EFFECTS OF *Acanthus montanus* (NEES) T. ANDERSON AQUEOUS LEAF EXTRACT ON HAEMATOLOGICAL INDICES IN STREPTOZOCIN-INDUCED DIABETIC RATS.

BY

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SR/2310/RPR/25/52

UNIVERSITY OF BENIN

FACULTY OF LIFE SCIENCES

DEPARTMENT OF PLANT BIOLOGY AND BIOTECHNOLOGY

NOVEMBER 2025

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A PROJECT REPORT SUBMITTED TO THE DEPARTMENT OF PLANT BIOLOGY AND BIOTECHNOLOGY, FACULTY OF LIFE SCIENCES, UNIVERSITY OF BENIN IN PARTIAL FUFILLMENT OF THE REQUIREMENTS FOR THE AWARD OF BACHELOR OF SCIENCE (HONOURS) DEGREE (BSc) IN PLANT BIOLOGY AND BIOTECHNOLOGY.

NOVEMBER 2025.

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CERTIFICATION

We certify that Eunice SOLOMON in the Department of Plant Biology and Biotechnology, Faculty of Life Sciences, University of Benin, Benin City, Nigeria, carried out this research work.

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Prof. B. Ikhajiagbe **Date**

(Head of Department)

DEDICATION

This work is dedicated to the Almighty God Jehovah for his continuous guidance, protection and support throughout the time of carrying out the research.

ACKNOWLEDGMENTS

I wish to convey my heartfelt appreciation to my supervisor, Professor Odaro Timothy for his valuable insights and support throughout the project period. I also want to thank Dr. Benjamin O. Gabriel for his expert guidance and assistance. I also appreciate the Head of Department, Professor Beckley Ikhajiagbe.

My deepest appreciation goes to my wonderful parents and elder brother for their financial and emotional support and their consistent words of encouragement. I am truly grateful.

I also extend my appreciation to the wonderful friends I made among my coursemates. I value all the support given. I also appreciate my project mates for their assistance during the various stages of the work and for their spirit of cooperation.

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ABSTRACT

This study examined the effect of aqueous leaf extract of *Acanthus montanus* on haematological indices in streptozotocin-induced diabetic rats. *Acanthus montanus* is a medicinal plant commonly used for its therapeutic properties. The leaves were collected, rinsed with water, air and oven dried, pulverized and extracted using cold water maceration techniques. 25 rats were divided into five (5) groups of five (5) rats each. Groups one, two and three received 25, 50 and 100 mg/kg of the aqueous extract respectively. The fourth group recieved 45mg/kg of streptozotocin and 10mg/kg of the standard drug glibenclamide while group five recieved 45 mg/kg of streptozotocin. Treatments were administered orally, once daily for 21 days. At the end of the experiment, blood samples were collected for analysis. The results obtained showed that at various doses, the plant extract has the ability to improve haematological indices. Different doses of the extract showed improved and stable effects on the various parameters such as the white blood cells, red blood cells and platelets. This research supports the potential use of the extract for alleviating haematological alterations associated with diabetes.

CHAPTER ONE

INTRODUCTION

1.1 Background of the study

Diabetes mellitus is a persistent and terrible metabolic disorder caused by persistent high blood sugar due to impaired insulin secretion or action or both (International Diabetes Federation, 2025). Diabetes arises as a result of the pancreas being unable to make insulin or the body inefficiency in using insulin. Its effects are fatal as it leads to low grade inflammation, disturbances in blood cell indices, kidney failure, limb amputation among others (Sharma and Bora, 2021; Ghasemi *et al*., 2023).

Globally, the incidence of Diabetes mellitus is increasing. It has been estimated that 589 million people lived with diabetes in 2024. This number is expected to rise to 853 million by 2050. (IDF, 2025). In Nigeria, as at the year 2024, a 3% prevalence in adults representing 2.99 million adults was recorded (IDF, 2024). This figure is the highest in the IDF region of Africa.

Streptozotocin or STZ for short is a naturally occurring nitrosourea compound and the most used diabetes causing chemical that is widely used to cause diabetes in animal models especially rodents e.g: rats in experimental research (Ghasemi and Jeddi, 2023). *Acanthus montanus* (Nees) T. Anderson in the Acanthaceae family is often known as the Bear's breech, mountain thistle and alligator plant. It is a tiny shrub used in traditional medicine for the treatment of wounds, furuncles, hepatitis, etc. The plant is a good source of secondary metabolites such as: flavonoids, alkaloids, tannins, steroids, glycosides, phenols and saponins. This makes it sufficient for the treatment of various ailments (Idu *et al*., 2022).

Haematology is the branch of medicine that focuses on the study of the blood, its cellular elements blood, its number and what it is made up of and using the information gotten to

diagnose diseases (Etim *et al.,* 2014). Haematological indices are extremely important for the evaluation of blood and its components and they provide information on the physiological and pathological status of an organism (Khan and Zafar, 2005). Haematological indices are useful for this research being carried out. This study will determine the effect of the aqueous leaf extract of *A. montanus* in managing diabetes-related hematological changes.

1.2 Overview of Diabetes mellitus

As mentioned earlier, diabetes mellitus is a long term metabolic disease that results from insufficient insulin release, action or both (International Diabetes Federation, 2025). Globally, it has emerged as one of the fast growing non-communicable diseases with an estimated 11.1% of adults affected and more than 40% remaining undiagnosed (International Diabetes Federation, 2025). The event of diabetes is higher in the urban areas than the rural areas and in high income countries than low income countries (Sharma and Bora, 2021).

Clinically, diabetes is categorized into: type 1 diabetes (evident by autoimmune damage of the pancreatic beta cells and total absence of insulin), type 2 diabetes (marked by an increasing malfunction of insulin release against a background of insulin resistance), and other types such as: gestational diabetes, monogenic diabetes, pancreatic and drug induced (World Health Organization, 2024; American Diabetes Association, 2025).

Effects of diabetes include cardiovascular disease, neuropathy, retinopathy, all of which reduce life expectancy and quality of life. According to the World Health Organisation in 2024, changes in haematological parameters including Haemoglobin (HB), packed cell volume (PCV), red blood cell count (RBC) and white blood cell count (WBC) are commonly associated with diabetes.

Diabetes carries serious health implications as well as socio-economic consequences such as death, strain on health care systems especially in developing countries. It also causes

financial strain on families as a result of expenses incurred during treatment. Diabetes is a medical concern that requires serious measures to combat.

1.3 Haematological Indices and Diabetes monitoring

Haematological indices are important parameters that are used to clinically investigate the presence of metabolites and other elements in the body of animals. They play an important role in the nutritional and pathological status of animals (Etim *et al.,* 2014).

Important parameters used in the evaluation of blood includes: red blood cell count, white blood cell count, haematocrit, lymphocytes, mean corpuscular volume, mean corpuscular haemoglobin. The red blood cell variables such as the haemoglobin and haematocrit are key elements in the diagnosis and classification of anaemia such as the sickle cell anaemia and other red blood cell disorders. Changes in these indices may indicate signs of nutritional deficiencies, haemolysis or prolonged systemic diseases (Asafa *et al*., 2025).

The White blood cell parameters such as neutrophil to lymphocyte (NLR) ratio provide valuable information into immune activity and inflammatory status. Another parameter is the platelet to lymphocyte ratio (PLR).

Diabetes mellitus disrupts hematological balance and results in anaemia, platelets disorders and leukocyte count variations. It has also been reported that reduced red blood cell count, haemoglobin, haematocrit are present in diabetic rats. These indicate systemic inflammation (Krisnamurti *et al*., 2022).

An increase in blood sugar levels causes oxidative stress, reduces red blood cells life span and damages the erythrocyte membranes and proteins (Szalai *et al*., 2023). In a research carried out by the Rivers State Teaching Hospital, it was reported that patients with Type 2 diabetes mellitus had raised hematological inflammatory indices. Elevated neutrophil to lymphocyte ratio and erythrocyte sedimentation rate were reported (Simeon *et al*., 2025).

This also agrees with another research carried out by Mansoori *et al*., (2023) where it was discovered that the white blood cell plays a significant role in predicting Type 2 diabetes mellitus.

Haematological indices are simple, cost effective markers in monitoring diabetes. They are used to track the progress of diabetic treatments be it Orthodox medicinal treatment or traditional medicinal plants. They are relevant for analysing inflammation in diabetes management. The blood constituents change in relation to the physiological status of an animal or human. The changes are important for assessing the response to treatment (Etim *et al*., 2014). The hematological values gotten are the references used for comparison during research.

1.4 Streptozotocin and its role in inducing diabetes mellitus

Streptozotocin or STZ is a nitroso-urea product of glucoseamine that is produced naturally by the soil-dwelling bacteria *Streptomyces achromogenes* (King *et al*., 2022). It originates from glucose and is commonly used to induce diabetes in laboratory animals. The nitrosoureas are a type of chemotherapy drugs that are under the larger family of alkylating agents. They are highly fat-loving and attaches alkyl group to DNA and RNA and are effective against tumors (Jackson and Harris, 2024).

Streptozotocin is the most commonly utilized chemical used to induce diabetes in rat models of both Type 1 and 2 diabetes (Ghasemi and Jeddi, 2023). It particularly damages the pancreatic beta cells, causes toxicity and leads to insulin deficiency and high blood sugar just like human diabetes (Hahn *et al*., 2020). Streptozotocin shares structural similarities to glucose, allowing its entry into the pancreatic beta cells through the glucose transporter GLUT2. This causes the pancreatic beta cells to die and results in a complete or partial loss of

insulin production affected by the streptozotocin dosage and the animal age at the time it is given (Marino *et al*., 2023).

Experimental Type 1 diabetes is induced by a single high dose of streptozotocin (usually at 200mg/kg body weight) in rats. It is suggested that streptozotocin at high doses could cause severe oxidative damage and cell death in pancreatic islet beta cells in rats, leading to a swift rise of blood sugar level within forty eight hours. Multiple low dose streptozotocin leads to a reduction of immune activities in pancreatic islets of treated animals. This is also linked to high blood glucose levels, less production and secretion of insulin from beta cells. Increased blood glucose levels have been observed in sixteen weeks following multiple low dose-streptozotocin administration (Zhu, 2022).

For the Type 2 diabetes, there are two commonly applied strategies: high fat diet added to streptozotocin and Nicotinamide added to streptozotocin. In the former, rodents are initially given a high fat diet that may or may not contain sugar for several weeks leading to obesity, insulin resistance and high insulin levels. The next step is the administration of streptozotocin, which decreases the beta cell numbers, a condition similar to late stage type two diabetes (Skovso, 2014). The latter involves administering of nicotinamide either shortly before or after streptozotocin. Nicotinamide reduces streptozotocin beta cell toxicity by blocking poly ADP-ribose polymerase 1(PARP 1), resulting in a partial loss of beta cells. It leads to insulin being partly reduced. The streptozotocin-nicotinamide model has been widely used to mimic human Type 2 diabetes (Yan, 2022).

Type 1 diabetic models uses high streptozotocin to cause severe beta cell loss, whereas Type 2 diabetic models combines factors like diet, low doses or nicotinamide to maintain partial beta cell function (Marino *et al*., 2023). Streptozotocin induced diabetes models are important in diabetes research advancements. They are used by researchers to test the safety of new drugs and treatments. The nicotinamide streptozotocin model is an efficient platform for

assessing anti-diabetic treatments (Yan, 2022). Streptozotocin models has also been used in a wide range of research including: studies of how to protect beta cells, how endocrine cells change into insulin producing cells, how immune cells enter the pancreas among others (Hahn *et al*., 2020). The streptozotocin induced diabetic models are essential in the study of diabetes.

1.5 Acanthus montanus

Acanthus montanus is commonly called the bear's breeches, alligator plant or mountain thistle. It is a member of the Acanthaceae family. Ethnomedicinally, *A.montanus* has a broad range of applications, ranging from wound healing to its use as a vermifuge. The plant possession of a rich composition of compounds and phytochemicals contributes to its importance in ethnomedicine (Idu *et al*., 2022). *A.montanus* leaf tissue is also a rich source of vitamin A and C.(Igwe and Eleazu, 2017).

Taxonomic Classification

Kingdom – Plantae

Division – Tracheophyta

Class – Magnoliopsida

Order – Lamiales

Family – Acanthaceae

Genus – *Acanthus L.*

Species – *montanus*

Binomial name – *Acanthus montanus* (Nees) T. Anderson

Source: Idu *et al.,*(2022)

1.6 Botanical description of *Acanthus montanus*

Acanthus montanus is a perennial herb with slender basal branches and clusters of glossy, deep-green oblong or lanceolate leaves. The leaves can grow up to 30 cm in length, the stem can grow up to 1.8 m tall and the whole plant can have a girth up to 61 cm. The leaves possess silver lines with wavy borders at the edges. The plant likes shady places with periodic deep watering, though it is also capable of tolerating hot and dry conditions. The leaves have spiny serration and can be shallow or deeply lobed, having a shiny dark green on the upper surface and a pale green underside. It also has elongated flowers ranging from pink to red. Its strong roots make it fit for slopy areas (Dressler *et al*., 2014). The leaf of *A. montanus* has the resemblance of an alligator and is tooth edged. Hence, it is also called the alligator plant (Idu *et al.,*2022).

Plate 1: Fresh leaves of *Acanthus montanus*

Source: Idu *et al.,* (2022)

1.7 Distribution and Cultivation of *Acanthus montanus*

Acanthus montanus originates from the tropical West and Central Africa countries such as: Angola, Congo, Benin Republic, Equatorial Guinea, Central African Republic, Gabon, Ghana, Niger and Togo (Igwe and Eleazu, 2017). It is widely distributed throughout Africa, the Balkans, Greece, Romania and the Eastern Mediterranean. In Nigeria, the plant is known with a number of local names. In Edo, the plant is known as "agamobo", in Ijaw as "edulee memen", in Yoruba as "ahon ekun", in Igbo as "agameebu" or "ogwudurun washishi" (Idu *et al*., 2022).

Acanthus montanus grows well in tropical areas with an annual rainfall of 1000-2500 mm and temperatures ranging from 20-35 degree Celsius. It prefers rich and well-drained soils that are loamy to sandy in nature. It is normally found in heights ranging roughly from 100-1500 mm above sea level (Anonymous, 2025). It can be grown from root cuttings or by seeds. Once it is grown, it is hard to remove because small root fragments can form rhizomes and create new plants, leading to invasive spread through creeping roots in loose soils.

1.8 Ethnomedicinal uses of *Acanthus montanus*

Acanthus montanus has been traditionally employed for treating conditions such as; wounds, boils, gonorrhea, heart disorders, syphilis, hepatitis among others. The paste made from combining the young twigs of the plant with sugar is applied on wounds to hasten their healing. Also, drinking a tea made from the leaves is used to alleviate indigestion and can act as a worm expeller and a remedy to simulate menstruation (Idu *et al*., 2022). In Southern Nigeria, it is taken by post-natal mothers to enhance recovery and strength. The people of Cameroon also use the preparations from the leaves for heart-related issues (Anonymous, 2025). The Igede people of Benue state and the Enugu-Ezike community of Enugu state use the root poultice to treat boils. They believe that the root paste makes the boil to 'ripen',

meaning if forms more pus, which means that the sickness is gone (Igoli *et al*., 2004). More studies need to be done in order to establish the safety of this plant in treating illnesses.

1.9 Phytochemistry

Phytochemistry is the study of the chemicals that comes from plants. These chemicals are gotten from the roots, leaves, seeds, fruits and flowers of the plant. *Acanthus montanus* contains numerous natural compounds including alkaloids and carbohydrates and small amounts of tannins, terpenoids, flavonoids, glycosides and saponins (Idu *et al*., 2022). Tests have shown that the methanol extract of *A. montanus* leaves has a lot of alkaloids, reducing sugars and phenols, while the chloroform extract is abundant in alkaloids, flavonoids, carbohydrates and phenols. The use of the Gas chromatography-mass spectrometry has discovered fatty acid and phenol products (Okenwa and Nnaji, 2014). The plant abundance of phytochemicals such as its alkaloids and polyphenols is its basis for its biological effects (Idu *et al*.,2022). These phytochemicals also protect the plant from pollution damage, drought and harmful ultra-violet rays (Vishnu *et al*.,2019).

1.10 Biological activities of *Acanthus montanus*

Further studies on the leaf and root extract of *Acanthus montanus* reveals that it contains a lot of biochemical compounds hence proving its pharmacological validity. It exhibits a range of biological activities such as: anti-inflammatory, antioxidant, anti-diabetic activities, etc.

1.10.1 Antioxidant

Tests shows that leaf extracts of *Acanthus montanus* are abundant in phenolic compounds and acts as anti-oxidants. Enitan *et al*., (2021) Different extracts such as: methanol, acetone and ethyl-acetate were tested and it was discovered that they were all active against DPPH (2,2-diphenyl,1,picrylhydrazyl) and nitric oxide radicals, with the acetone extract working the best.

Quantitative evaluations showed that the methanol extract contained the highest flavonol concentration while the acetone extract gave the strongest anti-oxidant effect. This shows that the type of solvent used changes both the amount of plant chemicals and their anti-oxidant strength (Enitan *et al*., 2021). In another research carried out, rats were exposed to oxidative stress. There was a significant drop ($p<0.05$) in red and white blood cells, packed cell volume and haemoglobin which led to anaemia. The methanol extract significantly ($p<0.05$) improved antioxidant enzyme activities, reduced high malondialdehyde which is a sign of lipid damage and restored blood measure to normal compared to untreated rats. This shows that methanol leaf extract of *Acanthus montanus* can protect the body from oxidative stress by stopping the damage of fats and restoring blood health. Animal tests also proved that in rats with liver injury caused by acetaminophen, the extract restored key anti-oxidant enzymes such as catalase and superoxide dismutase (Uroko *et al*.,2019).

1.10.2 Anti-inflammatory

Researchers tested the anti-inflammatory power of *Acanthus montanus* aqueous extract in mice with carrageenan-induced paw swelling. The extract at a dosage of 100-400mg/kg reduced swelling much like diclofenac which is a standard anti-inflammatory drug at 50mg/kg and L-Nitro arginine methyl ester (that blocks nitric oxide) at 100mg/kg. When the mice were given the nitric oxide precursor which increases nitric oxide in addition to the aqueous extract, it blocked most of the anti-inflammatory effects of the extract by 76%. This shows that the plant anti-inflammatory action works by reducing nitric oxide thus backing up its traditional use for treating inflammation (Foyet *et al*., 2008). In another test, the aqueous root extract of *Acanthus montanus* stopped the growth of the test organisms and significantly ($p>0.05$) reduced swelling in the mouse ear by 57%. It also lowered paw swelling in rats in a

dose-independent way (Okoli *et al*., 2008). Reviews of the genus of *Acanthus montanus* confirms its folk anti-inflammatory use (Matos *et al*., 2022).

1.10.3 Antimicrobial

A report was made that extracts from the leaves and roots of *Acanthus montanus* made with ethanol, ethyl acetate and n-hexane stopped the growth of both Gram-positive and Gram-negative bacteria as well as fungi, including *Candida albicans*, *Aspergillus* spp. and *Fusarium* spp. The extracts showed suppression zones between 14 and 34 mm. Tests also showed that the extracts contained high amounts of flavonoids between 53 to 86% and alkaloids between 3 to 11%. This is likely responsible for their antimicrobial properties and may explain their germ fighting ability. This supports the ethnomedicinal use of the plant in managing skin and urinary infections (Ndukwe *et al*.,2024). Okoli *et al*.,(2008) also showed that the aqueous root extract also showed mild antibacterial effects against germs that cause boils, especially *Pseudomonas aeruginosa* and *Staphylococcus aureus*.

1.10.4 Antimalarial

A recent study isolated a natural glycoside called verbascoside from the hydroethanolic extract of *Acanthus montanus* and tested it against malaria in mice. The crude extract and the acetone fraction reduced parasite levels by 37.83% and 41.57% respectively at 400mg/kg. Verbascoside was more effective, suppressing 48.65% of parasites at 5mg/kg and 61.19% at 10mg/kg in a 4-day test. In another test, it cleared up to 50% of the parasite at 5 and 10mg/kg respectively. Computer analysis showed that verbascoside binds well to plasmodium falciparum dihydrofolate reductase-thymidylate synthase and plasmepsin II stronger that chloroquine standard drug. These findings shows that *Acanthus montanus* is a promising malarial agent supporting its ethnomedicinal use against malaria (Olatunji *et al*.,2024).

1.10.5 Anticonvulsant and Sedative

A comparative study investigated the anticonvulsant and sedative properties of six medicinal plants, including *Acanthus montanus*, using several animal seizure models such as: Maximal electroshock (MES), picrotoxin (PIC), strychnine(STR), N-methyl-D aspartate (NMDA), pentylenetetrazol (PTZ), isonicotinic hydrazide aid (INH) induced convulsions as well as diazepam-induced sleep. *Acanthus montanus* demonstrated protective effects in 66.6% of mice against MES, PIC, and STR-induced seizures and in 83.3% of mice against PTZ-induced convulsions. It also showed sedative activity by prolonging diazepam-induced sleep. These results suggest that *Acanthus montanus* possesses both anti-convulsant and sedative properties, supporting its ethnomedicinal use in managing insomnia and epilepsy (Bum *et al*.,2009).

1.10.6 Analgesic

Methanol leaf extracts of *Acanthus montanus* have demonstrated significant, dose-dependent analgesic activity in multiple rodent pain models. The extract at 200-400mg/kg produced a dose-dependent and significant ($p<0.05$) increase in pain threshold and at 100-400mg/kg caused dose-dependent inhibition of squirming and suppression of both phases of the formalin test and a stronger effect in the late inflammatory stage. Although, it is less effective than morphine, these results indicate that the plant analgesic effects are moderated by both central and peripheral mechanisms (Adeyemi *et al*.,2004).

1.10.7 Anti-diabetic

To assess whether *A. montanus* can help lower blood sugar, the roots were extracted with methanol and separated into different chemical fractions: n-hexane, petroleum ether, ethyl acetate, diethyl ether and chloroform fractions. Ethyl acetate was selected for the analysis in

alloxan-induced diabetic rats. Thin layer chromatography was used for the separation and determination of the ethyl acetate constituents. The results showed that the extract lowered blood sugar in a dose dependent way. In diabetic rats, ethyl acetate reduced glucose levels by about 22%, 38% and 49% at doses of 100, 200 and 300mg/kg which was nearly effective as the standard drug glibenclamide. In normal rats, blood sugar was also lowered by 19 to 41% depending on the dose. There were no deaths even at doses up to 5000mg/kg indicating its safety. The extract had a dose dependent low blood sugar effect thus providing a pharmacological basis for the use of *A. montanus* in managing diabetes (Odoh *et al.,* 2013).

1.10.8 Insecticidal

A phytochemical screening of the alcoholic extract of *A. montanus* aerial parts identified 9 compounds, with 8 having different insecticidal activities against female *Aedes aegypti*. At doses of 1.25 g/mg and 0.63g/mg, sitosterol glucoside and palmitic acid emerged as the most active, causing up to 100% and 90% mortality. Other glycosides demonstrated moderate activities such as linasoride with 80% and acetoside with 70% (Elham *et al*., 2012). It has been previously reported that palmitic acid has the potential to kill the larva of *Culex quinquefasciatus, Anopheles stephensi* and *A. aegypti* (Rahuman *et al*., 2000).

1.10.9 Hepatocurative

The safety of methanol leaf extract of *A. montanus* in mice was studied with no harmful effect or death recorded. In rats that were induced with liver damage through acetaminophen, the extract lowered alanine aminotransferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase (ALP) and total bilirubin while significantly raising protein, albumin and direct bilrubin levels ($p<0.05$). It also improved liver structure. These results suggest that

A. montanus has liver protective properties, can reverse liver failure and can ease liver damaging side effects (Uroko *et al*., 2019).

1.10.10 Spermatogenesis

The aqueous leaf extract of *A. montanus* was discovered to have enhanced spermatogenic activity in the Swiss albino mice in a reversible manner. The activity was independent of the rise of *A.montanus* concentration and did not significantly alter or reduce body weight, the reproductive body weight or the gonado-somatic index. These findings indicate that *A. montanus* promotes sperm formation, suggesting potential for use in managing male infertility and spermatogenic disorders (Orlu and Obulor,2014).

1.11 TOXICITY STUDIES

The toxicological effects of *Acanthus montanus* leaf extracts were evaluated in male and female albino rats using a total of 90 rats (45 males and 45 females) distributed into 18 groups of five animals each. Animals were administered varying doses of 200, 400, 600 and 800mg/kg of either aqueous or methanol leaf extract, while control groups received only normal rat chow. There was a dose dependent and significant elevation in liver enzymes activities and a significant reduction in serum production and albumin concentration were also observed. When compared to the controls, there was no significant difference in total or direct bilirubin. Urea levels dropped and there was increase in kidney stress. When the tissues were checked, there was mild to serious liver and kidney abnormalities in groups treated with high doses of 800mg/kg aqueous extract and 400 to 800mg/kg methanol extract. These findings suggest that high dose administration of *Acanthus montanus* extracts may harm the liver and kidneys, so there should be neutralization before use (Iwueke *et al*., 2021).

1.12 Acute toxicological studies

The acute oral toxicity of aqueous lead extracts in Wistar rats was studied. The doses were 0, 500, 1000, 2000, 4000 and 8000mg/kg. Over seven days, it was revealed that there were no significant changes in behaviour or mortality at doses up to 4000mg/kg. However, animals treated with 8000mg/kg showed a lower reactivity to stimuli and less food and water intake. Further analysis also showed that there was an increase in serum proteins and kidney degenerations, but there were no defects in the liver and lungs (Paulin *et al*., 2007).

1.13 Sub-acute toxicological studies

Female Wistar rats were administered daily doses of 0, 125, 250, 500 and 1000mg/kg for 30 days. There were no signs of haematological changes or oxidative stress at doses of 125 to 500mg/kg. At higher doses more than 500mg/kg, kidney damaging effects and raised creatinine levels was observed which is lower than estimated human equivalent doses (Djami *et al*., 2011).

1.14 AIM AND OBJECTIVE OF STUDY

AIM:

This study is aimed at investigating the effect of aqueous leaf extract of *Acanthus montanus* on haematological indices in Streptozotocin- induced diabetic rats.

OBJECTIVES:

The objectives of the study are to:

1. Assess the effect of aqueous leaf extract of *A. montanus* on white blood cell count and differentials
2. Determine the impact of *A. montanus* aqueous leaf extract on red blood cell and components
3. Evaluate the effect of aqueous leaf extract of *A. montanus* aqueous leaf extract on platelet count and associated coagulation factors
4. Examine the impact of varying concentrations of the plant extract on haematological indices in diabetic rats.

CHAPTER TWO

MATERIALS AND METHODS

2.1 Chemicals and Reagents

Gentian violet paint, formalin, chloroform and distilled water.

2.2 Equipment and Apparatus

Digital weighing balance, conical flask, filter paper, syringes, surgical scissors, water bath, hot air oven, measuring cylinder, mortar and pestle, oral gastric tube, jars and plastic cages, EDTA vacutainer tubes, British milling machine and glucometer machine.

2.3 Plant Identification and Collection

The leaves of *Acanthus montanus* were identified and collected from the mature shrubs of individual plant species found in Bolorunduro in Akure, Ondo State, Nigeria. It was authenticated in the Herbarium unit of Plant Biology and Biotechnology, Faculty of life sciences University of Benin, Edo state, Nigeria with the voucher number: UBH-A312. .

2.4 Plant preparation

The leaves of *Acanthus montanus* were rinsed in clean water, chopped into pieces, air dried and further oven dried at 45°C for 24 hours. The dried leaves were pulverized using a British milling machine. The weight of the pulverized leaves was taken and the extraction process was carried out using aqueous solvent system. The pulverized leaves of *A. montanus* weighing 1.6 kg was extracted using cold maceration technique of 6.8 L of distilled water for a period of 72 hours with regular intervals of manual agitation (Adamu *et al.,* 2010). The

maceration process took place in conical flasks and jars. After soaking, the mixture was filtered using filter paper to separate the liquid extract from the plant residues.

2.5 Experimental Animals

A total number of twenty five (25) albino Wistar rats were obtained from the animal unit of the Department of Biochemistry, Faculty of Life Sciences, University of Benin, Benin city. They were housed in plastic cages in the Department of Plant Biology and Biotechnology, University of Benin. The animals weighed between 180-250 g. They were housed in the Phytomedicine Research Unit and maintained under standard laboratory conditions of room temperature, humidity and twelve-hour light and dark cycle. An acclimatization period of two weeks to the new environmental condition was allowed before the commencement of the experiment. All animals were maintained on standard pellet diet and clean water, and the cages were cleaned daily throughout the period of the experiment.

2.6 Preparation and Induction of diabetes

Streptozotocin was freshly prepared in 0.1M citrate buffer with 4.5 pH. Prior to induction, animals were fasted 24 hours and single dose of 45 mg STZ per kg body weight was administered intraperitoneally. Typical diabetic signs such as increased ingestion of water, food and recurrent urination rises in blood sugar level where observed. Diabetes mellitus was established 48 hours following STZ administration using Accucheck glucometer machine. Animals having plasma glucose concentration > 200 mg/dl were marked diabetic and integrated into the study as baseline (Gabriel and Idu, 2021).

2.7 Experimental Design

The twenty five (25) albino rats of both male and female gender were divided into five (5) groups of five (5) rats each. The rats were marked with gentian violet paint on different body parts according to the various groups for easy identification. The head, tail, back and legs were the body parts marked by the paint. Below are the various amounts of treatments they were given:

Group A: Received 45 mg/kg streptozotocin + 25 mg/kg of aqueous extract of *Acanthus montanus*

Group B: Received 45 mg/kg streptozotocin + 50 mg / kg of aqueous extract

Group C: Received 45 mg/kg streptozotocin + 100 mg/kg of aqueous extract

Group D: (Positive control) Received 45 mg/kg streptozotocin + 10 mg/kg glibenclamide

Group E: (Negative control) Received 45 mg/kg streptozotocin

The extract was administered to the animals orally, for a time period of three weeks (21) days.

2.8. Sample Collection and Haematological Analysis

At the end of the 21 days treatment period, the rats were sacrificed under mild chloroform anesthesia. This was to ensure that the rats do not feel pain during the blood collection procedure. Syringes were used to withdraw blood directly from the rats using cardiac puncture (Yakubu *et al*., 2007). Blood samples were immediately transferred into an anticoagulant tube (50 µl/vial, 2% EDTA). Haematological test was carried out with the analysis of: white blood cell (WBC), red blood cell (RBC), haemoglobin (HGB), hematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean

corpuscular haemoglobin concentration (MCHC), platelet count (PLT) and differentials of white blood cells such as the lymphocytes and granulocytes. The analyses were performed on the automated blood cell analyser CELL-DYN® 3700 System (Abbott) and ADVIA 2120i (Siemens Healthineers, USA).

2.9 Data Analysis

Values obtained from the experimental tests were subjected to statistical analysis, using statistical package for the social sciences (SPSS) version twenty two (22) with the determination of mean and standard error of mean. Analysis of variances (ANOVA) and Turkey's post-hoc was conducted to separate between the means. The results obtained were expressed as Mean±SEM and values were considered not significant at $P>0.05$.

CHAPTER THREE

RESULTS

The administration of *Acanthus montanus* had effects on white blood cell and differentials in STZ-induced Wistar rats. In Table 1, the white blood cell count showed no significant difference ($p > 0.05$) across all treated groups compared to the untreated control group. However, the lymphocyte percentage was significantly elevated across the 25, 50 and 100mg/kg doses of the treated groups with mean values of 64.33 ± 1.57 %, 67.93 ± 2.48 % and 77.23 ± 2.11% respectively when compared to the untreated control with a mean value of 57.50 ± 2.14 %. Other differentials were generally unchanged.

Table 1: Effect of *Acanthus montanus* aqueous leaf extract on white blood cell and its differentials in diabetic Wistar rats

Parameters	Untreated control 45 mg/kg STZ	Glibenclamide 10 mg/kg	A. *montanus* (25 mg/kg)	A. *montanus* (50 mg/kg)	A. *montanus* (100 mg/kg)
WBC 10^3/µL	11.90± 0.94[a]	8.53± 0.79[a]	9.43± 0.31[a]	5.60 ± 0.37[a]	5.37 ± 0.59[a]
LYM %	57.50 ± 2.14[a]	65.13 ± 2.41[b]	64.33 ± 1.57[b]	67.93 ± 2.48[b]	77.23 ± 2.11[b]
MID %	27.17± 1.91[a]	21.27 ± 0.73[a]	22.00± 0.27[a]	18.57 ± 0.21[a]	12.60 ± 0.62[a]
GRAN %	15.30 ± 0.52[a]	15.13 ± 0.11[a]	13.67 ± 0.18[a]	13.50 ± 0.15[a]	10.17 ± 0.81[a]
LYM 10^3/µL	5.20 ± 0.16[a]	5.80 ± 0.10[a]	5.60± 0.13[a]	3.70± 0.17[a]	4.13 ± 0.51[a]
MID 10^3/µL	2.57 ± 0.10[a]	1.40 ± 0.07[a]	2.30 ± 0.08[a]	1.10 ± 0.04[a]	0.67 ± 0.08[a]
GRAN 10^3/µL	1.77 ± 0.05[a]	1.33 ± 0.04[a]	1.53± 0.04[a]	0.80 ± 0.05[a]	0.57± 0.05[a]

The values are expressed in Mean ± SEM: n=5. Values with similar superscript across a row are not significantly different (p-*value* < a 0.05) compared to the untreated control according to Turkey post-hoc test.

KEY- WBC 10^3/µL: white blood cell count; LYM %: percentage of lymphocytes; MID%: percentage of monocytes; GRAN %: percentage of granulocytes; LYM 10^3/µL : lymphocyte count; MID 10^3/µL: monocytes absolute count; GRAN 10^3/µL: granulocytes count

In Table 2, there was significant increase of the red blood cells count across all three doses of the extract with mean values of 7.32 ± 0.45 10^6/µL, 7.23 ± 0.41 10^6/µL and 7.37 ± 0.53 10^6/µL against the untreated control with a mean value of 6.76 ± 0.57 10^6/µL. The haemoglobin concentration also exhibited significant increases progressively across the doses. The haematocrit had significant changes when compared to the untreated control with the highest increase recorded at the highest dosage of 100mg/kg. The other components were generally unchanged.

Table 2: Effect of *Acanthus montanus* aqueous leaf extract on red blood cell and components in diabetic Wistar rats

Parameters	Untreated control 45 mg/kg STZ	Glibenclamide 10 mg/kg	A. *montanus* (25 mg/kg)	A. *montanus* (50 mg/kg)	A. *montanus* (100 mg/kg)
RBC 10^6/µL	6.76 ± 0.57[a]	6.26± 0.41[a]	7.32 ± 0.45[b]	7.23 ± 0.41[b]	7.37 ± 0.53[b]
HGB g/dl	14.47 ± 1.11[a]	14.13 ± 0.63[a]	15.43± 0.48[b]	15.60 ± 0.52[b]	15.73 ± 0.79[b]
HCT %	37.10 ± 0.78[a]	36.33 ± 0.78[a]	39.97 ± 1.10[b]	39.60 ± 0.95[b]	39.33 ± 0.51[b]
MCV µM^3	55.03 ± 2.36[a]	58.37 ± 1.31[b]	54.73 ± 1.57[a]	54.87 ± 1.77[a]	53.43 ± 0.79[a]
MCH pg	22.57 ± 0.65[a]	24.70 ± 0.75[b]	21.07 ± 0.27[a]	21.53 ± 0.21[a]	21.27± 0.33[a]
MCHC g/dl	39.83 ± 0.31[a]	41.20 ± 0.99[b]	38.57 ± 0.72[a]	39.37 ± 0.94[a]	39.93 ± 0.72[a]
RDWS µM^3	33.43 ± 0.51[a]	42.73± 1.46[b]	34.90 ± 1.55[a]	32.03 ± 1.10[a]	32.03 ± 1.19[a]
RDWC %	15.20 ± 0.12[a]	17.80 ± 0.41[b]	15.97 ± 0.43[a]	14.63 ± 0.31[a]	14.90 ± 0.64[a]

The values are expressed in Mean ± SEM: n=5. Values with similar superscript across a row are not significantly different (p-*value* < a 0.05) compared to the untreated control according to Turkey post-hoc test.

KEY- RBC 10^6/µL: red blood cell count; HGB g/dl: haemoglobin concentration; HCT %: percentage of haematocrit; MCV µM^3: mean corpuscular volume; MCH pg: mean corpuscular haemoglobin; MCHC g/dl: mean corpuscular haemoglobin concentration; RDWS µM^3: Red cell distribution width

In Table 3, the platelet count of the treated controls had no significant changes ($p< 0.05$), although the mean values decreased when compared to the untreated control. The mean platelet volume had a significant change ($p> 0.05$) in the moderate dose of 50mg/kg with mean value of 7.83 ± 0.35 μM^3 against the untreated control of 7.20 ± 0.73 μM^3. The platelet distribution width also showed a significant increase in the 50mg/kg dosage against the untreated control.

Table 3: Effect of *Acanthus montanus* leaf extract on the platelet and components in Diabetic rats

Parameters	Untreated control 45 mg/kg STZ	Glibenclamide 10 mg/kg	A. *montanus* (25 mg/kg)	A. *montanus* (50 mg/kg)	A. *montanus* (100 mg/kg)
PLT 10^3/µL	558.17 ± 21.42[a]	718.00 ± 23.32[b]	415.33 ± 32.12[a]	457.00 ± 32.09[a]	435.33 ± 30.46[a]
MPV µM^3	7.20 ± 0.73[a]	7.67 ± 0.50[a]	7.67 ± 0.65[a]	7.83 ± 0.35[b]	7.33 ± 1.11[a]
PDW %	9.20 ± 0.51[a]	10.40 ± 0..40[b]	9.27 ± 0..83[a]	10.13 ± 0.60[b]	9.10 ± 0.90[a]
PCT %	0.40 ± 0.06[a]	0.61 ± 0.03[b]	0.31 ± 0.07[a]	0.35 ± 0.05[a]	0.32 ± 0.85[a]

The values are expressed in Mean ± SEM: n=5. Values with similar superscript across a row are not significantly different (p-*value* < a 0.05) compared to the untreated control according to Turkey post-hoc test.

KEY- PLT 10^3/µL: platelet count; MPV µM^3: mean platelet volume; PDW %: platelet distribution width; PCT %: plateletcrit

CHAPTER FOUR

DISCUSSION

The present study shows the effect of *Acanthus montanus* aqueous leaf extract on haematological indices in streptozotocin induced diabetic rats. The aqueous plant extract was graded at different doses: 25, 50 and 100 mg/kg respectively. The experiment was done to show the effect of the fore-mentioned doses on the different haematological parameters and their differentials such as white blood cells, red blood cells and platelets. It was also done to check for any significant changes (p- *value* < a 0.05) on the different blood parameters.

The white blood cells are immune cells that fight against infections. They are found in the blood and carry out immune responses (Tigner *et al.,* 2022). The white blood cell count is a count of the total number of white blood cells in a blood sample. In Table 1, the white blood cell count had a decrease across all doses of the plant extract administration which was not significant (p> 0.05) compared to the untreated control. This is in agreement with Okoroh *et al*., (2024) who discovered a similar result with the effect of ethanol extract of the fruiting bodies of *Pleurotus ostreatus* also known as the oyster mushroom. It was discovered that the level of white blood cells in the group of rats treated with the extract decreased. However, increased doses and longer time period of treatment could increase the white blood cell count (Odoh *et al*., 2013). Subacute toxicity studies have also reported no change in white blood cell count after 30 days of oral *A. montanus* exposure, which shows that a raised white blood cell count may depend on the dosage and time period context. (Djami *et al., 2011).* From this study, the white blood cell count of the untreated control was high. This is a common characteristic of inflammation caused by diabetes (Asgary, 2005). The reduction in white blood cell count with the administration of the extract proves *A. montanus* anti-inflammatory property (Okoli *et al.,* 2008).

The lymphocytes are a part of the adaptive immune system and they recognize and react to antigens. They are also active during autoimmune reactions. The granulocytes are involved in inflammatory response to bacterial infection and are the most cells present at the site of injury or infection (Tigner *et al.,* 2022). From this study there was a significant difference in percentage of lymphocytes after use of *A. montanus*. Mean values of 64.33 ± 1.57 %, 67.93 ± 2.48 % and 77.23 ± 2.11 % was recorded against the untreated control of 57.50 ± 2.14 %. This showed that the plant extract improved the lymphocyte levels. An increased lymphocyte level suggests that the extract has the ability to improve immune cell balance. The granulocytes percentage had a decrease which was not significant. This shows a little impact on the immunity. In contrast, Ofem *et al.,* (2012) discovered that the aqueous leaf extract of *Ocimum gratissimum* in rats reduced the lymphocyte level and increased the granulocytes. The finding of lower granulocytes may be as a result of the diabetes effect. An increase in lymphocytes and a non-significant change in granulocytes shows that the extract is safe for use as it shows improved immune regulation. This proves the immunostimulatory property of the plant extract (Okoli *et* al., 2008). The MID category includes the rare cells such as the monocytes, eosinophils and basophils (Tigner *et al.,* 2022). They carry out immune and inflammatory response. No significant changes took place with these cells upon administering the extract, suggesting that the *A. montanus* extract had no significant effects on these minor cell types. The administration of *A. montanus* aqueous leaf extract resulted in improvements in white blood cells and differentials. A significant increase in lymphocytes and a stabilizing effect on the white blood cell count proves that the extract improves immune cell levels.

Red blood cell count is the amount of red blood cells present per unit volume of blood. They are important in diagnosing anaemia (Brihi and Pathak, 2024). A reduced red blood cell count which was seen in the untreated control is associated with diabetes (Odoh *et al.,* 2013). In

Table 2, there was significant increase in the red blood cell count across all doses. Mean values of 7.32 ± 0.45 10^6/µL, 7.23 ± 0.41 10^6/µL and 7.37 ± 0.53 10^6/µL from the 25, 50 and 100 mg/kg doses of the plant extract were gotten against the untreated control with a mean value of 6.76±0.57 10^6/µL. This is in agreement with a study conducted where the methanolic leaf extract of *A. montanus* significantly increased total red blood cell count in Wistar albino rats (Izundu, 2024). The Haemoglobin is the primary carrier of oxygen in the blood and is an important measure of blood oxygen capacity (Rhodes *et al.,* 2022). The haemoglobin experienced significant increase compared to the untreated control. In a study conducted on the aqueous extract of *Azadirachta indica* on streptozotocin diabetic rats, a similar outcome was seen. The extract increased the haemoglobin concentration significantly (Ezeigwe *et al.,* 2020).A significant increase in the red blood cell count and haemoglobin shows that *A. montanus* aqueous leaf extract supports red blood cell formation. The haematocrit which is the percentage volume of red blood cells in the blood and reduces during anaemia (Brihi and Pathak, 2024) was also analysed. A significant increase was reported on the treated controls with the plant extract when compared with the untreated control. An increased haematocrit level shows that *A. montanus* reduces the effect of anaemia. The other haematological parameters such as the mean corpuscular volume which is the average volume of individual red blood cells (Brihi and Pathak, 2024) and the mean corpuscular haemoglobin were not significantly changed. The mean corpuscular haemoglobin concentration experienced a slight increase against the untreated control at the 100mg/kg dosage. Generally, there were no significant changes across all doses. This shows that the extract does not cause any significant changes in the red blood cell volume. This is in agreement with Djami *et al.,* (2011) who discovered that there no was no difference in these indices when a sub-acute test was done. The red cell distribution width had no significant changes across all doses of the extract used when compared to the untreated control. However, at the 25mg/kg dosage, there

was an increase, although not significant. This indicates that the plant extract does not have any major implication on the red blood cell size. Significant increase in the red blood cell count, haemoglobin and haematocrit suggests that *A. montanus* aqueous leaf extract has a positive effect on the red blood cells, reduces anaemia in diabetic rats and stimulates erythropoiesis (Uroko *et al*., 2020).

The platelets are blood cells that are small in size and they play essential roles in balance, tissue repair and assessment of bleeding risk (Tian *et al*., 2025). As seen in Table 3, *A. montanus* extract treated controls had a decrease that was not significant when compared to the untreated controls. This means that the platelets remained unchanged and a stable effect was shown. In contrast, Odoh *et al*., (2013) recorded an increase in platelet in a similar study. However, a higher dosage of up to 1000mg/ kg was used and the dosage period lasted up to eight weeks. This could suggest that different dosing yields different results. The mean platelet volume which indicates average size of platelets (Tian *et al*., 2025) had a significant increase at the 50mg/kg dosage of the plant extract with a mean value of 7.83±0.35 μM^3 against the untreated control of 7.20±0.73 μM^3. The platelet distribution width also had a similar result. The moderate dose had a significant increase compared to the untreated control. This suggests that the moderate dosage is able to increase mean platelet volume and distribution width. The plateletcrit had no significant changes. The decrease of the platelet count could suggest that the extract causes platelet aggregation which could reduce thrombiotic risks in diabetes (Elsharkawy *et al.,* 2025). Overall, these results suggest that *A.montanus* aqueous leaf extract has no harmful effect on the function and structure of the platelets but produces a stabilizing effect.

CONCLUSION

In conclusion, it can be seen that the aqueous leaf extract of *Acanthus montanus* has beneficial effects on haematological indices. It can improve white blood cells, thereby increasing immunity. It also increases red blood cell and haemoglobin formation, supporting more red blood cells formation and reducing the risk of anaemia and other infections arising from diabetes. It also provides a stable platelet count and maintains its normal function. These effects likely arise from the rich phytochemicals it possesses and support its antidiabetic, anti-inflammatory and antioxidant properties. It is recommended that further studies be carried out to isolate the phytochemical constituents responsible for these haematological improvements as this will create improved utilization of this useful plant part.

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