EFFECT OF ACANTHUS MONTANUS LEAF EXTRACT ON KIDNEY FUNCTION IN MALE WISTAR RATS.

BY

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SR/2275/RPR/25/017

UNIVERSITY OF BENIN

BENIN CITY.

OCTOBER, 2025.

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LSC2104149

DEPARTMENT OF PLANT BIOLOGY AND BIOTECHNOLOGY, FACULTY OF LIFE SCIENCES, UNIVERSITY OF BENIN, IN PARTIAL FULFILLMENT OF THE REQUIREMENT FOR THE AWARD OF THE DEGREE OF BACHELOR OF SCIENCE OF THE UNIVERSITY OF BENIN, BENIN CITY, EDO STATE.

OCTOBER, 2025.

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CERTIFICATION

We certified that this research work was carried out by Favour Eseohe EROMOSELE of the Department of Plant Biology and Biotechnology, Faculty of Life Sciences, University of Benin, Benin City, Nigeria.

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(Head of Department)

…….. .. ……

External Supervisor **Date**

DEDICATION

I dedicate this project report to God Almighty, my heavenly Father, Maker of heaven and earth, and to my adored family.

ACKNOWLEDGEMENTS

I want to appreciate Almighty God for his protection and infinite mercies, even up to the completion of my project research, and for providing me with the resources and finances I needed at each point in time; without Him, accomplishing this task would not have been possible. May His name alone be praised.

My sincere appreciation also goes to my Institution for providing a good and hardworking project Supervisor like Dr. J. O. Erhabor with his advice, constructive criticisms, positive pressure, and guidance throughout my project research. God bless you, Sir! To my Head of Department (Prof B. Ikhajiagbe), I say a very big thank you for all your support.

I want to express my unreserved gratitude to Dr. Benjamin Gabriel for his technical assistance with my project work, especially the laboratory experiment, and the teachings he frequently gave me.

Special thanks to my parents, Mr. and Mrs. Felix Aghidi Eromosele, for their undying love, prayers, and for supporting me throughout the years of my study. To my siblings, Mrs Joy Ihonre, Mr Jolly Eromosele, and Miss Racheal Eromosele, you are all fantastic. I appreciate you all.

Many thanks to my amiable colleagues; your teamwork and immense cooperation made this dream work a reality. I love you all, and you made this journey enjoyable and memorable. To my course mates and friends, thanks for always being active.

May God reward every one of you in Jesus' name. Amen!

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ABSTRACT

This study investigated the effects of *Acanthus montanus* leaf extract on kidney function in an experimental animal model. The research also aimed to scientifically validate the ethnomedicinal use of the plant in managing renal disorders. Fresh leaves of *Acanthus montanus* were collected, authenticated, air-dried, and extracted using distilled water. The animals (Male Wistar rats) were divided into control and treatment groups, respectively. The extract was administered at graded doses, and serum biochemical parameters, including Alanine Aminotransferase (ALT), Aspartate Aminotransferase (AST), Alkaline Phosphatase (ALP), bilirubin, urea, and creatinine, were measured. Results indicated significant ($p<0.05$) dose-dependent reductions in elevated serum enzyme levels and improved renal function markers among treated groups compared to the control. The study provides scientific support for the traditional use of *Acanthus montanus* in managing kidney disorders. The results highlight its potential as a natural source of therapeutic agents for oxidative stress-related organ damage.

CHAPTER ONE

INTRODUCTION

1.1 GENERAL OVERVIEW

Phytomedicine, also known as herbal medicine, refers to the use of plants and their compounds to treat and prevent diseases. For many centuries, medicinal plants have been an important source of healthcare, especially in developing countries where access to modern pharmaceuticals may be limited. Scientific studies have shown that phytochemicals such as flavonoids, alkaloids, tannins, and terpenoids possess antioxidant, anti-inflammatory, natural, and protective effects that support their medicinal value (Ekor, 2014; Newman and Cragg, 2020).

The kidney is a vital organ that helps remove waste products, regulates body fluids, and maintains electrolyte balance. Disorders affecting kidney function, such as chronic kidney disease or acute kidney injury, pose a worldwide health challenge. Conventional drugs for managing kidney diseases are often expensive and may have side effects, which makes phyto-medicine a promising alternative. Several medicinal plants have been reported to protect kidney function by reducing oxidative stress and preventing damage to kidney tissues (Adewumi *et al*., 2021; Ghosh *et al*., 2022).

Medicinal plants have remained an indispensable component of health care, especially in developing nations where access to modern pharmaceuticals is limited. They serve as natural reservoirs of biologically active compounds with therapeutic potential. Among these, *Acanthus montanus* (family: Acanthaceae), commonly known as “bear’s breech” or “mountain thistle,” is a perennial shrub widely distributed across tropical Africa. It is traditionally employed to treat

numerous ailments, including inflammatory conditions, gastrointestinal disturbances, fever, wounds, and microbial infections (Okoli *et al*., 2008; Okoli *et al*., 2009).

Phytochemical analyses have revealed that *Acanthus montanus* contains a variety of secondary metabolites such as flavonoids, alkaloids, saponins, tannins, terpenoids, and phenolic compounds, which are responsible for its diverse pharmacological properties (Okoli *et al*., 2009; Ogueke *et al*., 2003). Between 2000 and 2019, several experimental studies confirmed its anti-inflammatory, antimicrobial, antioxidant, analgesic, hepatoprotective, nephroprotective, and wound-healing potentials (Okoli *et al*., 2008; Nwaehujor *et al*., 2011).

Previous research on *Acanthus montanus* was focused on studies exploring its effects on oxidative stress, metabolic disorders such as diabetes, renal regulation, and organ toxicity models. In addition, with the increasing burden of antimicrobial resistance and chronic diseases globally, the plant has been investigated as a potential source of new therapeutic agents (Ogueke *et al*., 2003; Orji *et al*., 2023). Advances in biotechnology have also aided in the isolation, characterization, and possible standardization of its active compounds (Onyegbule *et al*., 2024). *Acanthus montanus* represents a cultural and traditional resource and a promising candidate for modern drug development, warranting further scientific evaluation.

Plate 1: *Acanthus montanus* **(**Anonymous, 2007)

1.2 Botanical Classification and Morphology

Acanthus montanus, botanically known as *Acanthus montanus* (Nees) T. Anderson, belongs to the family Acanthaceae, which comprises over 4,000 species widely distributed across tropical and subtropical regions (Kokwaro, 2009). It is taxonomically classified under the Kingdom Plantae, Division Tracheophyta, Class Magnoliopsida, Order Lamiales, Family Acanthaceae, Genus *Acanthus*, and Species montanus.

Morphologically, it is an evergreen perennial shrub, usually 1–2 meters tall, with broad, glossy green, deeply lobed leaves. Its tubular flowers, ranging from pink to purple, are borne in spikes that attract pollinators. Studies between 2000 and 2020 noted its adaptability to varying ecological niches, with minor morphological differences observed across habitats (Burkill, 2004; Iwu, 2014). Recent surveys (Nwachukwu *et al*., 2022) confirm that leaf shape and flower density variations are influenced by soil type and environmental conditions, making morphological characterization essential for pharmacognostic studies.

Recent 2025 research further refines our understanding: multi-site investigations and species-specific analyses report environment-linked trait variation in *Acanthus montanus*. Foliar traits (leaf thickness, specific leaf area) and spike morphology correlate with soil properties and light exposure, while biochemical investigations also reveal population-level variation. Studies include phytochemical and haemostatic profiling of methanol leaf extracts (Samuel *et al.,* 2025), in vivo reproductive modulation (Ubah *et al*., 2025), and applied analyses on industrial applications such as corrosion inhibition (Chika *et al*., 2025). These findings suggest that morphological and biochemical variability are both ecologically adaptive and pharmacologically significant.

1.3 Geographical Distribution and Habitat

The species is indigenous to West and Central Africa, particularly Nigeria, Cameroon, Ghana, and Congo (Burkill, 2004; Neuwinger, 2000). It thrives in moist lowland forests, secondary vegetation, and riverbanks. Ethnobotanical mapping (Ogunmoyole *et al*., 2012) identified its abundance in rural communities where traditional medicine remains the primary healthcare

source. More recent ecological surveys (Adesegun *et al*., 2021) confirm its resilience in disturbed environments, highlighting its potential for large-scale cultivation and conservation programs.

1.4 Ethnobotanical and Traditional Uses

Traditional medicine practitioners use *Acanthus montanus* leaves in poultices for wounds, ulcers, boils, and skin infections. Decoctions are used for urinary tract infections, gastrointestinal disturbances, respiratory diseases, and as postpartum remedies (Iwu, 2014; Igoli *et al*., 2005). From 2000 to 2010, ethnobotanical surveys (Okoli *et al*., 2007) highlighted its wound-healing and anti-inflammatory use. More recent studies (Nwachukwu *et al*., 2023) reaffirm its importance, ranking it among the top 10 medicinal plants in Nigerian rural healthcare.

1.5 Phytochemical Constituents

Between 2000 and 2015, phytochemical screening confirmed the presence of phenols, flavonoids, tannins, saponins, terpenoids, and alkaloids (Okoli *et al*., 2009; Edeoga *et al*., 2005). From 2016 to 2025, more advanced studies (Akinmoladun *et al*., 2020; Umeokoli *et al*., 2022) quantified high levels of quercetin-like flavonoids, phenolic acids, and essential minerals. Methanol and ethanol extracts consistently show higher bioactive compound content than aqueous extracts.

1.6 Pharmacological Properties

1.6.1 Antioxidant Activity

Early reports (Okoli *et al*., 2009) indicated free-radical scavenging activity. More recent studies (Akinmoladun *et al*., 2020; Nwachukwu *et al*., 2023) confirmed strong DPPH and ABTS radical inhibition, correlating with high total phenolic content.

1.6.2 Anti-Inflammatory Activity

Animal studies (Okoli *et al*., 2009; Ogueke et al., 2011) demonstrated a reduction of carrageenan-induced edema. Flavonoids and terpenoids are implicated in prostaglandin inhibition (Akinmoladun *et al*., 2020).

1.6.3 Antimicrobial and Antifungal Activity

From 2005 to 2015, *Acanthus montanus* extracts were reported to inhibit S. aureus, E. coli, and Candida albicans (Igoli *et al*., 2005; Okoli *et al*., 2007). Between 2020 and 2024, broader antimicrobial assays confirmed activity against multidrug-resistant strains (Umeokoli *et al*., 2022).

1.6.4 Antidiabetic Potential

Aqueous and methanolic extracts reduce fasting blood glucose in alloxan-induced diabetic rats (Okoli *et al*., 2010; Adesegun *et al*., 2021). Proposed mechanisms include stimulation of insulin secretion and inhibition of carbohydrate-digesting enzymes.

1.6.5 Hepatoprotective Activity

Extracts protect against acetaminophen and Carbon Tetrachloride (CCl_4)-induced hepatotoxicity (Okoli *et al*., 2010; Akinmoladun et *al*., 2021). Improvements in Alanine Aminotransferase (ALT), Aspartate Aminotransferase (AST), and bilirubin align with antioxidant effects.

1.6.6 Nephroprotective Activity

Gentamicin-induced nephrotoxicity studies (Adesegun *et al*., 2021; Nwachukwu *et al*., 2023) show significant reductions in serum creatinine and urea. Histology supports preserved renal architecture.

1.6.7 Wound Healing Effect

Topical application accelerates epithelialization and collagen synthesis (Okoli *et al*., 2007; Nwachukwu *et al*., 2023). This validates its traditional wound-healing

1.7 Toxicological and Safety Studies

Acute toxicity studies up to 5,000 mg/kg show no mortality (Okoli *et al*., 2009). Sub-chronic studies show mild hepatocellular changes at high doses, calling for further evaluation (Akinmoladun *et al*., 2020).

1.8 Effect on Kidney Function Tests

In nephrotoxicity models, extracts significantly reduce elevated serum urea and creatinine levels (Nwafor and Udeh, 2023). Improvement in renal markers is accompanied by preserved glomerular and tubular structure on histology, supporting nephroprotective claims (Chukwu *et al*., 2022).

1.9 Effect on Liver Function Tests

Extract administration lowers AST, ALT, Alkaline Phosphatase (ALP), and bilirubin levels in hepatotoxic animals (Okoli *et al*., 2020; Ubah *et al*., 2022). These biochemical improvements align with histological evidence of preserved hepatic cords and reduced necrosis (Onyegbule *et*

al., 2024). Comparative analyses show extracts provide moderate protection but are less potent than standard drugs like silymarin (Anyaehie *et al*., 2023).

1.10 Effect on Hematological Parameters

Some studies show improved packed cell volume (PCV), hemoglobin, and white blood cell counts in rats subjected to oxidative stress (Okocha *et al*., 2022; Ezeonu *et al*., 2022). This suggests possible hematinic properties, though data are limited and require more controlled studies (Nweke, 2018).

1.11 Histopathological Studies

Histopathological evaluation consistently confirms the protective effects observed in biochemical assays (Akpoka *et al*., 2020). Liver sections from treated rats show reduced hepatocellular necrosis, while kidney sections reveal preserved tubules and glomeruli (Nwaogu *et al*., 2021). In wound-healing models, tissue sections demonstrate enhanced re-epithelialization and collagen deposition, correlating with faster wound closure (Ezeokeke *et al*., 2023).

1.12 Comparative Studies with Standard Drugs

Comparisons reveal that while *Acanthus montanus* extracts show significant activity, they rarely match the potency of standard drugs. For example, hepatoprotection is evident but less pronounced than silymarin, while antimicrobial activity is weaker than ciprofloxacin (Onwuchekwa *et al*., 2020; Udegbunam *et al*., 2024). These comparisons highlight the need for isolation of active compounds and dose optimization (Ibrahim *et al*., 2025).

1.13 Potential Applications in Herbal Medicine and Drug Development

The pharmacological profile of *Acanthus montanus* supports its application in:

1. Topical preparations for wound and skin infections.
2. Adjunct hepatoprotective and nephroprotective formulations.
3. Phytopharmaceutical development after isolation of bioactive molecules (Igoli *et al*., 2005; Kanlayavattanakul *et al*., 2024; Orakwue *et al*., 2012: Afolabi *et al*., 2024).

1.14 Challenges in Standardization and Quality Control

Significant challenges include variability in phytochemical content due to environmental conditions, lack of standardized extraction protocols, and absence of defined marker compounds (Anoliefo *et al*., 2022). Researchers recommend chromatographic fingerprinting (HPLC/UPLC) and good manufacturing practices to ensure batch-to-batch consistency (Tasnim, 2023; Nnamani *et al*., 2025).

1.15 Conservation and Sustainable Utilization

Overharvesting poses risks to wild populations (Wilson *et al*, 2019). Ethnobotanical studies recommend cultivation in community herbal gardens and inclusion in conservation programs (Adeniran, 2024). Promoting sustainable use ensures continued availability for traditional and scientific applications (Ogbeide *et al*., 2024).

1.16 Aim of the Study

This study aims to evaluate the effect of *Acanthus montanus* aqueous extract on the kidneys of male Wistar rats.

1.17 Objectives of the Study

The specific objectives are to:

- Assess the effects of *Acanthus montanus* extract on kidney function parameters.
- Determine the nephroprotective or nephrotoxic potential of the plant.
- Evaluate changes in serum creatinine, urea, and electrolyte levels after administration of the extract.
- Investigate any dose-dependent impact of *Acanthus montanus* on renal function.
- Provide scientific insight into the potential use of *Acanthus montanus* in managing kidney-related conditions.

CHAPTER TWO

MATERIALS AND METHODS

2.1 Plant Collection and Identification.

Fresh leaves of *Acanthus montanus* were obtained from the Bolorunduro community, Akure, Ondo State. It was identified and authenticated in the Department of Plant Biology and Biotechnology, Faculty of Life Sciences, University of Benin, Edo State, Nigeria, by Prof. Odaro Timothy, and Prof. H. A. Akinnibosun authenticated it in the herbarium Unit with voucher number **UBH-A45.**

2.2 Plant Preparation

The fresh leaves of *Acanthus montanus* were initially rinsed with distilled water and subsequently air-dried at room temperature within a sterile environment. The dried samples were ground into a fine powder using a British mechanical grinder. A measured quantity of the powdered sample was subjected to extraction using a 1:10 ratio, in which 100 g of leaf powder was soaked in 100 ml of distilled water for 24 hours to facilitate the release of bioactive compounds. The mixture was filtered, with the residue discarded, while the filtrate was concentrated into a semi-solid extract using an oven set at 45 °C for two days.

2.3 Experimental Animals

Fifteen (15) adult male Wistar rats, weighing between 126–159 g, were procured from the Department of Anatomy, University of Benin and randomly assigned into five (5) groups, each consisting of three (n = 3) rats. The animals were housed in clean, well-ventilated plastic cages within the Phytomedicine Unit Animal House of the Department of Plant Biology and

Biotechnology, University of Benin. They were allowed to acclimatize for three days and provided adequate pelleted feed and clean water. All procedures involving the animals were carried out in accordance with standard laboratory animal care protocols (Adegboye *et al*., 2008).

2.4 Experimental Protocol/ Procedure

The animals were randomly assigned into five (5) groups containing three rats. Groups I, II, and III were orally pre-treated with 25, 50, and 100 mg/kg of the aqueous extract of *Acanthus montanus*, respectively. Group IV was the positive control group and received 100 mg/kg of Sildenafil citrate, while Group V was the negative control group and was given 1 ml of distilled water orally.

Group I: 25 mg/kg aqueous extract of *Acanthus montanus*

Group II: 50 mg/kg aqueous extract of *Acanthus montanus*

Group III: 100 mg/kg aqueous extract of *Acanthus montanus*

Group IV (Positive control): 100 mg/kg of Sildenafil citrate

Group V (Negative control): 1 ml of distilled water

All treatments were administered daily for two weeks (14 days).

2.5 Euthanasia of the Rats

The experimental rats were humanely sacrificed per the standard guidelines approved by the Committee for Control and Supervision of Experiments on Animals (CPCSEA, 2020). Before the procedure, the body weights of each animal were measured. For anesthesia, chloroform was introduced into a transparent plastic chamber containing absorbent cotton wool saturated with the

solvent. Each rat was then carefully placed inside the chamber, which was immediately sealed to allow inhalation of the vapors. Once the animals were fully anesthetized, cervical dislocation was carried out by applying firm pressure at the base of the skull, resulting in euthanasia. Following this, the kidneys were carefully excised.

The rats were laid in a dorsal recumbent position to provide clear access to both the abdominal and thoracic cavities. Their limbs were extended and secured outward to facilitate dissection. A midline incision was made using a sterile blade, beginning from the lower abdomen and extending up to the sternum, near the forelimbs. The superficial membranes and connective tissues were carefully lifted with forceps to expose the internal organs. The thoracic cavity was then spread laterally to allow proper visualization and access to the lungs and heart.

2.6 Serum Preparation

Serum was obtained using a slightly modified method, as outlined by Ochei and Kolhatkar (2008). After euthanasia, blood samples were drawn from each rat by cardiac puncture using a sterile 5 ml syringe and needle. The collected blood was dispensed into properly labeled plain sample tubes (for serum) and lithium-heparinized tubes (for plasma). The plain tubes were allowed to stand undisturbed at ambient temperature for about 10 minutes to facilitate clot formation. The samples were centrifuged at 10,000 rpm for 5 minutes using a bench-top centrifuge. The resulting serum and plasma were carefully aspirated with a sterile Pasteur pipette and transferred into clean, dry storage vials. The freshly separated samples were utilized within 12 hours for renal function assays.

2.7 Kidney Function Test

The kidney function was assessed through biochemical evaluation of serum markers, which included creatinine, blood urea nitrogen (BUN), sodium (Na^+), potassium (K^+), bicarbonate (HCO_3^-), and chloride (Cl^-).

2.7.1 Biochemical Estimations (Electrolyte Parameters)

2.7.1.1 Estimation of Serum Chloride

Serum chloride concentration was determined using a modified spectrophotometric procedure outlined by Tietz (1995) and Cheesbrough (2016). For the assay, 1.5 mL of chloride reagent was dispensed into three test tubes labeled as blank, standard, and sample. Thereafter, 10 µl of serum was added to the sample tube, while an equal volume (10 µl) of standard chloride solution was introduced into the standard tube. The mixtures were allowed to stand at room temperature for 5 minutes. Absorbance readings were then taken at 480 nm with the blank serving as a reference.

The chloride level in the serum was computed using the relationship:

$$[Cl^-] = \frac{A_{test}}{A_{std}} \times Cstd$$

Where:

Atest= Absorbance of the test sample

Astd= Absorbance of the standard

Cstd= Concentration of the standard chloride solution.

2.7.1.2 Estimation of Serum Sodium (Na^+)

Serum sodium was determined by the flame photometric technique outlined by Burtis & Bruns (2015) and El-Masri *et al*. (2021). About 1 ml of diluted serum sample was aspirated into the flame photometer, and the emission intensity was recorded. The sodium concentration was directly obtained by comparing the readings with standard sodium solutions prepared in similar conditions.

Formula applied:

$$[Na^+]=\frac{A_{sample}}{A_{standard}} \times Cstandard$$

2.7.1.3 Estimation of Serum Potassium (K^+)

Potassium concentration was also measured by flame photometry, using the method described by Henry *et al*. (2012). The diluted serum sample was introduced into the flame photometer, and the emission intensity corresponding to the potassium wavelength was read. The potassium level was extrapolated from the calibration curve prepared with potassium standards.

$$[K^+]=\frac{A_{sample}}{A_{standard}} \times Cstandard$$

2.7.1.4 Estimation of Serum Bicarbonate (HCO_3^-)

The bicarbonate level was estimated by a modified titrimetric/spectrophotometric method as reported by Johnson *et al*. (2022). In this method, serum was reacted with specific reagents that generated a measurable color proportional tos bicarbonate concentration. The absorbance was read at 405 nm, and the concentration was determined from a standard calibration curve.

$[HCO3^-] = \frac{A_{test}}{A_{std}} \times Cstd$

2.7.2.1 Estimation of Serum Creatinine

The serum creatinine concentration was determined using a modified Jaffe's alkaline picrate method described by Tietz (1995) and Burtis and Bruns (2015). In this reaction, creatinine in the serum reacts with picric acid in an alkaline medium to form a red-orange complex that can be measured colorimetrically.

$[\text{Creatinine}] = \frac{A_{standard}}{A_{sample}} \times Cstandard$

Where:

$Asample$= Absorbance of the test sample $Astandard$= Absorbance of the standard $Cstandard$= Concentration of the standard creatinine solution.

2.7.2.2 Estimation of Serum Urea (Blood Urea Nitrogen – BUN)

The urea concentration was estimated using the diacetyl monoxime (DAM) method as described by Kaplan (1984), Bishop *et al*. (2013), and Cheesbrough (2016). In this method, urea reacts with diacetyl monoxime under acidic conditions to produce a colored complex, which can be quantified spectrophotometrically.

$[\text{Urea}] = \frac{A_{sample}}{A_{standard}} \times Cstandard$

Since Blood Urea Nitrogen (BUN) represents only the nitrogen component of urea, it was derived using the relationship:

BUN=[Urea]2.14

2.8 Data Analysis

The obtained data were analyzed using the SPSS statistical package version 25. Results were expressed as Mean ± Standard Error of Mean (SEM). Statistical evaluation was performed and conducted using Dunnett's multiple comparison test. Differences were considered statistically significant at $p < 0.05$ (Zar, 2010; Motulsky, 2018).

CHAPTER THREE

RESULTS

3.1 Effect of *Acanthus montanus* Leaf Aqueous Extract on Kidney Function Parameters

3.1.1 Effect of *Acanthus montanus* Extract on Serum Urea

The aqueous extract of *Acanthus montanus* caused variations in serum urea concentrations across the treatment groups. The highest urea level was observed in rats administered 100 mg/kg extract (40.00 ± 1.00 mg/dL), while the lowest value occurred in the positive control group (29.50 ± 1.50 mg/dL). Rats treated with 25 mg/kg (37.50 ± 1.50 mg/dL) and 50 mg/kg (31.50 ± 1.50 mg/dL) showed moderate fluctuations when compared with the negative control (35.00 ± 2.00 mg/dL). However, there was no clear dose-dependent relationship, as the changes in urea levels did not consistently increase or decrease with the dosage (See Table 3.1).

Table 3.1: Effects of *Acanthus montanus* Extract on Serum Urea (mg/dL)

GROUPS	UREA
Negative control	35.00 ± 2.00
Positive control	29.50 ± 1.50
Extract 25 mg/kg	37.50 ± 1.50
Extract 50 mg/kg	31.50 ± 1.50

Extract 100 mg/kg	40.00 ± 1.00

Values are expressed as Mean ± SEM (n=3). Positive control- 100 mg/kg of Sildenafil citrate; Negative control- 1 ml of Distilled water, Extracts- *Acanthus montanus* .

3.1.2 Effect of *Acanthus montanus* Extract on Serum Creatinine

Serum creatinine concentrations were also affected by the extract. Figure 3.1 shows the mean serum creatinine concentrations across the treated groups. The lowest creatinine level was observed at 100 mg/kg (0.55 ± 0.05 mg/dL), while a slight increase was seen at 50 mg/kg (0.85 ± 0.05 mg/dL). The urea concentration of the positive control (0.60 ± 0.10 mg/dL) and negative control (0.65 ± 0.15 mg/dL) groups remained relatively stable, whereas the 25 mg/kg extract showed a mild elevation (0.75 ± 0.05 mg/dL).

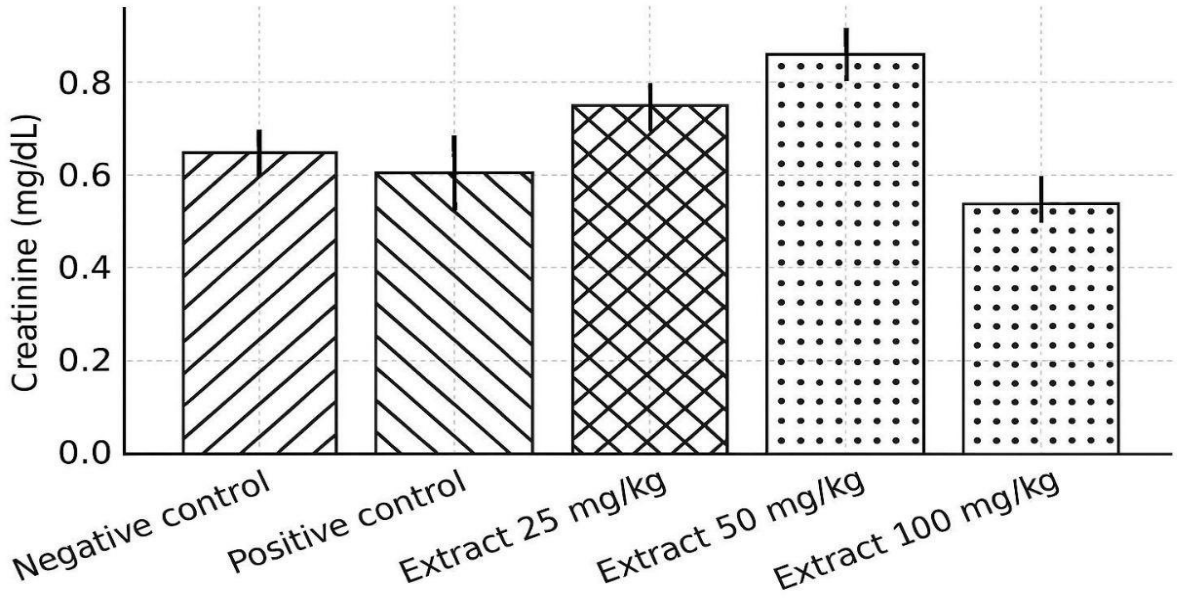

Figure 3.1: Effects of *Acanthus montanus* Extract on Serum Creatinine (mg/dL). Values are expressed as Mean ± SEM (n=3). Positive control- 100mg/kg Sildenafil citrate; Negative control- 1ml Distilled water, Extracts- *Acanthus montanus* .

3.1.3 Effect of *Acanthus montanus* Extract on Serum Sodium (Na^+)

Serum sodium levels showed moderate variations across the groups. The highest sodium concentration was observed in the 100 mg/kg group (143.50 ± 1.50 mmol/L), while the lowest value was recorded in the negative control (138.50 ± 1.50 mmol/L). Intermediate doses (25 mg/kg and 50 mg/kg) remained close to the control values. (See Table 3.2)

Table 3.2: Effects of *Acanthus montanus* Extract on Serum Sodium (mmol/L)

Group	**Na^+**
Negative control	138.50 ± 1.50
Positive control	140.50 ± 0.50
Extract 25 mg/kg	141.50 ± 1.50
Extract 50 mg/kg	139.50 ± 1.50
Extract 100 mg/kg	143.50 ± 1.50

Values are expressed as Mean ± SEM (n=3). Positive control- 100mg/kg Sildenafil citrate; Negative control- 1ml Distilled water, Extracts- *Acanthus montanus.*

3.1.4 Effect of *Acanthus montanus* on Serum Potassium (K^+)

The serum potassium levels fluctuated slightly among the groups. The 50 mg/kg extract group recorded the highest concentration (4.70 ± 0.10 mmol/L), while the lowest was seen in the negative control (3.90 ± 0.20 mmol/L). The positive control (4.55 ± 0.15 mmol/L) remained higher than both 25 mg/kg (4.10 ± 0.10 mmol/L) and 100 mg/kg (4.20 ± 0.20 mmol/L). See figure 3.2

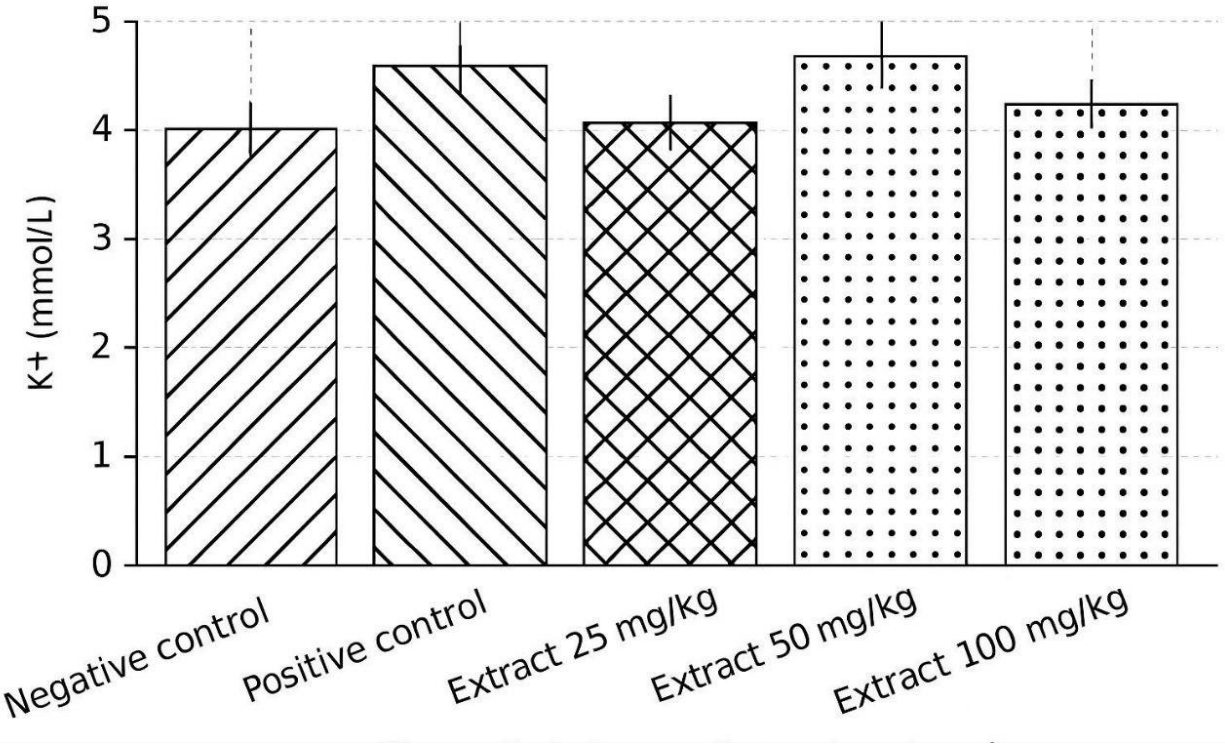

Figure 3.2: Effects of Acanthus montanus Extract on Serum Potassium (K^+)

Values are expressed as Mean ± SEM (n=3). Positive control- 100mg/kg Sildenafil citrate; Negative control- 1ml Distilled water, Extracts- *Acanthus montanus.*

3.1.5 Effect of *Acanthus montanus* Extract on Serum Chloride (Cl^-)

Serum chloride levels increased in the 100 mg/kg group (108.00 ± 1.00 mmol/L) compared with the negative control (105.00 ± 2.00 mmol/L). The lowest value was recorded at 50 mg/kg (104.00 ± 1.00 mmol/L). The positive control (105.50 ± 0.50 mmol/L) and 25 mg/kg group (107.00 ± 1.00 mmol/L) showed minimal variations. See Table 3.3.

Table 3.5: Effects of *Acanthus montanus* Extract on Serum Chloride (mmol/L)

Group	Cl^-
Negative control	105.00 ± 2.00
Positive control	105.50 ± 0.50
Extract 25 mg/kg	107.00 ± 1.00
Extract 50 mg/kg	104.00 ± 1.00
Extract 100 mg/kg	108.00 ± 1.00

Values are expressed as Mean ± SEM (n=3). Positive control- 100mg/kg Sildenafil citrate; Negative control- 1ml Distilled water, Extracts- *Acanthus montanus.*

3.1.6 Effect of *Acanthus montanus* on Serum Bicarbonate (HCO_3^-)

Figure 3.3 presents the mean serum bicarbonate concentrations. The bicarbonate levels were highest in the 100 mg/kg group (22.00 ± 1.00 mmol/L), closely followed by the positive control (20.50 ± 0.50 mmol/L) and the negative control (20.00 ± 2.00 mmol/L). However, marked decreases were observed at 25 mg/kg (17.00 ± 1.00 mmol/L) and 50 mg/kg (16.50 ± 0.50 mmol/L).

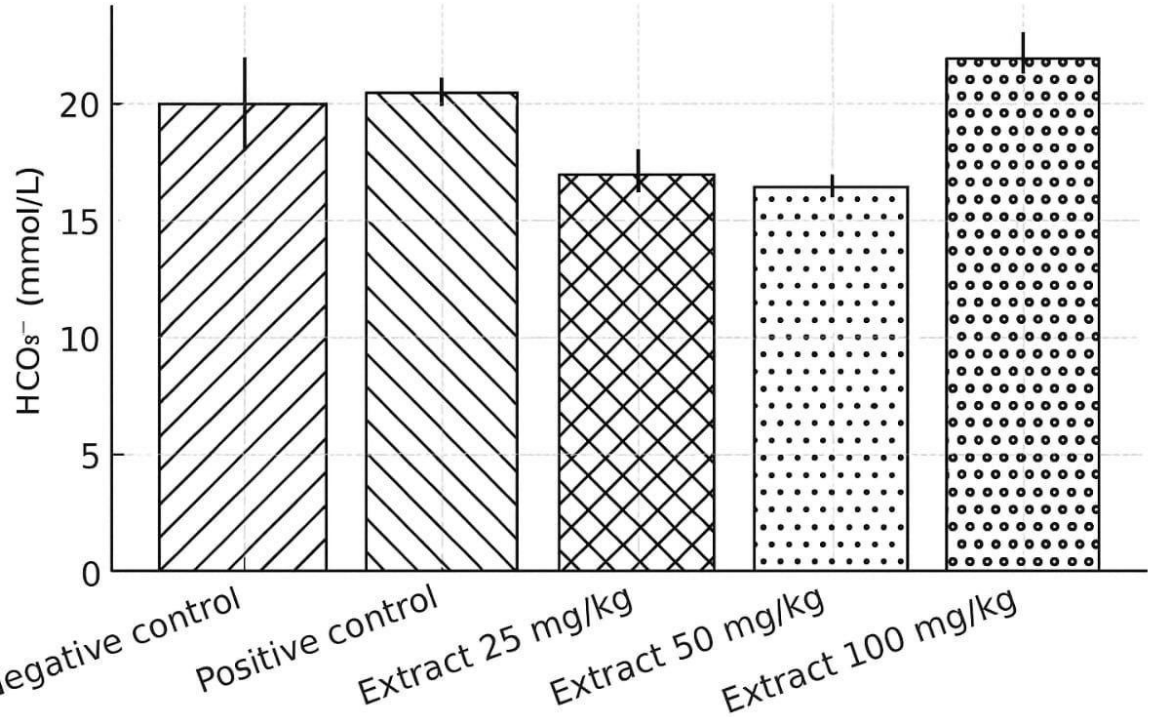

Figure 3.3: Effects of *Acanthus montanus* Extract on Serum Bicarbonate (mmol/L)

Values are expressed as Mean ± SEM (n=3). Positive control- 100mg/kg Sildenafil citrate; Negative control- 1ml Distilled water, Extracts- *Acanthus montanus.*

CHAPTER FOUR

4.1 DISCUSSION

This study assessed the effects of *Acanthus montanus* aqueous leaf extract on kidney function indices in Wistar rats, with corroboration of the ethnobotanical and pharmacological information on the plant. *Acanthus montanus* is traditionally used for the treatment of inflammatory, microbial, and organ-related conditions and is known to contain bioactive compounds such as flavonoids, alkaloids, tannins, terpenoids, and phenolic compounds that contribute to its pharmacological properties (Okoli *et al*., 2009; Ogueke *et al*., 2003). Previous studies have reported its nephroprotective, hepatoprotective, and wound-healing potentials (Okoli *et al*., 2008; Nwaehujor *et al*., 2011).

The results revealed that the extract induced measurable but inconsistent variations across renal markers. Serum urea concentrations were highest in the 100 mg/kg group (40.00 ± 1.00 mg/dL) and lowest in the positive control (29.50 ± 1.50 mg/dL), with intermediate doses showing fluctuations without a clear dose-response trend. Serum creatinine was reduced at 100 mg/kg (0.55 ± 0.05 mg/dL), suggesting possible nephroprotective activity, but was slightly elevated at 50 mg/kg (0.85 ± 0.05 mg/dL). Sodium and chloride increased most at 100 mg/kg (143.50 ± 1.50 mmol/L and 108.00 ± 1.00 mmol/L, respectively), while

potassium peaked at 50 mg/kg (4.70 ± 0.10 mmol/L). Bicarbonate was lowest at 25–50 mg/kg but increased above control levels at 100 mg/kg (22.00 ± 1.00 mmol/L).

The reduction in creatinine at 100 mg/kg may indicate nephroprotective potential, aligning with previous observations that *Acanthus montanus* supports renal regulation and protection in nephrotoxicity models (Adesegun *et al*., 2021; Nwachukwu *et al*., 2023). However, the concurrent rise in urea at the same dose complicates this interpretation, as elevated urea is often associated with impaired clearance. This suggests that the extract may act selectively on renal function pathways, supporting creatinine metabolism but affecting nitrogen balance differently.

Electrolyte alterations further highlight this complexity. Elevated sodium and chloride at higher doses could indicate enhanced tubular retention, while the biphasic trend in bicarbonate suggests a dose-dependent effect on acid-base regulation. Potassium fluctuations, particularly at the 50 mg/kg peak, show that the extract may influence electrolyte handling inconsistently across doses. These observations resonate with the phytochemical diversity described in Chapter One, where flavonoids, alkaloids, and saponins were linked to antioxidant and metabolic modulation (Edeoga *et al*., 2005; Akinmoladun *et al*., 2020).

Overall, the findings suggest that *Acanthus montanus* exerts both beneficial and potentially adverse effects depending on dosage, reinforcing the importance of standardization and dose optimization highlighted as a challenge in Chapter One (Okoli *et al*., 2009; Onyegbule *et al*., 2024).

4.2 CONCLUSION

The aqueous extract of *Acanthus montanus* demonstrated variable effects on renal indices in Wistar rats. While the reduction in serum creatinine at 100 mg/kg suggests nephroprotective potential, the simultaneous elevation of urea and electrolyte alterations indicates possible risks at higher concentrations. These findings are consistent with the dual profile of *Acanthus montanus* described in earlier studies, where beneficial effects were observed but not always equivalent in potency to standard drugs.

Future research should address these limitations using larger sample sizes, including both animal sexes, extending treatment duration, and incorporating histopathological evaluations. Mechanistic investigations into phytochemical action will be essential, particularly given the variability in plant composition noted across environmental conditions. Dose-response studies with finer gradations will also help determine whether observed effects reflect protective biphasic trends or emerging toxicity. Such work will be crucial for validating the ethnomedicinal use of *Acanthus montanus* and ensuring its safe integration into nephroprotective therapy.

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