokinase, and pyruvate kinase that support the pathways involved in the storage and utilisation of glucose. Insulin, on the other hand, inhibits gluconeogenic enzymes that are typically active during the post-absorptive state, including pyruvate carboxylase, fructose bisphosphatase, and phosphoenolpyruvate carboxykinase. (Figure 2.11)

2.6.9 Effect of Insulin on Muscle

Insulin increases amino acid transport and ribosome activity to promote protein synthesis. In order to restore glycogen reserves lost by muscular exercise, it also promotes the synthesis of new glycogen. Glycogen synthase is activated, glycogen

phosphorylase is inhibited, and glucose transport into muscle cells is increased during this process [87].

2.6.10 Effect of Insulin on Adipose Tissue

Through three main pathways, insulin decreases blood levels of free fatty acids and increases triglyceride storage in adipocytes: 1. It triggers the strong hydrolysis of circulating lipoproteins to liberate triglycerides by activating lipoprotein lipase. 2. Insulin promotes the absorption of glucose into cells, leading to the metabolic byproduct of glycerophosphate synthesis. This makes it easier to esterify the fatty acids produced by hydrolyzing lipoproteins. 3.Insulin decreases intracellular lipase activity, which in turn prevents stored triglycerides from being lipolyzed within cells [86].

2.6.11 Complications of Insulin Therapy

Oral hypoglycemic agents and insulin are central to diabetes treatment, effectively managing hyperglycemia. However, they are associated with significant side effects and do not substantially alter the progression of diabetic complications [87].

The following are the main side effects of insulin therapy:

1. Hypoglycemia can be brought on by skipping meals, engaging in intense physical activity, or taking more insulin than is necessary at the moment. The primary autonomic warning signals of hypoglycemia and insulin excess are symptoms related to poor central nervous system function, which might include abnormal behaviour, disorientation, and possibly even coma. Regular insulin use can cause rapid-onset hypoglycemia, which can lead to autonomic hyperactivity. This hyperactivity can include sympathetic symptoms (tachycardia, palpitations, sweating, tremors) and parasympathetic symptoms (nausea, hunger). If left untreated, this hyperactivity can escalate to convulsions and coma.

2. Insulin allergy, immunological insulin resistance caused by the development of anti-insulin antibodies, and injection site lipodystrophy are among the immunopathological.

2.7 Phytocompounds

Plant secondary metabolites, or phytochemicals, have been the subject of much research due to their possible benefits in the treatment of type 2 diabetes. Various investigations have provided compelling evidence that these bioactive compounds, found in medicinal plants, can modulate insulin signaling pathways. Notably, the administration of methylswertianin and bellidifolin phytochemicals led to improved insulin sensitivity in individuals with diabetes. The mechanism underlying the action of these phytochemicals involves the upregulation of key proteins in the insulin signaling pathways, namely IR, IRS-1, and PI3K.

Furthermore, both phytochemicals increased the activity of glucose-6-phosphatase (G6Pase) and decreased the activity of glucokinase (GK), which stimulated the release of insulin by pancreatic beta cells. Similar to this, a different study showed that giving two gallotannins raised the expression of GLUT4 and PI3K mRNA.

Furthermore, the bioactive substance 3β-taraxerol worked by improving glucose transport, sustaining glucose consumption, and encouraging the synthesis of glycogen. This study demonstrated that the triterpenoid induced glucose transport via GLUT4 translocation, which was made possible by the AKT protein's PI3K-dependent activation [44].

Astragalus polysaccharide was found to improve insulin sensitivity and glucose homeostasis in the skeletal muscle of people with type 2 diabetes in a previous study. This increase in insulin sensitivity was attributed to the translocation of GLUT4 and the regularisation of insulin-stimulated PKB-Ser473 phosphorylation [25]. In human adipocytes, cyanidin-3-O-β-glucoside and protocatechuic acid showed insulin-like action. By facilitating GLUT4 translocation and increasing adiponectin secretion, these plant chemicals increased glucose absorption and may

have enhanced PPAR-γ activity. Adenosine monophosphate-activated protein kinase (AMPK) was activated by daifzein, which led to the migration of GLUT4 to the muscle cell's plasma membrane. Catalpol, specioside, and verminoside are examples of iridoid chemicals that significantly activated GLUT4 in the cell's outer layer, allowing intracellular glucose uptake [45].

Furthermore, it was demonstrated that oleanolic acid and ursolic acid were competitive inhibitors of PTP1B, enhancing the absorption of glucose and increasing the expression of the phosphorylated insulin receptor. Vanillic acid and berberine significantly increased GLUT4 translocation through an AMPK-dependent mechanism, while arecoline had the same effect through the PPAR-γ pathway. Moreover, the fungal metabolite dimethyl asterrriquinone-B directly stimulated the AKT, ERK, and IRTK pathways, producing glucotropic actions similar to those of insulin without inducing mitosis [46].

In addition to the bioactive compounds mentioned earlier, numerous other phytochemicals, distinct from flavonoids, have been studied for their anti-hyperglycemic properties in previous research. Extracted from Piper retrofractum, alkaloid substances including piperine, pipernonaline, and dehydropipernonaline have been shown to activate the PPAR-γ protein and the AMPK signalling pathway. Another xanthone chemical that has been found to be an anti-hyperglycemic plant biomolecule is mangiferin.

Mangiferin has been demonstrated in experiments employing Salacia oblonga extract to upregulate GLUT4 protein expression and enable its translocation to the cell surface in L6-myocytes and 3T3-L1 adipocytes, hence improving these cells' absorption of glucose. These signalling pathways are essential for controlling the diabetic pathophysiological circumstances.

Bioactive compounds found in food, often present in small amounts, such as polyphenols, omega-3 fatty acids, carotenoids, organic acids, vitamins, phytosterols, and nucleotides, have the ability to activate one or more signaling pathways [47].

2.8 *Adhatoda vasica*

Adhatoda vasica is a member of the Acanthaceae family and goes by a number of common names, including Baker, Malabar Nut, and Vasaka. It is an evergreen shrub with an awful odour and a bitter flavour that grows to a height of 1.0 to 2.5 metres. Significant antioxidant activity has been observed for phenolic compounds found in *A. vasica*, which effectively scavenge free radicals. There is a great deal of interest in researching the plant's phytochemicals and active ingredients in hopes of finding new drugs because of its therapeutic qualities [48].

2.8.1 Plant Description

The stem is herbaceous above and woody below, with huge, lance-shaped leaves. The leaves are not stipulated and are positioned opposite one another. The flowers are tiny, irregular, zygomorphic, bisexual, hypogynous, and form spikes or panicles [49]. The plant produces capsular fruits with four seeds. Purple and white are the two colour options for flowers. Its Sanskrit name is where its common name, Vasaka, comes from [50]. The inflorescences are densely flowered, borne in axillary spicate cymes, and have short peduncles and broadly oval, foliaceous bracts. Many people use leaves, blossoms, fruits, and roots to treat whooping cough, colds, chronic bronchitis, and asthma. They are also used for their sedative, expectorant, and antispasmodic qualities [51].

2.8.2 The Role of Bio-active Components of *Adhatoda vasica*

A recent study employed a variety of analytical approaches, including phytochemical screening, thin-layer chromatography, column chromatography, protease activity evaluation, and antioxidant, antidiabetic, and anti-inflammatory activity, to investigate the fractions obtained from *Adhatoda vasica* leaves. Alkaloids, flavonoids, coumarins, terpenoids, steroids, emodin, and quinones were found in

the plant extract according to the results of the phytochemical screening. Purification of the bioactive chemicals was achieved by column chromatography. When various solvent solution with varied polarity were used for thin-layer chromatographic experiments, clear bands were formed at 254 nm and 366 nm.

Significant quantities of protease activity were also detected in the fraction that had been purified. The highest anti-inflammatory activity was found in fraction 3 (75%), followed by fraction 5 (62.73%), in vitro studies using albumin denaturation. In the membrane stabilization experiment, fraction 5 (88%) and fraction 7 (87.68%) had the highest levels of proteinase inhibitory activity and activity, respectively, at 500 μg/ml; fraction 6 (80.23%) and fraction 3 (64.65%) displayed the highest levels of activity. Aspirin, a common drug, has an anti-inflammatory impact of 90.87%. Fractions 4 (79.05%) and 5 (77.05%) had the highest levels of in vitro antidiabetic efficacy at 500 μg/ml using the alpha-amylase inhibition assay. In the reducing power experiment, which measured antioxidant activity, fraction 2 had the highest absorbance (1.04), at 500 μg/ml. Subsequently, the fraction that was extracted from the column had greater absorbance (0.93) in comparison to the standard ascorbic acid [52].

According to research performed by study, *Adhatoda vasica* leaf and flower extracts had higher concentrations of bioactive substances. Following chemical analysis, *Adhatoda vasica* leaves showed increased levels of total antioxidants (651% DPPH inhibition), total carotenoids (1987 mg/100 g), catalase (4716μg/g), ash content (16.72%), total phenolic compounds (71.32 mg GAE/g), and the enzymes peroxidase (1322μg/g) and superoxide dismutase (4566μg/g). *Adhatoda vasica* flower extract contained a high concentration of flavonoids (0.87 mg/100 g) and organic matter (89.99%). Complete randomized design (CRD) and factorial configurations were used to evaluate the data collected for each parameter. *Adhatoda vasica* and Calotropis procera were compared on a mean basis at a 5% probability level using the LSD test. Their research revealed that these phytochemicals may lend credence to the hypothesis that these medicinal plants possess the ability to generate novel pharmaceuticals and can be employed as herbal remedies to address a variety of viral and cancerous illnesses. These substances are advantageous for the treatment of cancers as well [52].

2.8.3 Phytochemistry

The extensive variety of pharmacological applications of *Adhatoda vasica* is believed to be attributed to its high content of alkaloids [53, 54]. *Adhatoda vasica* leaves contain the primary quinazoline alkaloid, vasicine. *Adhatoda vasica* leaves and roots also contain alkaloids, such as l-vasicinone, deoxyvasicine, maiontone, vasicinolone, and vasicinol, in addition to vasicine. Studies indicate that these compounds are responsible for the bronchodilatory effect of *Adhatoda vasica* [55, 56]. (Figure 2.12)

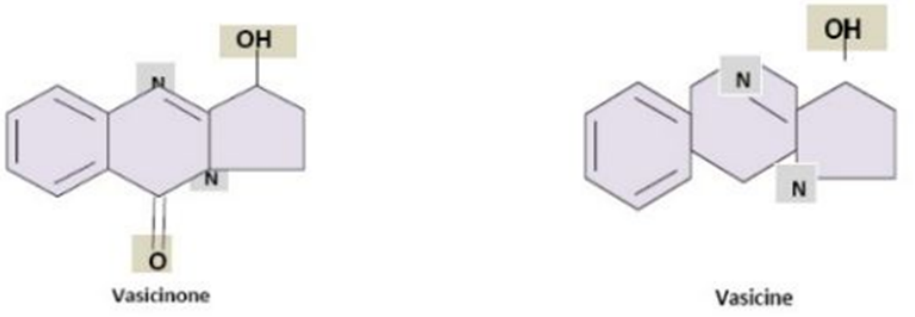


FIGURE 2.12: Vasicinone and Vasicine's Chemical Structure in *A.vasica*[55]

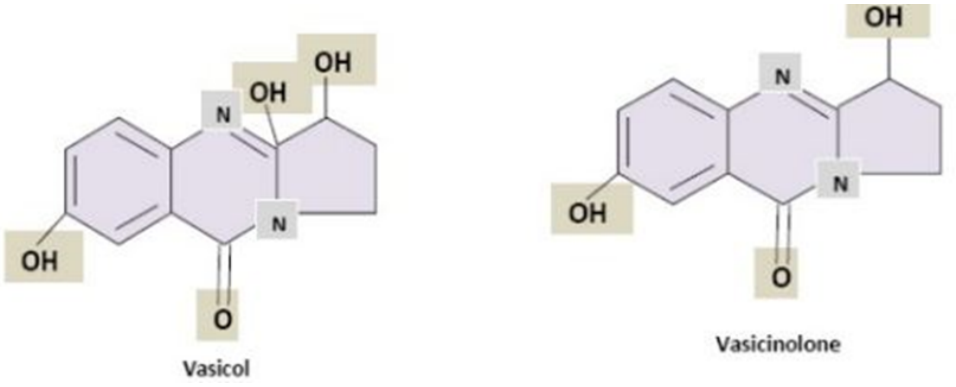


FIGURE 2.13: Chemical Structure of Vasicol and Vasiciolone in *A.vasica* [55]

Alkaloids, tannins, saponins, phenolics, steroids, reducing sugars, and flavonoids are among the substances present has been identified through phytochemical screening of *A. vasica*. The main alkaloids included in *A. vasica* are quinazoline alkaloids, which include vasicine, vasicoline, vasicol, vasicinone, vasicinol, adhatodine, adhatonine, adhavascinone, etc. The abundance of these bioactive phytochemicals confers various pharmacological activities to *A. vasica*, including antimicrobial, insecticidal, antipyretic, hepatoprotective, respiratory ailment-fighting, anti-diabetic,

anti-tubercular, anti-cancer, radioprotective, and anti-ulcer properties [57]. (Figure 2.13)

2.9 Pharmacological Activity

2.9.1 Anti-asthmatic and Bronchodilator Activity

Adhatoda vasica is a traditional medicinal herb that has been used historically to treat respiratory ailments. The two primary alkaloids in *Adhatoda vasica*, vasicine and vasicinone, are known to have beneficial benefits on respiratory conditions. *Adhatoda vasica* leaf and root extracts are useful in treating bronchitis, lung and bronchiole diseases, and common colds and coughs [58].

2.9.2 Anti-ulcer Activity

Studies looked into *Adhatoda vasica*'s ability to prevent ulcers brought on by aspirin, pylorus, and ethanol. In experimental rats, *Adhatoda vasica* leaf powder showed a substantial anti-ulcer effect when compared to control groups [59].

2.9.3 Anti-allergy Action

At a dosage of 5 mg, the extract, which contains 20% vasicine and the vascinol alkaloid, reduced ovalbumin-induced allergic responses by about 37%. [60].

2.9.4 Antitubercular Action

Adhatoda vasica alkaloids include vasicine, which is the precursor of the widely used mucolytics ambroxol and bromhexine. These two substances have an inhibitory effect on Mycobacterium TB growth that is pH-dependent. *Adhatoda vasica* may play a major role as an adjuvant in the treatment of tuberculosis because of its

indirect effects on the disease, which include enhanced levels of lysozyme and rifampicin in lung tissue, sputum, and bronchial secretions [61].

2.9.5 Anti-bacterial Activity

By employing paper disc and dilution methods, the leaf extract was used for in vitro screening. The outcomes shown significant impacts on strains of Gram-positive bacteria such *Streptococcus faecalis*, *Staphylococcus aureus*, and *Staphylococcus epidermidis*, as well as the Gram-negative bacterium *E. coli*, and considerable antibacterial activity, especially against *Pseudomonas aeruginosa* [62]. Moreover, research indicates that *Adhatoda vasica* have strong antibacterial properties, particularly against *Pseudomonas aeruginosa* infections. Phenols, terpenoids, alkaloids, steroids, and saponins were found in the plant extract after preliminary phytochemical screening. A study was conducted to examine the antibacterial properties of ethanol and leaf extracts from *Adhatoda vasica*. *Pseudomonas aeruginosa* has the biggest zone of inhibition, according to the data. Furthermore, the biggest zone of inhibition was seen in the antifungal activity against *Aspergillus clavatus*. According to the findings, *Adhatoda vasica* can be utilised as a bioactive chemical source to create new drugs, especially ones that are intended to cure microbial infections [62].

2.9.6 *Adhatoda vasica*'s Protective Role in Liver Damage Caused by Anti-TB Drugs

A study found that the herb *Adhatoda vasica* Nees, which is commonly used in traditional medicine, has shown promise in the treatment of several illnesses, including infections of the upper respiratory tract. It is well renowned for its capacity to fight tuberculosis and shield the liver from harm from specific drugs. On the other hand, medications used to treat tuberculosis, such as isoniazid, rifampicin, and pyrazinamide (H-R-Z), might damage the liver. We still don't fully understand all of these medications' metabolic processes, despite the fact that we have a wealth of information regarding their negative effects. In this study, rats were

given medicines H, R, and Z over a period of 25 days, creating a model of liver injury. Additionally, they gave two separate dosages of an ethanolic *Adhatoda vasica* Nees leaf extract (200 and 300 mg/kg body) to investigate its protective effect on the liver. They used FTIR and RP-HPLC techniques to confirm the extract's chemical composition and provide evidence for their conclusions. They also used a DPPH assay to evaluate the antioxidant capabilities of the extract [88]. The study assessed a number of indicators associated with oxidative stress in the liver, antioxidants, alterations in liver tissue, and gene expressions linked to the body's metabolism of foreign substances. CYP2E1, CYP7A1, NAT, NR1I2, and UGT1A1 were among these genes. The RP-HPLC method yielded a concentration of 134.519 $\pm$ 0.00269 μg/10mg for vasicine, while the DPPH assay showed that it could scavenge free radicals at a rate of 47.81 μg/mL.

In addition to noticeable alterations in liver structure, the H-R-Z therapy group saw considerable changes in enzyme, thiobarbituric acid, and GSH levels. The H-R-Z group also showed much lower expression of the CYP2E1 gene, negligible expression of the NR1I2 and UGT1A1 genes, and significantly greater levels of the NAT and CYP7A genes. On the other hand, a larger dosage of A. vasica extract successfully mitigated these effects by lowering oxidative stress and raising antioxidant levels. It also acted as a dual activator of both phase II (NAT and UGT1A1) and phase I (CYP2E1) enzyme systems. When anti-TB medications are used, their own metabolism can be affected, which can result in the generation of toxic metabolites that damage the liver by upsetting the equilibrium between oxidants and antioxidants. By examining A. vasica's possible defenses against these negative consequences, this study shed light on liver damage associated with the metabolism of foreign chemicals [88].

2.9.7 *Adhatoda vasica*'s Role in Alzheimer's Disease Treatment

Researchers are searching for novel molecules for the treatment of Alzheimer's disease (AD), a neurodegenerative condition marked by memory and cognitive

impairment, as there are currently no viable treatments for AD. *Adhatoda vasica* Nees (AV) leaves, probably due to their high amount of pyrroloquinazoline alkaloids, have shown promise in the fight against AD. However, not much is understood about the specific anti-AD mechanisms that these alkaloids utilize. To investigate the potential anti-AD benefits of the active pyrroloquinazoline alkaloids, the study separated them from AV using a bioactivity-guided fractionation approach.

The researchers employed column chromatography to isolate alkaloids from the dichloromethane (DCM) fraction of the methanolic AV extract. Various techniques, including ATR, HRMS, NMR, TLC, HPLC, and ATR, were utilized to characterize the isolated chemicals. In silico studies were conducted to assess how these compounds interact with cholinesterase enzymes (AChE and BuChE). Additionally, in vitro tests were performed to evaluate their potential to inhibit AChE, BuChE, and Aβ aggregation. The propidium iodide displacement assay confirmed the compounds' selectivity at the AChE PAS site. Furthermore, in vivo tests were conducted on rats with scopolamine-induced amnesia and Aβ-induced neurotoxicity to evaluate improvements in memory and cognitive function [42]. By using bioactivity-guided fractionation, the study found that the main substances causing cholinesterase inhibition in the DCM fraction were vasicinone (VAS) and vasicine (VA).

Both VAS and VA established strong linkages in the active regions of AChE and BuChE, according to in silico studies. These substances successfully prevented the aggregation of AChE, BuChE, and Aβ by dislodging propidium iodide in the AChE PAS site, as shown by in vitro tests. VAS and VA significantly improved both memory and cognitive function in in vivo trials on rats with scopolamine-induced amnesia and Aβ-induced cognitive and memory deficits. Moreover, VAS and VA were found to be safe while helping AD-affected rats' hippocampal cell density to return. Based on this research, VAS (from *Adhatoda vasica* Nees) is an effective anti-AD medication that shows similar effectiveness to VA in reducing the memory and cognitive impairments linked to AD. It is highly promising to continue preclinical research on VAS and VA as natural treatments for Alzheimer's disease [63].

2.9.8 The Role of Bio-active components of *Adhatoda vasica*

A recent study employed a variety of analytical approaches, including phytochemical screening, thin-layer chromatography, column chromatography, protease activity evaluation, and antioxidant, antidiabetic, and anti-inflammatory activity, to investigate the fractions obtained from *Adhatoda vasica* leaves. Alkaloids, flavonoids, coumarins, terpenoids, steroids, emodin, and quinones were found in the plant extract according to the results of the phytochemical screening. Utilizing column chromatography, bioactive compounds were purified. When various solvent solutions with varied polarity were used for thin-layer chromatographic experiments, clear bands were formed at 254 nm and 366 nm. Significant quantities of protease activity were also detected in the fraction that had been purified. The highest anti-inflammatory activity was found in fraction 3 (75%), followed by fraction 5 (62.73%), in vitro studies using albumin denaturation. In the membrane stabilization experiment, fraction 5 (88%) and fraction 7 (87.68%) had the highest levels of proteinase inhibitory activity and activity, respectively, at 500 μg/ml; fraction 6 (80.23%) and fraction 3 (64.65%) displayed the highest levels of activity. Aspirin, a common drug, has an anti-inflammatory impact of 90.87%. Fractions 4 (79.05%) and 5 (77.05%) had the highest levels of in vitro antidiabetic efficacy at 500 μg/ml using the alpha-amylase inhibition assay. In the reducing power experiment, which measured antioxidant activity, fraction 2 had the highest absorbance (1.04), at 500 μg/ml. Subsequently, the fraction that was extracted from the column had greater absorbance (0.93) in comparison to the standard ascorbic acid [64].

According to research performed by [44] study, *Adhatoda vasica* leaf and flower extracts had higher concentrations of bioactive substances. Following a chemical analysis of the samples, *Adhatoda vasica* leaves showed greater levels of total antioxidants (651% DPPH inhibition), total carotenoids (1987 mg/100 g), catalase (4716μg/g), ash content (16.72%), total phenolic compounds (71.32 mg GAE/g), superoxide dismutase (4566μg/g), and peroxidase (1322μg/g). *Adhatoda vasica* flower extract contained a high concentration of flavonoids (0.87 mg/100 g) and

organic matter (89.99%). Complete randomized design (CRD) and factorial configurations were used to evaluate the data collected for each parameter. *Adhatoda vasica* and Calotropis procera were compared on a mean basis at a 5% probability level using the LSD test. Their research revealed that these phytochemicals may lend credence to the hypothesis that these medicinal plants possess the ability to generate novel pharmaceuticals and can be employed as herbal remedies to address a variety of viral and cancerous illnesses. These substances are advantageous for the treatment of cancers as well [65].

2.9.9 Nature's Defense: *Adhatoda vasica* as an Antioxidant Solution

The goal of the study was to look into naturally occurring plant-based antioxidants as possible therapies for illnesses linked to oxidative stress brought on by free radicals and reactive oxygen species inside living cells [66]. Specifically, the research focused on *Adhatoda vasica*'s ethanolic leaf extract (ELEAV), evaluating its phytochemical content, antioxidant capacity, and free radical scavenging abilities using various assessment techniques. Phytochemical analysis revealed that ELEAV contained phenols, steroids, alkaloids, flavonoids, terpenoids, and saponins. ELEAV exhibited strong antioxidant properties, demonstrating a 69.23% inhibition of the 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical. It also showed significant inhibitory effects on scavenging nitric oxide (65.24%), superoxide (61.54%), and hydroxyl radicals (61.24%). Furthermore, ELEAV shown a concentration-dependent capacity to efficiently prevent lipid peroxidation in bovine brain extract caused by ferric ions (68.26%). These results highlight the potential of A. vasica as a naturally occurring source of antioxidants with notable biological activity [66].

2.9.10 Alpha-Amylase Inhibition by *Adhatoda vasica*

Microvascular complications such retinopathy, neuropathy, nephropathy, and stroke are more common in diabetics and can cause serious health issues or even death.

Despite the fact that there are many synthetic anti-diabetic medications available, they can be expensive and have undesirable side effects. This led to the conduct of a study that examined the molecular composition of an ethanol-based extract derived from *Adhatoda vasica* leaves and assessed the extract's ability to inhibit alpha-amylase both in vitro and in silico. Ethanol and the Soxhlet extraction method were used to extract the leaves of *Adhatoda vasica*. Acarbose, sitagliptin, and the ethanolic extract were all manufactured at various concentrations between 0.1 and 1 mg/ml. Alpha-amylase-blocking potential of each concentration was evaluated using a spectrophotometric technique. For molecular docking, SToPToX was utilized, and for toxicity profiling, Autodock Vina. The plant extract exhibited the highest alpha-amylase inhibition (56.763±0.0035) at a dose of 1 mg/ml [67]. This inhibitory effect was confirmed by the in-silico investigation. When *Adhatoda vasica*'s two active components, vitexin (C9) and vitecol (C5), interacted with the alpha-amylase enzyme (PDBID: 4W93), they showed the greatest binding energies, at 8.0 and 8.3 Kcal/mol, respectively. This study used dynamic simulations to confirm that these ligands attach to the protein's active site. The plant extract's safety was indicated by the toxicity assessment. In conclusion, the bioactive substances found in *Adhatoda vasica* leaves regulate blood sugar. It is possible that the identification, purification, and isolation of these chemicals will result in the creation of novel drugs with fewer adverse effects [67].

2.9.11 Anti-inflammatory Attributes of *Adhatoda vasica*

In a study, researchers first dissolved 1.08 grams of plant powder in 100 milliliters of distilled water to test the zinc nanoparticles generated from *Adhatoda vasica* for their ability to reduce inflammation and increase antioxidant activity. After the solution was heated for ten minutes and filtered, the resulting plant extract was used for the green synthesis. They combined 50 milliliters of *Adhatoda vasica* extract with 50 milliliters of zinc nanoparticles and used a UV-Vis spectrometer to analyze the nanoparticles. The study aimed to assess the anti-inflammatory and antioxidant properties of these zinc nanoparticles enhanced by *Adhatoda vasica*,

comparing their effectiveness with traditional drugs across concentrations ranging from 10 μl to 50 μl. The findings highlighted the significant anti-inflammatory and antioxidant effects of zinc nanoparticles influenced by *Adhatoda vasica*. The study also revealed that these effects were more pronounced at higher concentrations. Therefore, this natural medicine-based approach holds promise as an effective treatment for various ailments [68].

2.9.12 *Adhatoda vasica*'s Anti-Inflammatory Role in Diabetic Wound Healing

In response to harmful stimuli, the body releases extra immune cells and plasma into the injured tissue as part of its initial inflammatory response. By initiating a signaling cascade that includes growth factors and cytokines, this ultimately encourages the regeneration of wounded tissue at the site, especially involving blood vessels and immunological markers. Chronic conditions like diabetic foot ulcers, which may need limb amputation or even result in death, can be significantly influenced by persistent and unrelenting inflammation. Nonetheless, this led to a study into the mechanism of action of *Adhatoda vasica*'s alkaloid fraction ALK-F to lower nitric oxide formation as determined by the Griess assay. Moreover, ELISA has been used to measure TNF-α and IL-6 expression. Moreover, the expression of COX-2 and iNOS in LPS-stimulated RAW 264.7 macrophages has been studied using western blotting and RT-PCR. Vasicine, a quinazoline alkaloid, was found to be present in ALK-F by means of high-performance liquid chromatography (HPLC). DCFH-DA probing is used. The total amount of intracellular ROS was determined by the study. When triggered by LPS in a dose-dependent manner, ALK-F from A. vasica was found to dramatically inhibit nitrite synthesis (13.2 $\pm$ 1.06 μM), iNOS, and COX-2 (2.6 and 3.3-fold, respectively). At the highest dosages tested (1 μg and 10 μg), ALK-F from A. vasica also significantly reduced the overall creation of intracellular ROS and the production of pro-inflammatory cytokines TNF-α (1102 $\pm$ 1.02 pg/mL) and IL-6 (18 $\pm$ 0.87 ng/mL). It was discovered that the crude alkaloid fraction contained 12% of the quinazoline alkaloid vasicine through the use of gradient elution in HPLC analysis.

Collectively, the research results indicate that A. vasica inhibits nitric oxide and modifies genes associated with inflammation, two important mechanisms via which it mitigates inflammation and offers therapeutic advantages for the treatment of diabetic wounds [69].

2.9.13 Cytotoxic Effects of *Adhatoda vasica* Compounds on Cancer Cells

The National Institute of Cell Sciences in Pune conducted research on Vasicine Acetate, a synthetic derivative of Vasicine from *Adhatoda vasica*, to explore its antibacterial, antioxidant, and cytotoxic properties using the human adenocarcinoma cell line A549. The full DMEM medium with 10% foetal bovine serum, 2 mM L-glutamine, and antibiotics (about 100 μg/mL streptomycin and 100 IU/mL penicillin) was used to cultivate these cells. The pH was then adjusted to 7.2. To assess cytotoxicity, a modified version of the technique was used. In particular, 96-well plates were seeded with 5 $\times$ 10$\wedge$3 cells per well at different test sample doses (2000, 1000, 500, 250, 125, and 62.5 μg/mL). After that, the cells were incubated at 37^{o}C in an environment with 100% relative humidity, 95% air, and 5% CO2. Following the designated periods of incubation, 100 μL of a medium containing 1 mg/mL of 3- (4, 5-dimethylthiazol-2-yl) -2,5- diphenyltetrazolium bromide (MTT) was added to each well. Following a 4-hour incubation period, 100 μL of DMSO was added after aspirating the solution. Using an ELISA reader, the absorbance at 540 nm was measured to determine the cytotoxicity against cancer cells. The IC50 value, or the test sample concentration that results in a 50% inhibition of cell growth, was calculated using the average of three separate trials. The outcome of this study suggests that vasicine acetate has cytotoxic effects on the lung adenocarcinoma cancer cell line A549 [89].

2.9.14 Effects of *Adhatoda vasica* on Ovarian Cancer

More women die from ovarian cancer than from any other malignancy that affects the reproductive system. The main cause of this is the lack of trustworthy

screening methods, which results in diagnosis at a later stage. Less than thirty percent of patients make it to the five-year mark, and conventional treatments like chemotherapy and surgery frequently have ineffective outcomes. Additionally, an increasing amount of evidence indicates that flavonoids, which are naturally occurring substances present in diets based on plants, may have anticancer effects due to their antioxidative, antiestrogenic, antiproliferative, and anti-inflammatory qualities [90]. In an attempt to reduce it, a study was conducted to investigate the possible anticancer properties of crude ethanol extracts derived from *Adhatoda vasica* leaves. The researchers found the LC50 value of A. vasica using a cell-based assay, and it showed anticancer potential, opening the door for more research. The metastatic properties of PA1 cells were assessed 0 hours, 24hours, and 48 hours after A. vasica extracts were given to the cells at the LD50 value. To assess the expression of crucial genes including p53, p21, and GAPDH, the mRNA extracted from teratocarcinoma PA1 cells treated with A. vasica extract was reverse transcribed into cDNA and amplified. A. vasica extract is efficient in preventing ovarian cancer, as demonstrated by an examination of the changes in gene expression between treated and untreated control cells. By showing A. vasica's antiproliferative and antimetastatic actions on PA1 cells, this study highlights the plant's potential as a therapeutic agent for ovarian cancer [90].

2.9.15 *Adhatoda vasica* Anti-Thrombolytic Action with Herbal Tea Bag Formulation

In the study "Formulation of a herbal tea bag with potential in vitro thrombolytic activity using *Adhatoda vasica* Linn (Pawatta), Vitex negundo Linn (Nika), and Caesalpinia bonduc Linn (Kumburu)," three medicinal plants were combined to make a herbal tea. The assay for clot lysis was then used to ascertain the tea ability to dissolve blood clots in vitro." The selected therapeutic plants' phytochemical profiles were identified. Microcentrifuge tubescontaining pre-weighed blood clots were supplemented with 100 μL of aqueous extracts (AE)obtained from the leaves of each plant, at varying concentrations 125-500 mg/mL Blood clots that had dissolved were removed from the supernatants after 90 minutes at 37 oC of incubation.

After reweighing the tubes, the percentage of clot lysis was computed. The positive control used in this experiment was streptokinase, and the negative control was distilled water. After examining the thrombolytic effect of different combinations of these plants, a tea bag was made using the combination that worked best. It was possible to identify proteins, amino acids, carbohydrates, cardiac glycosides, flavonoids, diterpenoids, tannins, and alkaloids. At 500 mg/mL, 500 mg/mL, and 125 mg/mL, respectively, AE from the leaves of C. bonduc, V. negundo, and A. vasica showed 33.32% (p=0.001), 28.16% (p=0.007), and 22.02% (p=0.031) of the strongest thrombolytic effect. The tea bag, streptokinase, and the most effective mixture (1:4:4) all demonstrated clot-dissolving activity of 88.50% (p=0.000), 31.01% (p=0.003), and 13.35% (p=0.04), respectively. This investigation revealed that the AE of A. vasica leaves exhibited a moderate level of thrombolytic action [91].

2.9.16 Hepato-protective Effects of *Adhatoda vasica* in Liver Damage

Studies have indicated that *Adhatoda vasica* Nees, a component of Tibetan herbal treatments for liver problems, may be used as an adjuvant therapy for liver disorders. The use of active chemicals from medicinal plants has received attention since oxidative stress is a major factor in the development of liver disorders and can cause damage to the liver. The active components of A. vasica were identified, and their ability to prevent tert-Butyl hydroperoxide (t-BHP) damage was evaluated. The stimulation of the AMPK/p62/Nrf2 pathway was the main focus of the researchers' investigation into the mechanism behind this protection. In order to do this, the researcher's examined the extract's chemical makeup using Ultra Performance Liquid Chromatography (UPLC).

They used a variety of assays to evaluate the fractions' antioxidant qualities and adjusted the extraction conditions of flavonoids from a particular subfraction. They determined which chemicals had the strongest antioxidant action using UPLC-MS analysis. The researchers utilized the CCK-8 assay to evaluate cell viability and employed various markers to monitor changes in oxidative stress and

hepatic enzyme activity induced by t-BHP. They also assessed cell apoptosis and reactive oxygen species (ROS) generation. To gain insights into the mechanism, the researchers examined protein expression of the AMPK/p62/Nrf2 pathway using western blotting [49]. Ultimately, they found that the 70% ethanol extract of A. vasica leaves contained the highest concentration of active compounds, while the ethyl acetate fraction of the extract (AVEA) demonstrated potent abilities in combating oxidative stress, exhibiting strong reducing power and effective scavenging of free radicals.

Chemical analysis revealed that AVEA contained 17 components, including flavonoid - C - glycosides, flavonoid-O-glycosides, and a quinazoline alkaloid. Optimized conditions included a temperature of 65^{o}C, a solid-liquid ratio of 1:14, and a 73% ethanol content for extracting flavonoids from AVEA. It has been demonstrated that AVEA protects liver cells by increasing their viability, regaining the activity of their enzymes, and lowering oxidative stress. Moreover, it stopped cell apoptosis. The process involved AMPK/p62/Nrf2 pathway activation, which resulted in the production of genes related to antioxidant defenses. In conclusion, the flavone and alkaloids-rich AVEA was found to be the active ingredient in A. vasica. By turning on the AMPK/p62/Nrf2 pathway, it provided defense against oxidative stress brought on by t-BHP. By supporting the maintenance of a healthy in liver cells, AVEA may prove to be a viable therapy option for liver illnesses linked to oxidative stress [70].

Adhatoda vasica exhibits a wide range of biological activities, as evidenced by numerous experimental studies. It belongs to a category of herbal medicines with both a robust traditional foundation and solid experimental support for its usage. Consequently, this plant holds significant promise for development within the pharmaceutical industry [71].

Medications that are currently on the market, including as insulin, metformin, and sulfonylureas, have side effects. Methodology for addressing diabetes is symptomatic that also have many side effects. Therefore, current focus of this research is to discover novels compounds or phytocompounds that can trigger insulin in diabetic patient body.

Throughout history, our traditional system has relied on a variety of plant-based formulations for treating diabetes mellitus. Continuing research into discovering novel bioactive compounds derived from plants has the potential to significantly impact the pharmaceutical sector or existing dietary supplements in the near future, potentially sparking a revolutionary change.

Chapter 3

Methodology

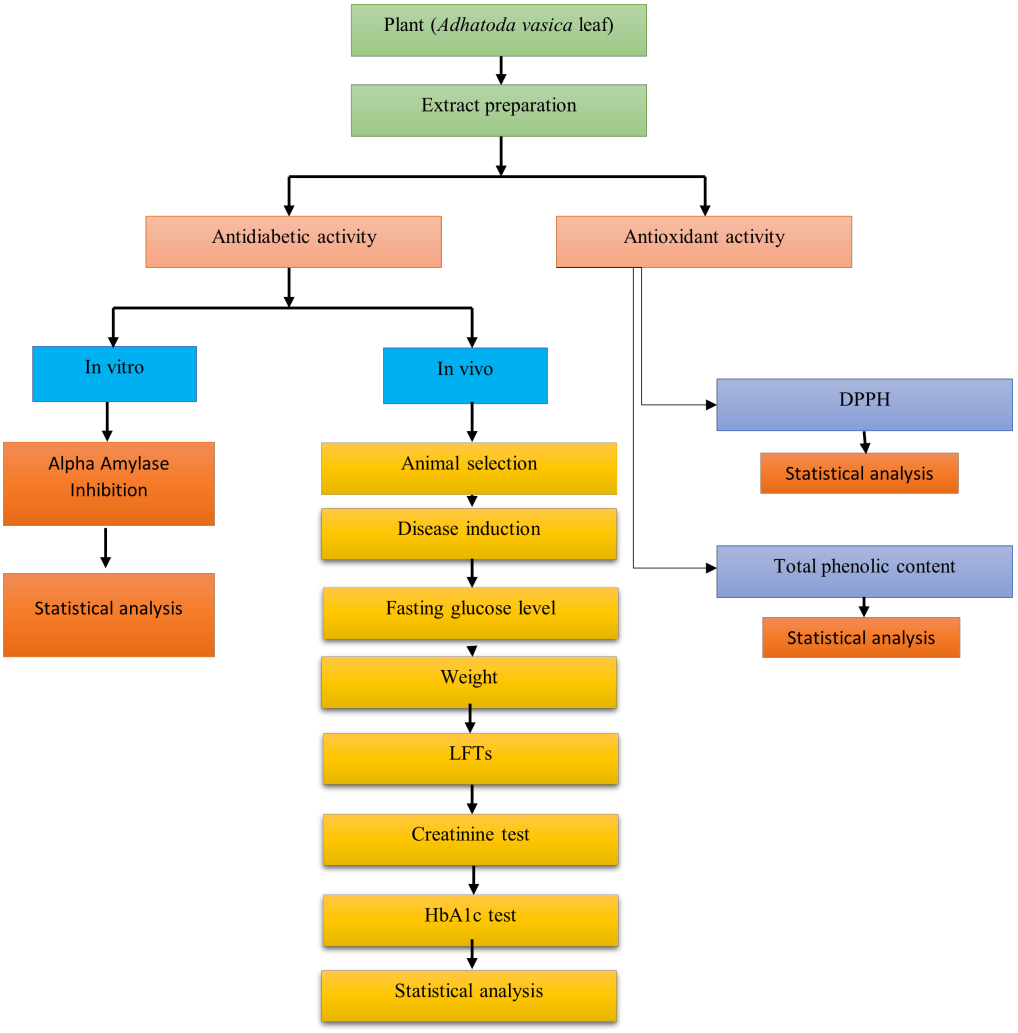


FIGURE 3.1: Research plan concept

The Methodology for plant extraction and its application in antioxidant and antidiabetic activity in Figure 3.1.

3.1 Procurement of Raw Materials

The leaves of *Adhatoda vasica* obtained from the local markets as well as from Kashmir [72]. Ethanol, Alpha-amylase, starch, 3, 5. Salicylic acid dinitro (DNSA), gallic acid, 2,2-diphenyl-1-1picrylhydrazyl reagent (DPPH), ethanol, sodium carbonate (NaCO3), Folin-Ciocalteu reagent (FCR) and were all available in Capital University of Science and Technology's laboratory.

3.2 Equipment

- Spectrophotometer
- Incubator
- Weighing balance
- Water bath
- Microwave
- pH meter

3.3 Apparatus

- Petri Plate
- Beaker
- Conical Flask
- Micropipette
- Cotton Swab

- Measuring Cylinder
- Spatula
- Dropper
- Test Tubes

3.4 Drying of Medicinal Plants

Fresh leaves of the *Adhatoda vasica* were taken from the field and thoroughly washed with tap water. The leaves were cleaned and then allowed to dry for 15–17 days at room temperature. To get a uniformly coloured powder, dried leaves were pulverised using a grinder. (Figure 3.2).

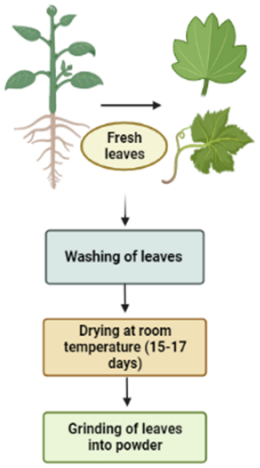


FIGURE 3.2: Procedure of drying medicinal plant leaves

3.5 Optimization of Parameters for Extraction

The effect of three extraction process factors; the ethanol concentration, powder concentration and extraction time was evaluated. The protocol criteria were taken into consideration when selecting the factorial levels. The methanol/water ratio, solute/solvent ratio, and extraction time were adjusted to the following ranges; 40:60-90:10, 80-100 (g/ml) and 12–40 h respectively.

3.6 Preparation of *Adhatoda vasica* Leaves Extracts

The grounded sample was taken in a beaker with the addition of solvent, and the beakers were placed at room temperature for the given period of time. Then supernatants (extracts) were separated from the pellet after the centrifugation at 4000 rpm for 10 minutes. Up to the time of additional testing, extracts were kept at -4^{o}C, and each test was run in triplicate.

3.7 Antioxidant Activity

3.7.1 Total Phenolic Compound Determination

TPC was calculated using the previously published spectrophotometric F-C reagent method [73]. 270 μl of the ethanolic extract of various strengths (20ppm, 50ppm and 100ppm) and 1.36 ml of the 10% F-C reagent were added in the aluminum-foil-covered falcon tubes. After 5 minutes, 1.36 ml of 7.5% sodium carbonate was added, and the mixture was then mixed and allowed to sit at 45^{o}C in an incubator with distilled water for 45 minutes. There were three solutions of each sample. Experiment was performed in triplicates.

The absorbance was determined with a spectrophotometer set at 765 nm. TPC was determined using gallic acid concentrations (0 – 120 ppm) as the standard and a calibrated curve (R2 = 0.895).

3.7.2 Radical Scavenging Assay 2, 2-Diphenyl - 1 - Picryl-hydrazyl

With just minor adjustments, the DPPH test, which was previously reported by [74], was utilised to assess the extracts' anti-oxidant activity. The experiment was conducted by using 0.004% (w/v) DPPH solution prepared in ethanol. Solutions

of 25ppm, 50ppm, 100ppm, 150ppm, 200ppm, 250ppm and 300ppm were prepared to note absorbance at 0 and after 30 minutes. There were three samples of each concentration.

Experiment was performed in triplicates. A sample of extracts (25μl) was mixed with 0.1 molar tris base HCL buffer (250μl) and then the DPPH reagent (1 ml) was added. At 517 nm, the absorbance was determined with a spectrophotometer. This formula was utilised to determine the activity of scavenging free radicals:

$$Scavenge\ percentage = \frac{(A_{blank} - A_{sample})}{(A_{blank})} \times 100\%$$

A_{sample} of the treatment with plant extract absorbance is at 517 nm. A_{blank} is the sample's absorbance at 517 nm without plant extract.

3.7.3 Inhibition of Alpha-amylase Activity

The medicinal plant extract's inhibitory activity for alpha-amylase was determined by using previously reported methods with a few modifications [75]. Solutions of 20ppm, 50ppm, 100ppm, 150ppm, 200ppm, 250ppm, 300ppm, 350ppm, 400ppm, 450ppm, 500ppm and 5000ppm were prepared. There were three samples of each concentration. Experiment was performed in triplicates.

The inhibitory effect of extracts-amylase activity was quantified using di-nitro salicylic acid. For this purpose, 0.5% alpha-amylase solution was prepared in distilled water. To maintain the pH of the mixture, 20 mM sodium phosphate buffer (pH 6.9) was added after 100 µl of plant extracts in various concentrations and combinations (20 ppm, 50 ppm, 100 ppm, 150 ppm, 200 ppm, 250 ppm, 300 ppm, 350 ppm, 400 ppm, 450 ppm, 500 ppm, and 5000 ppm) were added to the 100 µl alpha-amylase solution. Ten minutes at 37^{o}C were dedicated to pre-incubating the solution.

A 1% starch solution was made with distilled water, and 200 µl of this solution was added to the pre-incubated solution. Before adding 1 ml of 1% DNSA, the solution was once more incubated for 10 minutes at 25 ^{o}C. To stop the process, one

millilitre of di-nitro salicylic acid was added, and the mixture was then brought to a boil for five minutes.

The reaction mixtures were diluted 1:5 with water and allowed to cool to room temperature before their absorbance was measured at 540 nm using a spectrophotometer. The following formula was used to determine the enzyme's inhibition percentage:

$$Alpha\ amylase\ inhibition\ percentage = \frac{(A_{control} - A_{treatement})}{A_{control}} \times 100\%$$

$A_{treatement}$ is defined as the absorbance of the plant extract treatment at 540 nm. An absorbance of the control sample without plant extract at 540 nm is called a control. Additionally, a control sample was run in triplicate.

3.8 Antidiabetic Effect

3.8.1 Extract Drying

After extraction, the ethanolic extract was dried. The extracts were placed on petri dishes and then left to dry openly at room temperature to obtain greenish-black colored residue. The extracts were tightly packed and stored at 4^oC temperature for further use.

3.8.2 Experimental Animals

The animals used in the study were obtained from the Animal house within the Department of Pharmacy at CUST. The research involved adult male Sprague Dawley (SD) rats, weighing between 150 to 200 grams [98] divided into five groups: A, B, C, D and E, each with 3 rats. All the rats were acclimatized to the laboratory condition for one week before commencing the experiments. The animals were housed in 12 hours light and dark cycle at room temperature. Streptozotocin was administered intraperitoneally to the rats of all groups. Group A served

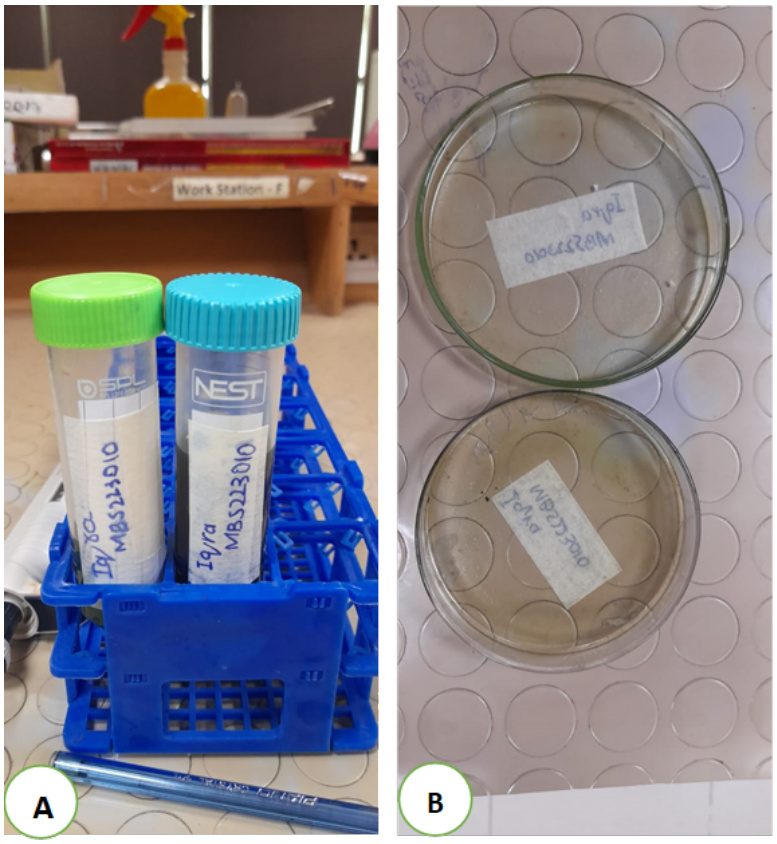


FIGURE 3.3: (A) Ethanolic extract (B) Extract drying

as the negative control without any treatment, while group B was treated with metformin, therefore serving as the positive control. Groups C, D, and E were treated with different concentrations of plant extracts. Rats were provided with 20% glucose water except negative control group. It was necessary to avoid sudden hypoglycemia after injection.

3.8.3 Experimental Design

These experimental rats were randomly divided into five groups consisting of three male rats in each group. The first group was normal rats, and the next four were diabetic rats.

- Group A: will serve as the negative control, without any treatment.
- Group B: STZ-induced diabetic rats that served as diabetic positive control was treated with 30mg/kg metformin [99].

- Group C: STZ-induced diabetic rats were treated with 100 mg/kg of Adhatoda vasica ethanolic leaves extract.
- Group D: STZ-induced diabetic rats were treated with 200 mg/kg of Adhatoda vasica ethanolic leaves extract.
- Group E: STZ-induced diabetic rats were treated with 400 mg/kg of Adhatoda vasica ethanolic leaves extract.

FIGURE 3.4: Grouping of rats

The treatment had been continued for 14 days.

3.8.4 Induction of Diabetes Mellitus in Experimental Rats

Rats were given a dose of 20 g/kg of glucose before the injection of STZ [100]. Each rat received a single low dose of STZ (30 mg/kg), administered intraperitoneally after being dissolved in 0.1 M sodium citrate buffer at pH 4.4 [101]. Despite STZ showing maximum stability at pH 4, it undergo degradation within 15–20 minutes

in the citrate buffer. Hence, the solution needed to be prepared just before injection and utilized within 5 minutes of dissolution to prevent degradation. Moreover, due to STZ's sensitivity to light, tubes containing the solution had to be covered with aluminum foil [102]. Blood samples were taken from the tails of rats three days following STZ injection and measured using a EasyGluco glucometer. Diabetic rats were included in this study if their FBG levels were greater than 200 mg/dL.

FIGURE 3.5: STZ injection administered via intraperitoneal route for disease induction

3.8.5 Measurement of Body Weight

After treating with streptozotocin, the body weight of the rats was measured. Body weight was an important parameter to monitor in diabetic rats. Regularly weighing diabetic rats and tracking changes over time helped in assessing their overall health and response to treatment. The body weight of each animal was measured on a weekly basis throughout the experiment using an electronic weighing balance.

3.8.6 Blood Glucose Measurement

The glucometer strip was inserted into the glucometer. With gloved hands, the rat was carefully picked up, and its tail was pricked as minimally as possible using a lancet. A single drop of blood from the tail was placed onto the glucometer strip, and a reading was taken. Blood glucose levels were measured as a baseline (0) and then on the 7^{th} and 14^{th} days of treatment.

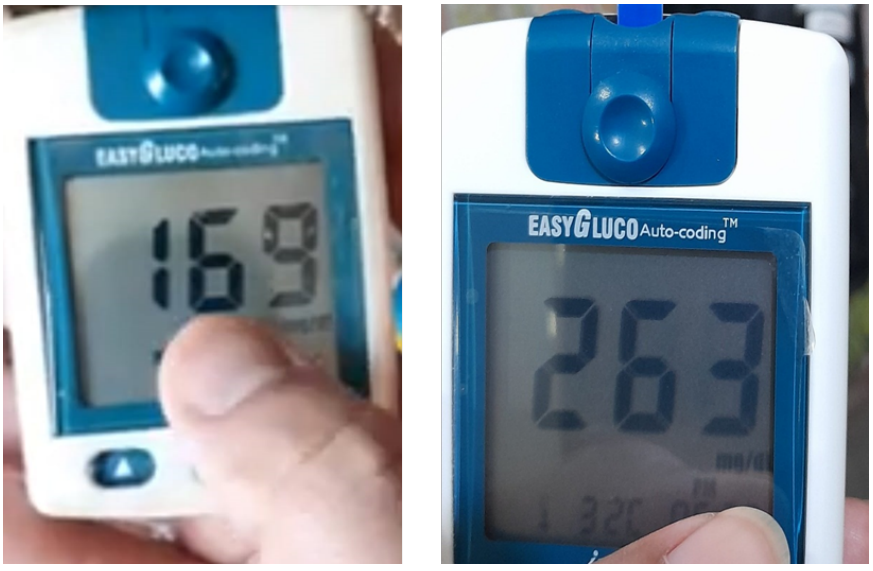


FIGURE 3.6: Blood glucose level of STZ induced diabetic male rat

3.8.7 Serum Creatinine, Liver Function Test(LFT) & HbA1c Test

The rats were gently sedated using an inhalation anesthetic to immobilize them before being placed in a rat holder. A 1 mL blood sample was then obtained from each rat through cardiac puncture using a 23-gauge needle and a 1 mL syringe [103]. The blood samples were collected in anticoagulant tubes. The Liver Function Test (LFT) and HbA1c test was conducted at the Center for Animal Diagnostics (CADx) in Lahore.

FIGURE 3.7: A, B The blood sample obtained from each rat through cardiac puncture. C. The blood samples collection in anticoagulant tubes

3.9 Statistical Analysis

Statistical differences were tested using one-way analysis of variance (ANOVA) followed by Duncan multiple range test. A difference in the mean p-value ¡0.05 was considered statistically significant.

Chapter 4

Results and Discussion

4.1 2, 2-Diphenyl-1-Picrylhydrazyl Radical Scavenging Assay

The established mechanism by which antioxidants prevent oxidation is through the scavenging of free radicals. Rapid assessment of the antioxidant capacity of particular compounds or extracts can be accomplished by the scavenging of stable DPPH free radicals technique [76]. The technique used to measure the antioxidant activity of the ethanolic extract of *Adhatoda vasica* leaves is called the DPPHH (2, 2 diphenyl-1-1 picrylhydrazyl) test. Ethanol was used as control. Scavenging activity is shown in (Table 4.1). Ethanolic leaf extract demonstrated the ability to scavenge free radicals. Scavenging activity is shown in (Table 4.1). Ethanolic leaf extract demonstrated the ability to scavenge free radicals.

A straightforward, quick, and sensitive technique to assess the antioxidant activity of a particular drug or plant extracts is to use the DPPH stable free radical assay [77]. The strongest absorption happens when free-radical DPPH interacts with an odd electron at 517 nm (purple hue). DPPHH, which has less hydrogen than DPPH and is hence less absorbent, is produced when DPPH and an antioxidant that scavengers free radicals unite. Unlike the DPPH-H state, this radical form decolorizes (becomes yellow) as the number of electrons it gathers increases (Figure 4.1)

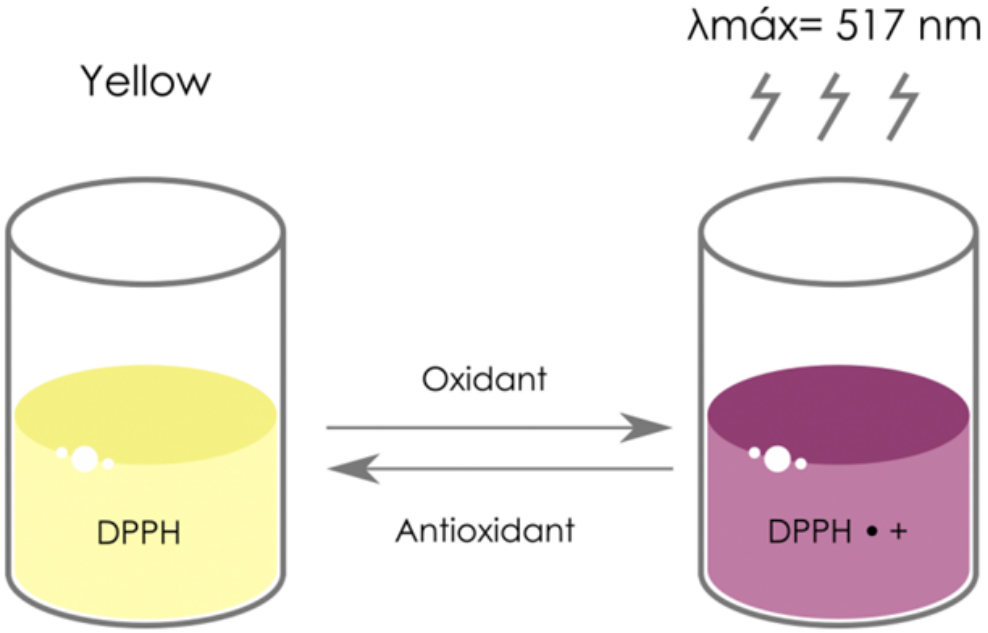


FIGURE 4.1: Demonstrating how an antioxidant and free-radical scavenger combine to produce DPPHH from DPPH

The reduction of DPPH's purple colour in test samples indicates the antioxidant action. Hence, by donating electrons or supplying a hydrogen atom, antioxidant molecules can neutralize DPPH free radicals. As a result, the colourless, stable molecule 2,2-diphenyl-1-hydrazine is formed, which decreases the absorbance of the solution at 517 nm. The DPPH solution was left undisturbed for ten minutes in order to verify its stability.

The DPPH solution was left undisturbed for ten minutes in order to verify its stability. Throughout the experiment, the solution's colour remained unchanged, indicating that DPPH's maximal stability had occurred.

The DPPH solution was left undisturbed for ten minutes in order to verify its stability. Throughout the experiment, the solution's colour remained unchanged, indicating that DPPH's maximal stability had occurred. At 517 nm, the absorption intensity was measured. The ethanolic leaf extract of *Adhatoda vasica* was

gradually added to the DPPH solution, resulting in a steady drop in the absorption peak strength at 517 nm and a gradual shift in the solution's colour from deep violet to pale yellow.

TABLE 4.1: %age scavenging of *Adhatoda vasica*'s ethanolic leaf extract at 0 minute and after 30 minutes

Extract concentrations (ppm)	**Scavenging activity at 0 minutes**	**Scavenging activity after 30 minutes**
25	17.13% ± 4.21	39.09% ± 3.82
50	39.87% ± 2.68	44.86% ± 0.76
100	40.02% ± 0.42	45.15% ± 0.35
150	38.47% ± 4.9	39.861% ± 5.57
200	37.88% ± 4.98	39.28% ± 1.94
250	30.61% ± 1.11	36.87% ± 4.7
300	37.76% ± 4.98	38.40% ± 4.9

The data are shown as mean ± S.D. and each value in the table was derived by averaging the results of three tests. The antioxidant activity of the ethanolic leaf of *Adhatoda vasica* was calculated using DPPH. The antioxidant activity percentage was calculated as [(Ac - As) / Ac] × 100.

4.1.1 Initial Scavenging (0 Minutes)

At 0-minute absorbance was higher than 30 minutes' absorbance observed through spectrophotometer. Lower concentrations (25, 50 and 150 μg/mL) exhibited moderate to high scavenging activity 17.13% ± 4.21, 39.87% ± 2.68 and 38.47% ± 4.57 with low to moderate variability. The scavenging activity at 100 μg/mL shows significant variability 40.02% ± 0.42, indicated by a high standard deviation. Higher concentrations (200, 250 and 300 μg/mL) showed decreased scavenging activity with considerable variability 37.88% ± 1.94, 30.61% ± 4.7, 37.76% ± 4.9. Percentage Scavenging of *Adhatoda vasica*'s ethanolic leaf extract at 0 minute shown in figure 4.2.

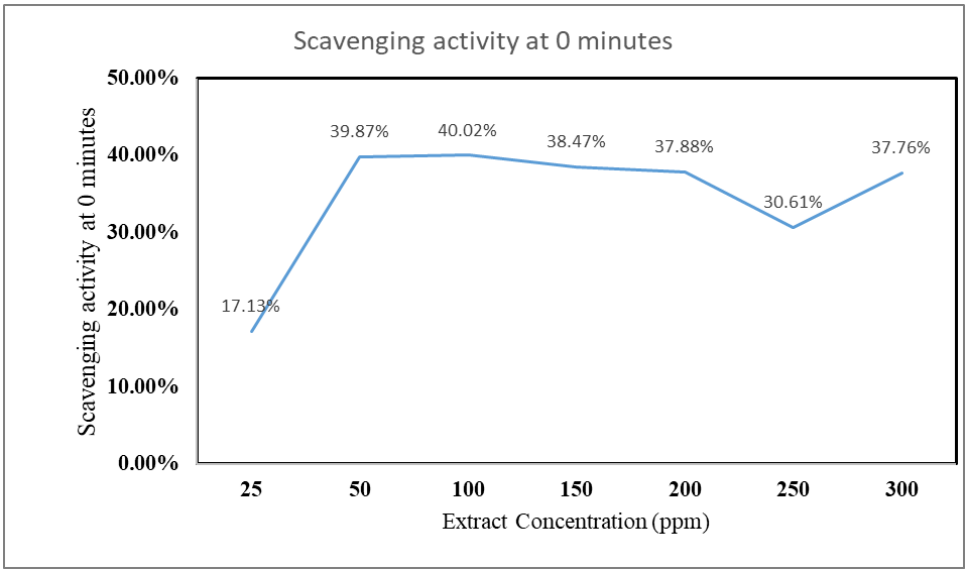


FIGURE 4.2: % Age Scavenging of *Adhatoda vasica*'s ethanolic leaf extract at 0 minute

4.1.2 Scavenging After 30 Minutes

After 30 minutes' absorbance was lower than 0 minute' absorbance observed through spectrophotometer. The scavenging activity generally increased over time for all the concentrations. Since the scavenging activity of DPPH relies on the hydrogen-donating capacity of the 85 tested compound, comparisons are not quantitative because the reaction with DPPH depends on the compound's structural conformation [78]. At concentration 25 μg/mL, there was an increase from 17.13% to 39.09%.

At concentration 50 μg/mL, there was an increase from 39.87% to 44.86%, At concentration 100 μg/mL, there was an increase from 40.02% to 45.15%. At concentration 150 μg/mL, there was an increase from 38.47% to 39.86%. At concentration 200 μg/mL, there was an increase from 37.88% to 39.28%. At concentration 250 μg/mL, there was an increase from 30.61% to 36.87%. At concentration 300 μg/mL, there was an increase from 37.76% to 38.4%, indicating an improvement in antioxidant activity over time. Percentage Scavenging of *Adhatoda vasica*'s ethanolic leaf extract after 30 minutes shown in figure 4.3.

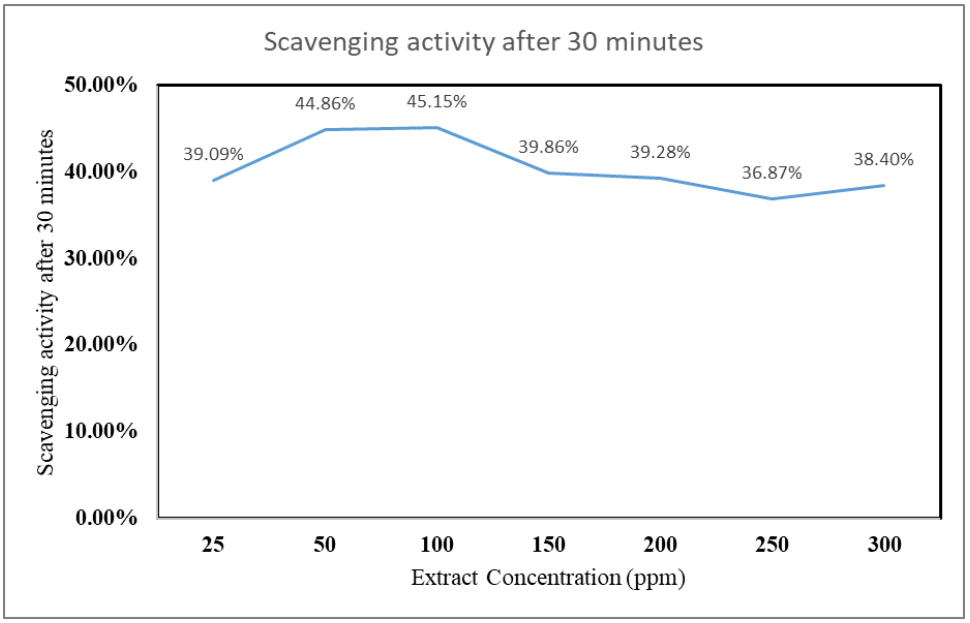


FIGURE 4.3: %Age The scavenging activity of *Adhatoda vasica*'s ethanolic leaf extract after 30 minutes

These findings suggested that the scavenging activity of *Adhatoda vasica*'s ethanolic leaf extract demonstrates concentration-dependent antioxidant activity with a tendency to stabilize over time. (Figure 4.4)

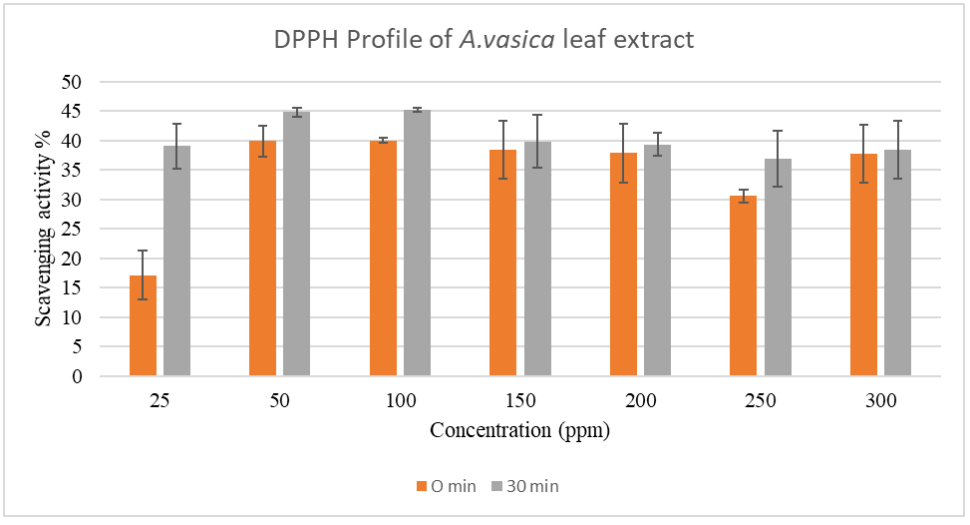


FIGURE 4.4: Comparing scavenging activity at 0 min. and after 30 min

The scavenging activity generally increased over time for all concentrations, indicating an improvement in antioxidant activity. This time-dependent increase is consistent with findings from other studies. For instance, [92] observed a similar

trend where plant extracts' capacity to scavenge DPPH radicals grew over time., suggesting a gradual release or interaction of antioxidant compounds.

Notable increases in scavenging activity were seen at all concentrations after 30 minutes, with the highest increase at 25 μg/mL from 17.13% to 39.09%. This indicates that even at lower concentrations, the extract exhibits potent antioxidant properties over time. According to [93] the scavenging activity of phenolic compounds in plant extracts is largely dependent on their capacity to donate hydrogen. The observed increase in scavenging activity over time suggests a strong hydrogen-donating capacity of the compounds in *Adhatoda vasica* extract. The impact of antioxidants on DPPH radicals is believed to be due to their ability to donate hydrogen [94]. Engaging in radical scavenging activities is essential to halting the damaging effects of free radicals in a variety of illnesses, such as diabetes. Antioxidants are known to reduce lipid peroxidation by the DPPH free radical scavenging technique. Because this approach requires relatively little processing time, it is widely utilised to predict antioxidant activity. The ability of substances to transfer hydrogen atoms to exhibit antioxidant activity is measured by the DPPH assay. The highest absorbance of DPPH+, a purple-hued, stable radical cation, is measured at 517 nm. The solution turns discoloured when antioxidants that can donate an electron to DMPD+ are present. This is a quick reaction that is proportionate to the sample's antioxidant capacity. This study demonstrated consistent with existing literature on the behavior of phenolic compounds and their interaction with DPPH radicals. The implications of this study highlight the potential applications of *Adhatoda vasica* extract in health-related fields, emphasizing the importance of time in optimizing its antioxidant properties.

4.2 Total Phenolic Concentration

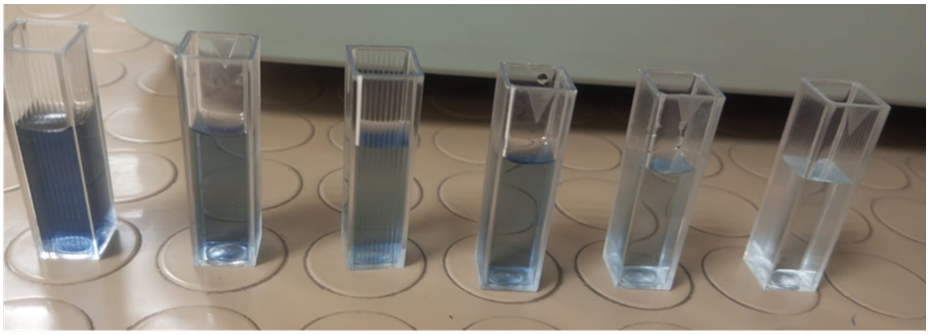
The study discovered a high correlation between the amount of phenolic compounds in plant materials and their antioxidant activity [79]. Therefore, it is crucial to take into account how the overall phenolic content affects the antioxidant activity of extracts from mushrooms.

It has been suggested that polyphenols are significant phytochemicals with strong antioxidant properties as well as other powerful therapeutic properties. It has been shown that the main plant chemicals with antioxidant activity are phenolic compounds, and that their redox characteristics are what give them this action. One family of antioxidant agents that may both adsorb and neutralize free radicals are phenolic compounds [80].

The FC method was used to determine the total phenolic content of the plant extract, and a calibration curve was created using gallic acid. The standard curve was used to create a regression equation, which was then used to quantify the amount of gallic acid in the *Adhatoda vasica* ethanol extract: y= 0.0632x+0.2211, R2 = 0.8958, where x is the equivalent gallic acid (mg/ml) and y is the absorbance.

The absorbance values (0.189, 0.321, 0.592, 0.489, 0.479, 0.653, 0.691, 0.654, 0.778, 0.784, 0.896, 1.057) increase as the concentration of Gallic acid increases (0 to 12 mg/ml). This implied that the amount of light absorbed at 765 nm and the concentration of gallic acid in the solution were directly correlated. Gallic acid curve shown in figure 4.6.

a.



b.

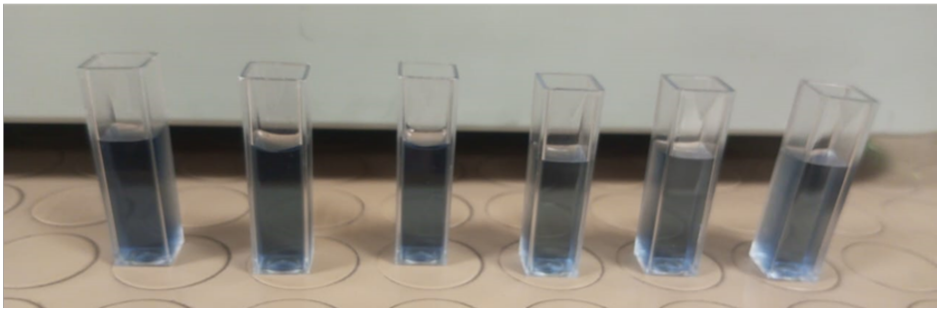


FIGURE 4.5: a, b Gallic acid concentrations

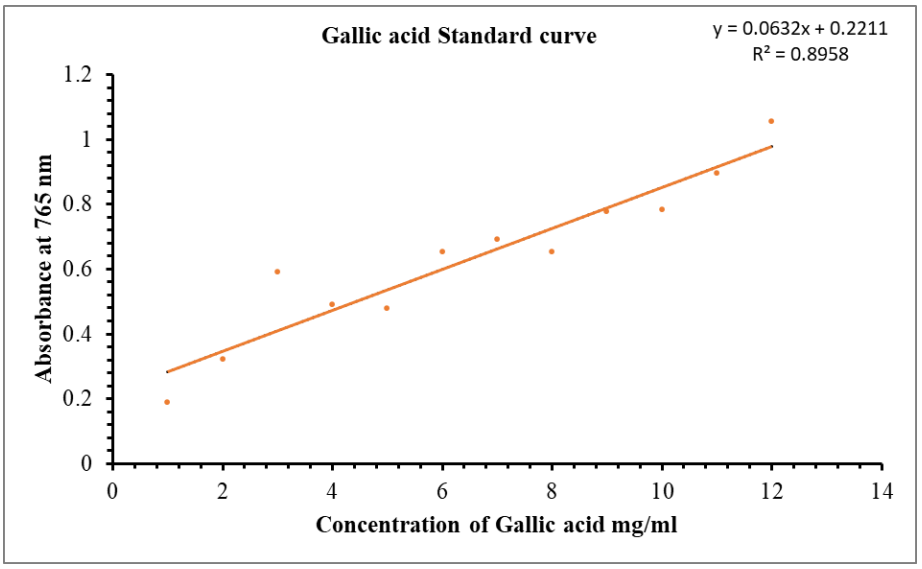


FIGURE 4.6: Graph plot of Gallic acid results.

Phenolic concentrations were observed in the ethanolic leaf extract of *Adhatoda vasica* that was being studied (figure 4.5a,b). The kind of extract that is, the polarity of the solvent used in extraction—determines the overall phenolic levels of *Adhatoda vasica* plant extracts. When phenols are extracted using polar solvents, the resulting extracts contain high concentrations of these compounds due to their high solubility in these solvents [81]. At 100ppm concentration, the *Adhatoda vasica*'s ethanolic leaf extract exhibited absorbance up to 0.809. At 50ppm concentration, the *Adhatoda vasica*'s ethanolic leaf extract exhibited absorbance up to 0.718. At 200ppm concentration, the *Adhatoda vasica*'s ethanolic leaf extract exhibited absorbance up to 0.328. As the extract content grew, the absorbance values increased as well, as seen in table 4.2. This implied a favourable relationship between the extract's phenolic component concentration and absorbance measured at the specific wavelength used (typically around 765 nm for phenolic compounds).

TABLE 4.2: Absorbannce at 765nm of 20ppm, 50ppm, 100pp concentrations of *Adhatoda vasica*'s ethanolic leaf extract

Sample	**Absorbance at 765 nm**		
Adhatoda vasica's ethanolic leaf extract	20ppm	50ppm	100ppm
	0.328	0.718	0.809

Each value in the table was obtained by calculating the average of three experiments and data are presented as mean ± S.D.

The curve shown in figure. 4.6, helps quantify the total phenolic content in Adhatoda vasica's ethanolic leaf extract. By measuring the absorbance of the extract sample at the same wavelength (765 nm) and comparing it to the standard curve, the concentration of phenolic compounds in Adhatoda vasica's ethanolic leaf extract could be estimated. When the absorbance values of 0.328, 0.718, and 0.809 for concentrations of 20 ppm, 50 ppm, and 100 ppm, respectively, of The ethanolic leaf extract of Adhatoda vasica was calibrated using the Gallic acid standard curve. (Figure 4.4), they indicated the concentration of phenolic content. It was found that the phenolic concentration in the extract at 20 ppm with an absorbance of 0.328 was 1.8 mg/ml. Similarly, the phenolic concentration in the extract at 50 ppm with an absorbance of 0.718 was 8 mg/ml, and the phenolic concentration in the extract at 100 ppm with an absorbance of 0.809 was 9.6 mg/mlThe extracts with the highest content of phenols also exhibit the strongest antioxidant activity (Figure 4.7). (Table 4.3). These results also shown in table 4.3.

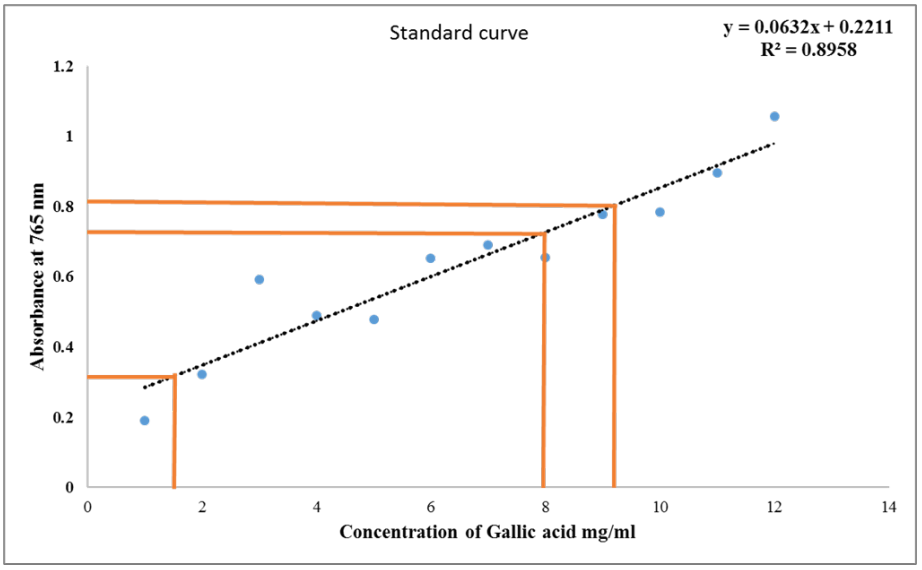


FIGURE 4.7: Phenolic concentration in Adhatoda vasica's ethanolic leaf extract

TABLE 4.3: Phenolic concentration in Adhatoda vasica's ethanolic leaf extract

Sample	Absorbance at 765nm	Phenolic concentration mg/ml
20ppm	0.328	1.8
50ppm	0.718	8
100ppm	0.809	9.6

The data are shown as mean ± S.D. and each value in the table was derived by averaging the results of three tests.

The study's findings are consistent with previous research, demonstrating that the phenolic content of Adhatoda vasica ethanolic leaf extract positively correlates with its antioxidant activity. Higher concentrations of phenolic compounds were associated with greater absorbance and, consequently, higher antioxidant activity. For instance, the extract at 100 ppm exhibited the highest absorbance (0.809) and the highest phenolic concentration (9.6 mg/ml), indicating potent antioxidant activity. The results imply that the ethanolic leaf extract of Adhatoda vasica may be a useful natural antioxidant source. This is significant for its potential application in managing oxidative stress-related diseases. The medical benefits of phenolic compounds are well-known, and they include anti-inflammatory, anti-cancer, and cardioprotective qualities. Therefore, the high phenolic content in Adhatoda vasica could translate into multiple health benefits. Compared to other plants studied for their phenolic content and antioxidant activity, Adhatoda vasica shows competitive or even superior potential. For instance, green tea, which is renowned for its high phenolic content and antioxidant properties, has been shown to have phenolic concentrations similar to those found in this study.

4.3 Inhibition of Alpha Amylase

Alpha-amylase is an enzyme that breaks down starch molecules to release reducing sugars, such as maltose. These reducing sugars are recognised by adding DNSA, which is reduced (Figure 4.7) to 3-amino-5-nitrosalicylic acid (ANSA) in the presence of maltose. The vivid orange-red compound ANSA can be detected spectrophotometrically at 540 nm [95]. The amount of ANSA generated will decrease when α-amylase activity is inhibited.

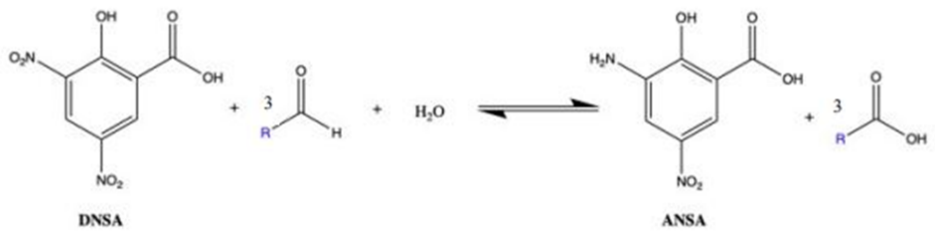


FIGURE 4.8: The conversion of an orange red ANSA to yellow DNSA in the presence of a reducing sugar [95]

One of the important enzymes involved in the digestion of starch, glycogen, and the metabolism of carbohydrates is alpha amylase. One method for treating problems involving the absorption of carbohydrates, such as diabetes and obesity, is to inhibit it. Because it is involved in the metabolism of carbohydrates, blocking it will lower blood sugar levels after meals [82]. Many medicinal plants and their preparations are utilized in both traditional medicine and ethnomedicine to treat diabetes because their main bioactive ingredients have strong antioxidant and alpha amylase inhibitory qualities [83]. Alpha-amylase is a crucial enzyme involved in starch digestion, and its inhibition can have significant implications for various applications, including diabetes management and food processing. We looked into the inhibitory effects in this study, of multiple samples of Adhatoda vasica's ethanolic leaf extract across a range of concentrations on alpha-amylase activity. The following formula was used to get the inhibition percentage:

$$Alpha\ amylase\ inhibition\ percentage = \frac{(A_{control} - A_{treatement})}{A_{control}} \times 100\%$$

TABLE 4.4: Presentation of data showing the percentage of inhibition for each sample at different concentrations.

S.No.	**Concentrations of *Adhatoda vasica*'s ethanolic leaf extract (ppm)**	**Percentages of inhibition (%)**
1	20	13.33 ± 3.03ab
2	50	14.33 ± 3.86ab
3	100	22.00 ± 2.08bc
4	150	28.67 ± 2.65c
5	200	31.00 ± 3.15c
6	250	27.33 ± 1.53c
7	300	27.67 ± 1.53c
8	350	29.33 ± 2.16c
9	400	32.33 ± 2.86c
10	450	30.33 ± 3.1c
11	500	26.67 ± 1.15c
12	5000	5.67 ± 1.52a

Values are mean ± SEM, n = 3, by using one way ANOVA followed Duncan multiple range test.

Sample 12 of 5000ppm, exhibited the percentage inhibition 5.67% ± 1.52a. Sample 11 of 500ppm, exhibited the percentage inhibition 26.67% ±1.15c. Sample 10 of 450ppm, exhibited the percentage inhibition 30.33% ± 3.1c. Sample 9 of 400ppm, exhibited the percentage inhibition 32.33% ± 2.86c. Sample 8 of 350ppm, exhibited the percentage inhibition 29.33% ± 2.16c. Sample 7 of 300ppm, exhibited the percentage inhibition 27.67% ± 1.53c. Sample 6 of 250ppm, exhibited the percentage inhibition 27.67% ± 1.53c. Sample 5 of 200ppm, exhibited the percentage of inhibition 31% ± 3.15c. Sample 4 of 150ppm, exhibited the percentage inhibition 28.67% ± 2.65. Sample 3 of 100ppm, exhibited the percentage inhibition 22% ± 2.90bc. Sample 2 of 50ppm, exhibited the percentage inhibition 14.33% ± 3.8ab. Sample 1 of 20ppm, exhibited the percentage of inhibition 13.33% ± 3.03ab. Alpha amylase percentage inhibition of Adhatoda vasica's ethanolic leaf extract shown in table 4.4.

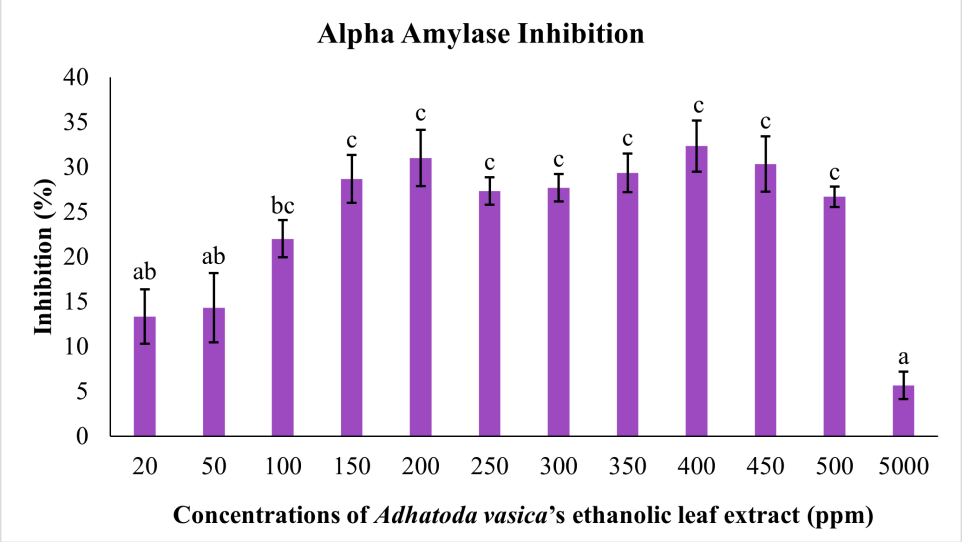


FIGURE 4.9: Percentages of Alpha Amylase Inhibition in Adhatoda vasica's ethanolic leaf extract of different strength

The alpha-amylase inhibition test findings (figure 4.9) showed that the samples have the capacity to suppress the alpha-amylase enzyme's activity. The recorded p-value of 0.05 indicates the significance of these results. This was supported by the fact that several components of the extract, such as phenols, flavonoids,

saponins, steroids, alkaloids, and terpenoids, were known to be potent -amylase inhibitors.

These findings were consistent with earlier research, which found a favorable correlation between total polyphenol content and the capacity to inhibit pancreatic α-amylase [84, 85].

High Concentration (5000 ppm): Sample 12 exhibited minimal inhibition 5.67 $\pm$ 1.52a. This suggested that at very high concentrations, the extract may not be effective in inhibiting alpha-amylase. This could be due to the saturation effect, where too much of the extract might interfere with its own activity or cause other interactions that reduce its efficacy.

Alpha-amylase inhibitors evaluated against standard inhibitors like acarbose, which is a pharmaceutical alpha-amylase inhibitor used in managing diabetes. The in vitro inhibitory action of the ethanolic leaf extract of *Adhatoda vasica* on a-amylase in comparison to the commercially available anti-diabetic medication acarbose. Acarbose belongs to the class of oral hypoglycaemics known as α-glucosidase inhibitors since it inhibits both α-amylase and α-glucosidase. It was established that acarbose functions as a combined α-glucosidase inhibitor [96] and a competitive inhibitor of α-amylase [97]. Typically, acarbose shows inhibition in the range of 50%-70% at therapeutic concentrations. The samples showing 26%-31% inhibition suggest that the extract is a moderately effective inhibitor when compared to acarbose. Samples with 15%-25% inhibition are less effective but still demonstrate a noteworthy ability to inhibit alpha-amylase. Samples with inhibition <15% are relatively weak compared to standard inhibitors.

The results indicate that certain concentrations of *Adhatoda vasica* ethanolic leaf extract have a significant potential to inhibit alpha-amylase, particularly at 500 ppm, 450ppm,400ppm, and 200 ppm. This suggests that the extract, particularly at these concentrations, could be beneficial in managing blood sugar levels, potentially offering a natural alternative or supplement to pharmaceutical inhibitors. The detected inhibitory effects may be caused by active phytochemicals found in

the extract, such as phenols, flavonoids, saponins, steroids, alkaloids, and terpenoids. Further research and comparison with standard inhibitors like acarbose would help in understanding the practical applications and efficacy of the extract.

4.4 Biomarkers Assessment

4.4.1 Blood Glucose Levels

This research aimed to assess the potential impact of Adhatoda vasica extract on diabetic rats, specifically investigating its effects on parameters like body weight and concentrations of glucose in the blood. A total of 15 rats after acclimatization for 1 week were divided randomly into 5 groups.

TABLE 4.5: Fasting blood glucose level and body weight before disease induction

Groups	**Negative Control**	**Positive Control**	**Extract Group 100mg/kg**	**Extract Group 200mg/kg**	**Extract Group 400mg/kg**
Fasting glucose level	95±0.82	103±1.63	104±3.86	94.67±7.72	101.67±3.30
Body weight	213.33±16.35	201±3.65	181.67±11.89	206.33±9.67	203±5.10

Values are mean ± SEM, n = 3, by using one way ANOVA followed Duncan multiple range test.

The drug group displayed the most significant rise in blood glucose levels before the induction of the disease compared to other groups. Simultaneously, the highest increase in weight was also observed within the same drug group. The recorded p-value of < 0.05.

Conversely, values denoted by identical letters or superscripts indicate no statistically significant differences between them. Duncan's multiple range test is a statistical method used to compare multiple groups or treatments simultaneously. When the values are assigned different letters (e.g., a, b, c), it implies that those

groups are significantly different from each other at a significance level of $p < 0.05$, meaning there is a meaningful distinction between those particular groups in the experiment or study. On the other hand, when values share the same letters or superscripts, it suggests that these groups do not have statistically significant differences among them. On the other hand, when values share the same letters or superscripts, it suggests that these groups do not have statistically significant differences among them. This indicates that, based on the test's results, those specific groups are statistically similar or equivalent in the context of the analyzed parameters or variables.

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Table 4.6: Changes in fasting blood glucose level (mg/dl)

Negative Control	**Positive Control**	**Extract Group 100mg/kg**	**Extract Group 200mg/kg**	**Extract Group 400mg/kg**
208.0 ± 3.77a	220.0 ± 20.00a	252.0 ± 2.25b	237.0 ± 1.73b	205.0 ± 1.04a
212.5 ± 4.58ab	212.2 ± 24.7ab	253.0 ± 2.29c	233.0 ± 7.94bc	204.7 ± 1.15a
223.5 ± 12b	186.0 ± 15a	248.0 ± 7.54c	224.0 ± 12b	201.0 ± 5.29a

Values are mean ± SEM, n = 3, by using one way ANOVA followed Duncan multiple range test.

Table 4.6, displays the fasting blood sugar levels of diabetic rats over 14 days of treatment. After disease induction, the fasting blood sugar levels in STZ-induced diabetic rats ranged from 208 to 263 mg/dl. The Adhatoda vasica ethanolic extract really helped lower blood sugar levels in rats with diabetes caused by STZ. After the 14-day treatment, both metformin and Adhatoda vasica ethanolic extract led to significant decreases in blood glucose levels compared to the negative control group.The recorded p-value of ¡0.05 indicates the significance of these results.

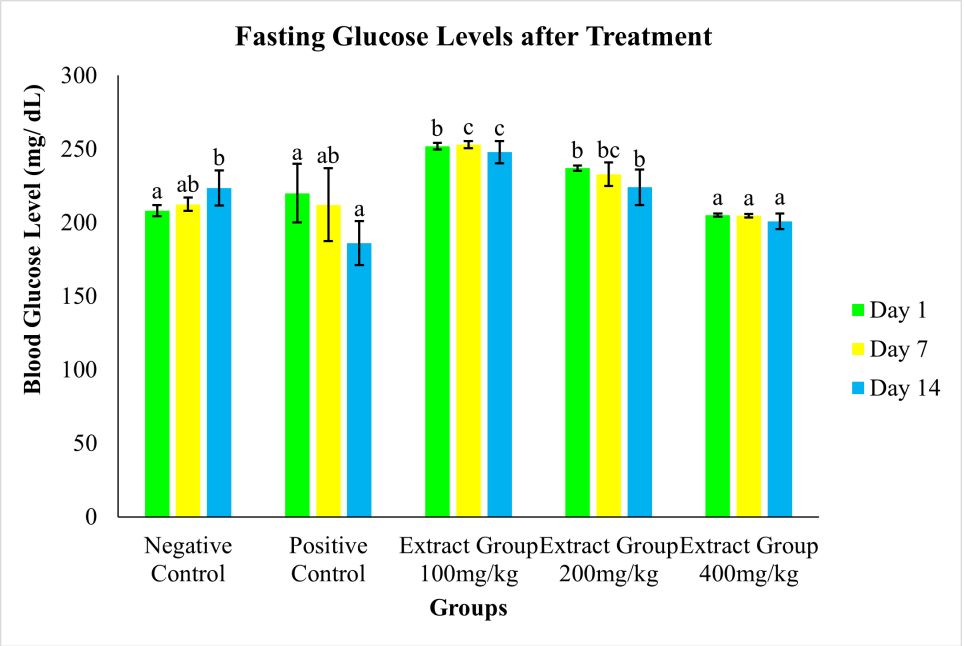

FIGURE 4.10: This figure presents the variations in fasting blood glucose levels (measured in mg/dL) observed among different experimental groups of male Sprague Dawley rats over the course of the study

In figure 4.10, the results demonstrate a notable decrease in blood glucose levels within the drug-administered group, indicating a significant impact of the drug on reducing glucose levels. Furthermore, the group treated with the extract exhibits a comparable trend to the drug-administered group, suggesting a similarity in the reduction of blood glucose levels. These findings imply a potential correlation between the administered extract and the observed decrease in blood glucose levels, similar to the effects observed with the drug. Moreover, the group treated with the extract exhibited the most noteworthy drop in blood sugar levels by the 14tht day of treatment, while the metformin showed a significant reduction by the 7th day. These observations highlight the effectiveness of both the extract and the standard drug treatment in lowering blood glucose levels in diabetic rats. The use of Adhatoda vasica ethanolic extract was observed to lower blood sugar levels in diabetic rats over a 14-day period of treatment. The blood glucose levels in diabetic rats notably decreased following the administration of Adhatoda vasica ethanolic extract.

The anti-diabetic potential of Adhatoda vasica leaf extract was evaluated by assessing its hypoglycemic effect in STZ induced diabetic rats. This indicates that

the leaf extract of Adhatoda vasica, at the specified dosage, demonstrated a notable impact on lowering blood glucose levels in diabetic rats. The findings suggest a potential antidiabetic effect of the Adhatoda vasica leaf extract, as evidenced by its significant influence on glucose regulation compared to the metformin treatment in this study. In the present study, the leaf extract of Adhatoda vasica at a dosage of 100 mg/kg, 200 mg/kg and 400 mg/kg as well as metformin at a dose of 30mg/kg, were administered separately. Remarkably, both treatments resulted in a significant reduction in blood glucose concentrations among diabetic rats induced with streptozotocin (STZ). This suggests that both the Adhatoda vasica leaf extract and metformin exhibited notable antidiabetic effects in the studied rat model.

This supports the hypothesis that the leaf extract from Adhatoda vasica can lower blood sugar levels. While the exact mechanism underlying Adhatoda vasica hypoglycemic effect remains unknown, the fact that metformin and Adhatoda vasica extract both significantly decreased the blood glucose concentrations of STZ induced diabetic rats raises the possibility that Adhatoda vasica may be responsible for the hypoglycemic effect.

4.4.2 Body Weight Measurements

Throughout the study, the body weight of the subjects was regularly assessed using electronic weigh balance at weekly intervals. There were noticeable patterns in the variations in body weight across the various experimental groups by the 14th day of treatment.

TABLE 4.7: Changes in body weight

Week	**Negative Control**	**Positive Control**	**Extract Group 100mg/kg**	**Extract Group 200mg/kg**	**Extract Group 400mg/kg**
Day 1	174.67 ± 2.52c	174 ± 1.73c	118 ± 2.00a	162.33 ± 1.53b	175 ± 1.73c
Day 7	168.33 ± 2.08b	182.67 ± 3.79c	124.67 ± 9.07a	164.33 ± 3.51b	179.08 ± 3.21c

Table 4.7 continued from previous page

Week	Negative Control	Positive Control	Extract Group 100mg/kg	Extract Group 200mg/kg	Extract Group 400mg/kg
Day 14	157.33 ± 6.43b	188.33 ± 1.15d	130.67 ± 8.39a	168 ± 4.00c	181.67 ± 1.50d

Values are mean ± SEM, n = 3, by using one way ANOVA followed by Duncan multiple range test.

The standard drug group and extract treatment group displayed the most significant increase in body weight. In contrast, the diabetic negative group exhibited weight loss during the second week of treatment, decreasing from 174.67±2.52c to 168.33±2.08b. The recorded p-value of 0.05 indicates the significance of these results.

After the 14-day treatment period, noteworthy changes were observed in the body weight among various groups. The effects of Adhatoda vasica ethanolic extract on the body weight changes of diabetic rats are shown in table 4.7. The standard drug-treated group, and the extract treatment group showed a significant increase in body weight. This increase suggests potential effects related to the treatments administered. The diabetic negative control group, lost weight, which was different from the other groups gaining weight. This shows that standard drug and extract treatments exhibit positive results.

The plant extract and anti-diabetic drug were thought to influence metabolism differently in diabetic rats compared to healthy ones. The severity of diabetes in the treated rats might have differed from the negative control group. This variation could have led to different responses to the treatments administered, influencing weight changes in distinct ways. Rats receiving the plant extract or anti-diabetic drug were on a diet rich in nutrients to support their recovery, potentially leading to weight gain. The disease itself could have impacted body weight differently in diabetic and non-diabetic rats.

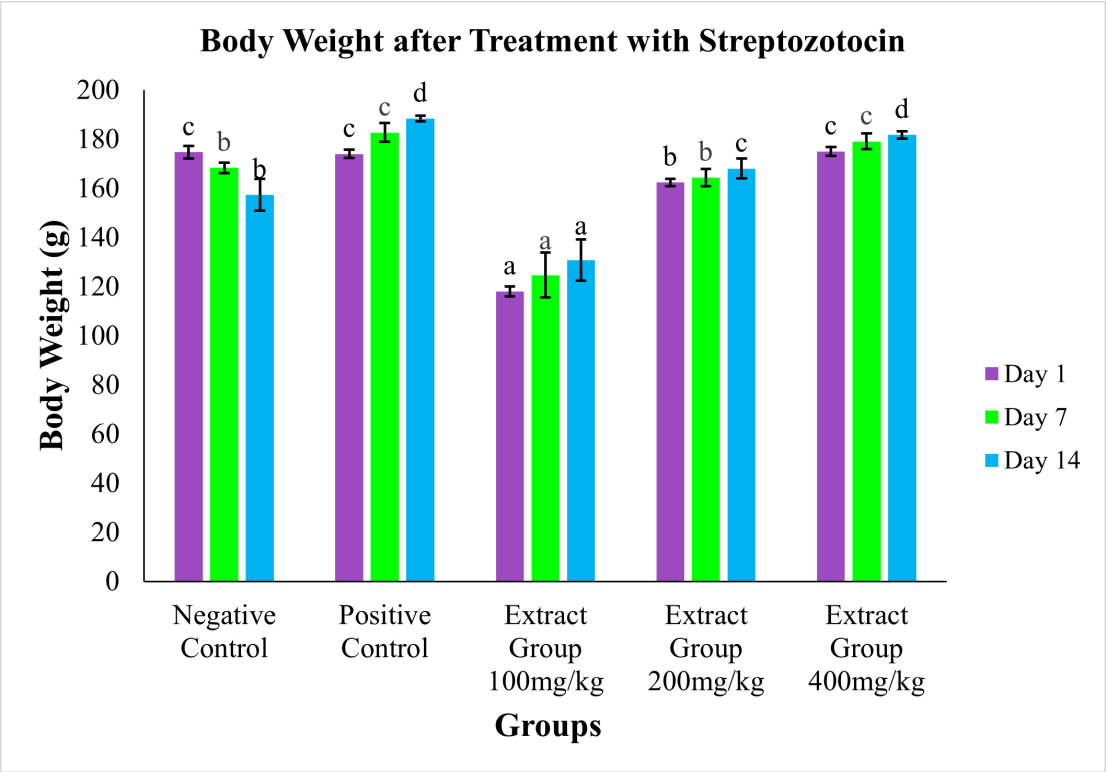

FIGURE 4.11: The changes in body weight of male Sprague Dawley rats across various experimental groups were monitored over the study period. Data were collected weekly to assess the impact of treatments on body weight in STZ induced diabetic rats.

After the 14-day treatment period, noticeable alterations in body weight were observed across different groups. The impact of the Adhatoda vasica extract on the body weight changes of diabetic rats is detailed in Table 4.7. The positive control group and the extract treatment group displayed a significant increase in body weight, indicating potential effects associated with the administered treatments.

Conversely, the diabetic negative control group experienced weight loss, setting them apart from the other groups that exhibited weight gain. This difference suggests that treatments may influence body weight differently in individuals with diabetes. Rats receiving either the plant extract or the anti-diabetic drug were on a nutrient-rich diet, potentially contributing to the observed weight gain.

A study carried out by Ramdas B. Pandhare and B. Sangameswaran, involving diabetic rats induced by STZ, an increase in body weight was noted in treatment groups [103].

4.4.3 Serum Creatinine Test

Serum creatinine test in both diabetic STZ-induced rats and treated rats aims to investigate and compare the impact of diabetes on renal function. In diabetic rats induced with streptozotocin (STZ), the study seeks to understand the alterations in renal function parameters caused by hyperglycemia. This includes assessing marker such as serum creatinine to see how much the kidneys might be affected.

TABLE 4.8: Serum creatinine test results

Groups	**Serum Creatinine**
Negative control	1.12 ± 0.03b
Positive control	1.00 ± 0.1ab
Extract group 100mg/kg	1.09 ± 0.09ab
Extract group 200mg/kg	1.08 ± 0.03ab
Extract group 400mg/kg	0.98 ± 0.03a

Values are mean ± SEM, n = 3, in positive control group n=2, by using one way ANOVA followed by Duncan multiple range test.

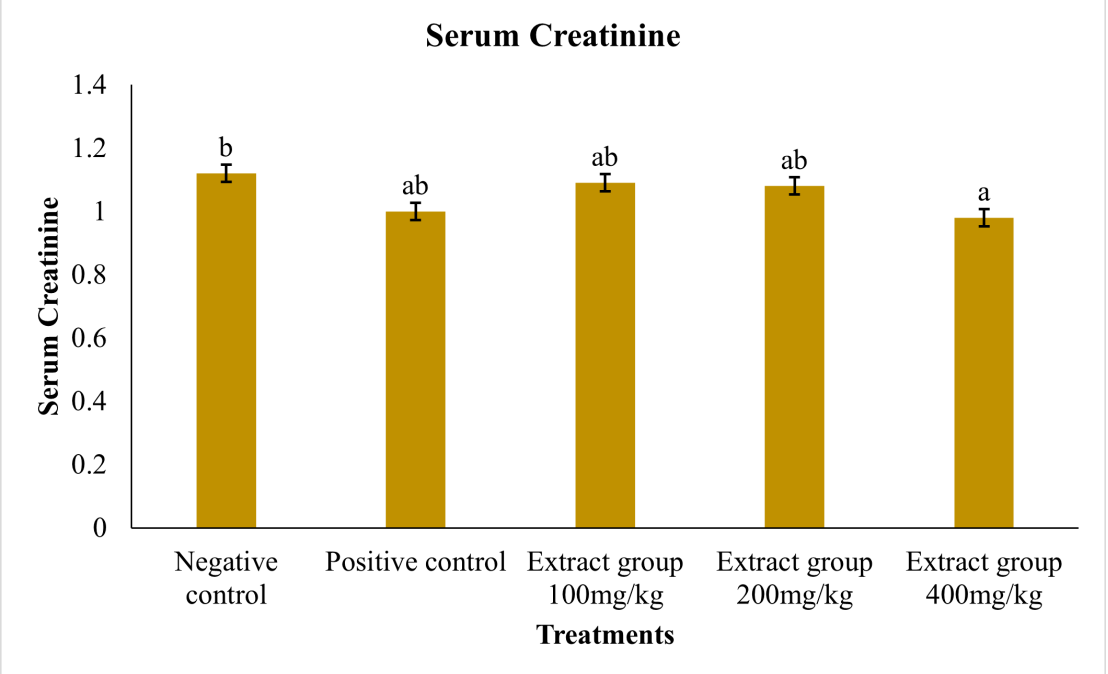

FIGURE 4.12: Graph of Serum Creatinine Levels in Diabetic Male Sprague Dawley Rats with and without Treatment After 14 Days of Treatment

After the experiment, Serum creatinine test were conducted to analyze the impact of diabetes on the kidney. The recorded Serum creatinine level was higher at 1.12±0.03b in the negative control group, which consists of untreated diabetic rats. In contrast, a lower serum creatinine level of 0.98±0.03a was observed in the group treated with the Adhatoda vasica extract concentration 400mg/kg.

The higher serum creatinine in untreated diabetic rats may indicate compromised kidney function, while the lower serum creatinine in the plant extract group suggests a potential protective effect on the kidneys. The plant extract might have properties that positively influence kidney function, helping to regulate serum creatinine levels. The impact of diabetes on the kidneys can vary among individuals.

The impact of diabetes on the kidneys can vary among individuals. Some people may experience kidney complications earlier or more severely than others. This experiment has captured significant changes in creatinine levels. Increased blood glucose levels in diabetes can affect the kidneys' ability to filter effectively, leading to a buildup of urea in the bloodstream. Diabetic individuals may experience increased thirst and urination, potentially resulting in dehydration. Dehydration can concentrate urea in the blood, contributing to higher recorded levels.

The lower serum urea levels in the positive group are expected because their kidneys are functioning normally, efficiently filtering and excreting waste products, including urea. Each rat may respond differently to the diabetic condition or the absence of diabetes. Each rat may respond differently to the diabetic condition or the absence of diabetes.

4.4.4 Liver Function Test (LFT)

LFT (Liver Function Tests) in both diabetic STZ-induced rats and treated rats aims to investigate and compare the impact of diabetes on liver function.

In diabetic rats induced with streptozotocin (STZ), the study seeks to understand the alterations in liver function parameters caused by hyperglycemia. This includes assessing markers such as A.L.T (S.G.P.T) and A.S.T (S.G.O.T) to see how much the liver might be affected.

TABLE 4.9: Liver function test results

Groups	A.L.T (S.G.P.T)	A.S.T (S.G.O.T)	Bilirubin
Negative control	129 ± 2.00d	670 ± 5.00d	0.20 ± 0.10a
Positive control	45 ± 2.00a	199 ± 2.00a	0.20 ± 0.02a
Extract group 100mg/kg	71 ± 2.00c	461 ± 3.00e	0.23 ± 0.06a
Extract group 200mg/kg	67 ± 2.00c	322 ± 3.00b	0.20 ± 0.01a
Extract group 400mg/kg	52 ± 2.00b	342 ± 3.00c	0.20 ± 0.01a

Values are mean ± SEM, n = 3, in positive control group n=2, by using one way ANOVA followed by Duncan multiple range test.

The negative group showed a high level of A.L.T (S.G.P.T) at 129±2.00d, with a recorded p-value >0.05, indicating significance. In the positive control drug group, the A.L.T (S.G.P.T) remained within the normal range. On the other hand, extract treated group reported low levels of A.L.T (S.G.P.T). Bilirubin is elevated in all groups, suggesting possible liver issues, but the levels are identical across the groups, indicating uniformity in liver response or condition.

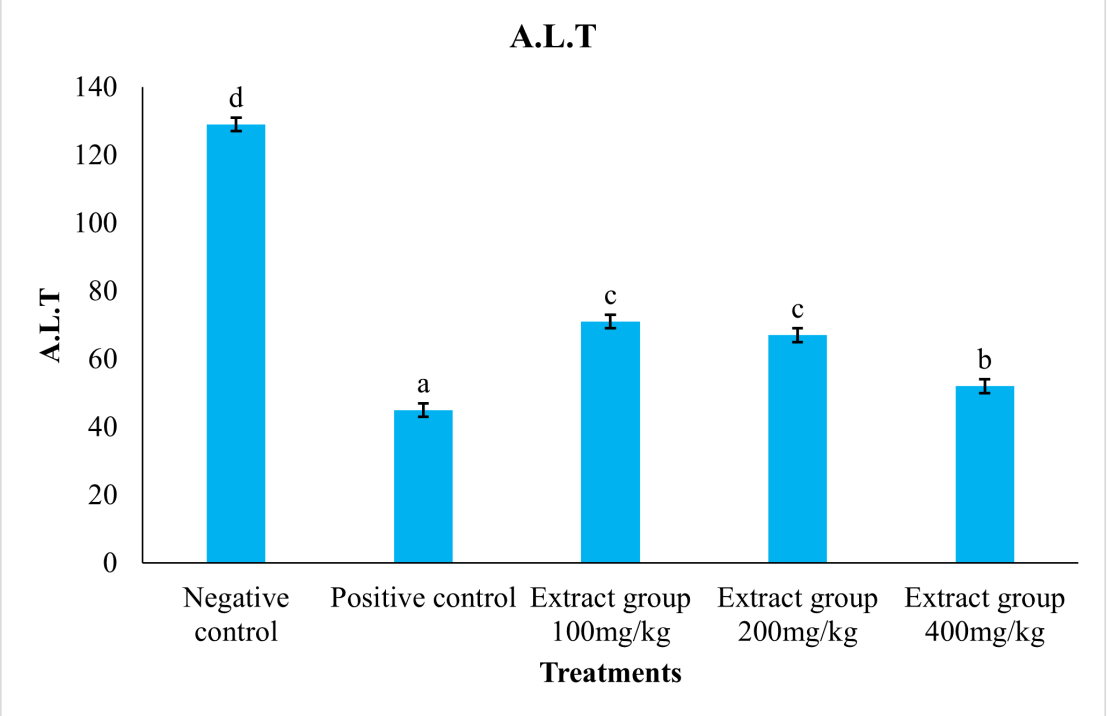

FIGURE 4.13: Graph of ALT Levels (LFTs test) in Diabetic Male Sprague Dawley Rats with and without Treatment After 14 Days of Treatment

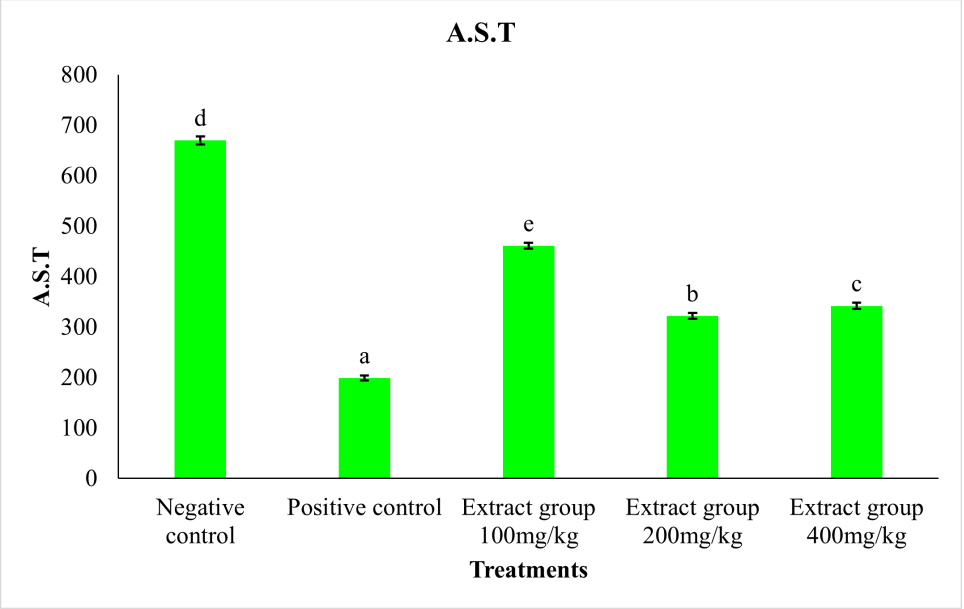

FIGURE 4.14: Graph of AST Levels (LFTs test) in Diabetic Male Sprague Dawley Rats with and without Treatment After 14 Days of Treatment

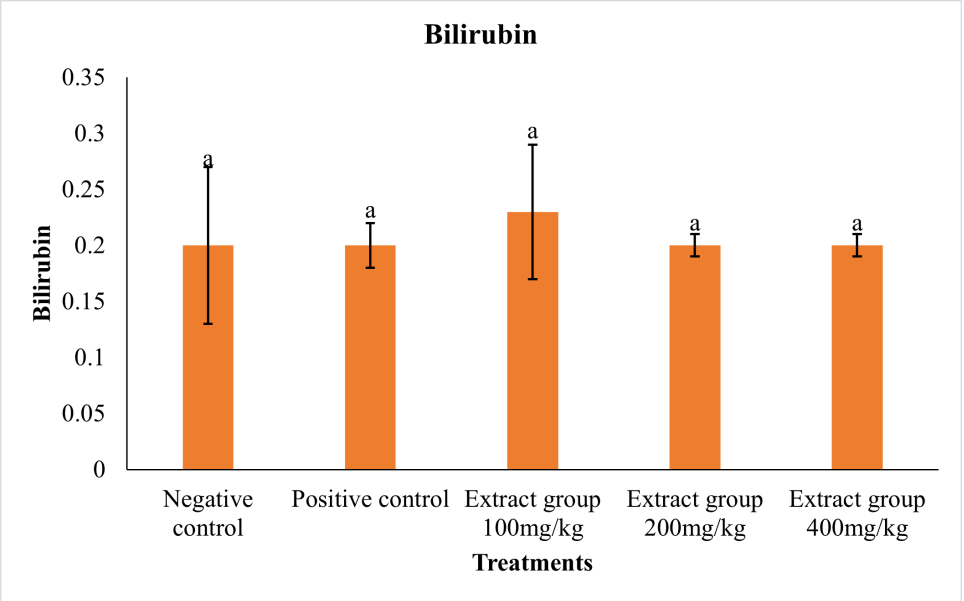

FIGURE 4.15: Graph of Bilirubin Levels (LFT test) in Diabetic Male Sprague Dawley Rats with and without Treatment After 14 Days of Treatment

The negative control group, showed high levels of liver enzymes at 129±2.00d and 670±5.00d, with a recorded p-value of >0.05 indicating significance. Elevated A.L.T (S.G.P.T) and A.S.T (S.G.O.T) levels may indicate impaired liver function in diabetic rats. Diabetes can cause changes in metabolism, leading dysfunctional of liver enzymes.

After the experiment, Liver Function test (LFTs) were conducted to analyze the impact of diabetes on the liver. Bilirubin is elevated in all groups, suggesting possible liver issues, but the levels are identical across the groups, indicating uniformity in liver response or condition.

A.L.T (S.G.P.T) is high, recorded at 129±2.00d, in the negative control group representing untreated diabetic rats indicating possible liver inflammation. In contrast, it is low at 45±2.00a in the positive control group and at 52±2.00b in the extract treated group concentration 400mg/kg. A.S.T (S.G.O.T) is high, recorded at 670±5.00d, in the negative control group representing untreated diabetic rats. In contrast, it is low in the extract treated group and positive control group.

4.4.5 Glycosylated Hemoglobin (HbA1C)

After the experiment, HbA1c test were conducted to analyze the impact of long-term overview of blood sugar control.

Table 4.10: HbA1c test results

Groups	**HbA1c**
Negative control	6.43 ± 0.03e
Positive control	4.30 ± 0.10a
Extract group 100mg/kg	5.26 ± 0.02d
Extract group 200mg/kg	4.81 ± 0.02c
Extract group 400mg/kg	4.71 ± 0.02b

Values are mean±SEM, n = 3, in positive control group n=2, by using one way ANOVA followed by Duncan multiple range test.

The diabetic group without any treatment showed the highest HbA1c value. This result aligns with expectations, as untreated diabetes generally leads to poorly controlled blood glucose levels, reflected in elevated HbA1c values. The diabetic group treated with metformin showed a significant reduction in HbA1c levels, reaching near-normal values.

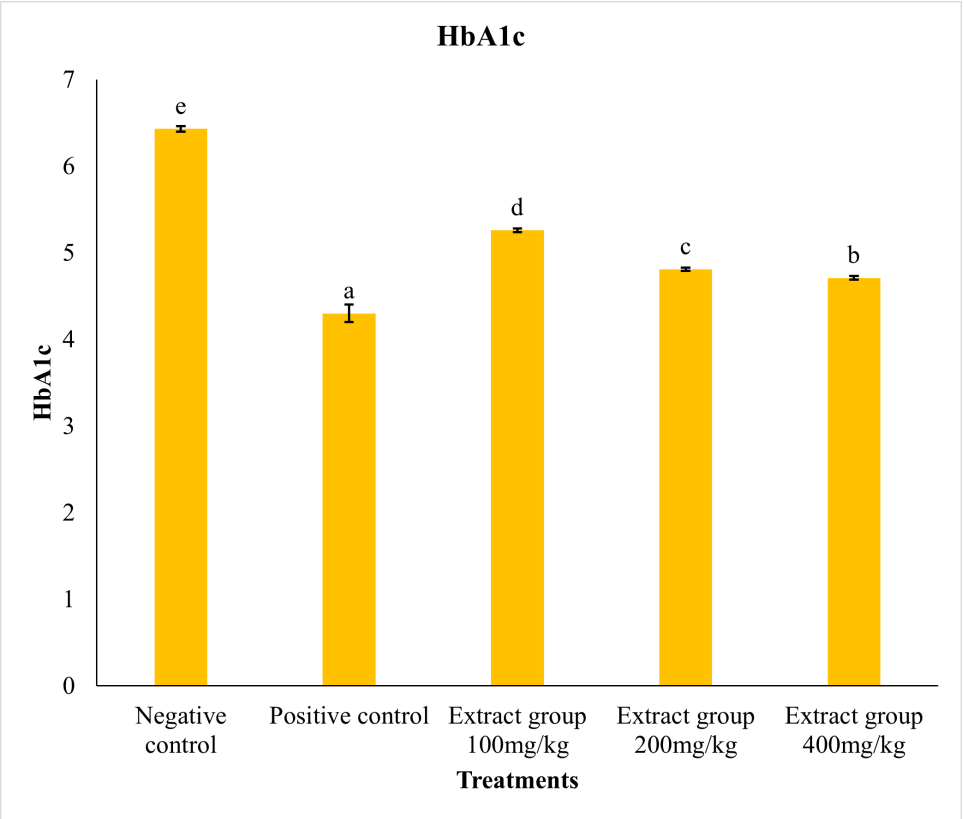

FIGURE 4.16: Graph of HbA1c test in Diabetic Male Sprague Dawley Rats with and without Treatment After 14 Days of Treatment

Metformin is a well-established treatment for managing diabetes, and this result supports its efficacy in controlling blood sugar levels. Extract group of concentration 100mg/kg shows a moderate reduction in HbA1c levels compared to the negative control, indicating some hypoglycemic effect of the extract, though less potent than metformin. The HbA1c level decreases further at this higher extract dose 200mg/kg, showing a dose-dependent response. The value is closer to the metformin-treated group, suggesting improved glycemic control at this dosage. The lowest HbA1c in the extract-treated groups is observed in extract group of concentration 400mg/kg. The effect is near that of the metformin group, indicating a strong glucose-lowering effect at this higher dosage.

The extract showed a dose-dependent effect, with the highest dose (200 mg/kg) approaching the efficacy of metformin in reducing HbA1c levels. The results suggested that the extract may be effective in controlling blood glucose levels in a manner comparable to metformin, particularly at higher doses. Further research could explore the active components of the extract, its mechanism of action, and

potential side effects compared to established treatments like metformin.

Chapter 5

Conclusion and Future Perspective

The study focused on assessing the in vitro hypoglycemic effect of phenolic compounds found in the *Adhatoda Vasica* plant, which contains essential bioactive compounds such as flavonoids, quinolines, and essential oils. The research aimed to determine the antidiabetic role of candidate plants against diabetes mellitus through in vitro testing. The results implied that investigating alternative therapeutic approaches, such as herbal remedies and related active ingredients, might be more economical and could result in fewer negative side effects. The study highlighted the importance of scientifically validating traditional herbal medicines used to treat Type 2 Diabetes Mellitus (T2DM) for better efficacy and safety. Future research could focus on conducting in vivo studies to further validate the hypoglycemic effects of the phenolic compounds from the *Adhatoda Vasica* plant. Investigating the mechanisms of action of the bioactive compounds in *Adhatoda Vasica* could provide insights into their potential as antidiabetic agents. Collaboration between traditional medicine practitioners and modern scientists could lead to the development of novel and effective treatments for T2DM. Continued research in this area may lead to the discovery of new therapeutic options for managing T2DM with improved outcomes and reduced side effects. This study investigated the antioxidant and alpha-amylase inhibitory activities of *Adhatoda Vasica*'s ethanolic leaf extract. The findings demonstrated a concentration-dependent antioxidant

activity, with scavenging activity increasing over time across all tested concentrations. The total phenolic content of the extract, which was determined using the Folin-Ciocalteu method and calibrated against gallic acid, showed a favourable connection with its antioxidant activity. The extract's varied absorbance values at different doses showed a strong relationship between antioxidant capability and phenolic content. The extract exhibited a notable antioxidant activity due in large part to the presence of phenolic chemicals, which are recognised for their redox characteristics. The extract's potential as an alpha-amylase inhibitor was demonstrated by the alpha amylase results, which may help control blood sugar levels and cure diseases including diabetes and obesity. The ethanolic leaf extract of *Adhatoda Vasica* contains bioactive substances that may have contributed to its alpha-amylase inhibitory activity, including phenols, flavonoids, saponins, steroids, alkaloids, and terpenoids. These results are consistent with earlier research that demonstrated a beneficial correlation between total polyphenol content and the capacity to inhibit pancreatic alpha-amylase. The study's encouraging findings provide a number of directions for further investigation and advancement. The primary goal of future studies should be to identify and isolate the particular bioactive substances causing the alpha-amylase inhibitory action. Sophisticated methods such as mass spectrometry (MS), high-performance liquid chromatography (HPLC), and nuclear magnetic resonance (NMR) spectroscopy can be used to identify and quantify these compounds. Detailed mechanistic studies are necessary to understand the precise mode of action of the identified compounds. Investigating how these compounds interact with the alpha-amylase enzyme at the molecular level can provide insights into their inhibitory mechanisms and help optimize their efficacy. While in vitro studies provide valuable initial insights, it is crucial to evaluate the effects of *Adhatoda Vasica*'s ethanolic leaf extract in vivo. Animal models of diabetes and obesity can be used to assess the extract's efficacy in reducing postprandial blood glucose levels and its long-term impact on metabolic health. Based on the bioactivity of the extract, formulations can be developed for therapeutic use. These could include oral supplements, functional foods, or nutraceuticals designed to manage blood sugar levels in diabetic patients or those at risk of metabolic disorders. Clinical trials are necessary to verify the

safety and effectiveness of the extract in human subjects after successful in vivo research. Such trials should aim to determine optimal dosages, potential side effects, and long-term benefits of using *Adhatoda Vasica*'s ethanolic leaf extract as an alpha-amylase inhibitor. Investigating the synergistic effects of *Adhatoda Vasica*'s extract with other known antidiabetic and antioxidant compounds could enhance its therapeutic potential. Combining it with other medicinal plants or conventional antidiabetic drugs might provide more effective treatment options. Ensuring sustainable sourcing of *Adhatoda Vasica* and standardizing the extraction process is vital for the consistent quality and potency of the extract. Developing guidelines for the cultivation, harvesting, and processing of the plant will support large-scale production and clinical application. Ensure sustainable sourcing of *Adhatoda Vasica* and standardize the extraction process to maintain consistent quality and potency of the extract. Developing guidelines for the cultivation, harvesting, and processing of the plant will support large-scale production and clinical application. Explore other potential medicinal properties of *Adhatoda Vasica*'s ethanolic leaf extract, such as anti-inflammatory, antimicrobial, and anti-cancer activities. Comprehensive studies on these aspects could expand the therapeutic applications of the extract.

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