RADINKA JOURNAL OF HEALTH SCIENCE

ISSN: 3025-7751

https://www.rjupublisher.com/ojs/index.php/RJHS

HISTOLOGICAL EFFECT OF ANTIDIABETIC HERBAL TEA ON THE KIDNEY OF ALBINO RATS INDUCED WITH *ALLOXAN* (DIABETES)

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Abstract

Background: The kidneys are central to the human excretory system, crucial for maintaining homeostasis. Diabetes mellitus, a prevalent metabolic disorder, commonly leads to serious complications, including diabetic nephropathy. This study investigated the protective effects of an antidiabetic herbal tea on the kidneys of alloxan-induced diabetic albino rats, exploring its potential as a complementary treatment. Methods: Thirty albino rats were randomly assigned to five groups. Group A served as the non-diabetic control. Group B was the untreated diabetic group. Groups C, D, and E were diabetic rats that received varying doses of the herbal tea for 28 days. Body weights were monitored, and kidney tissues were examined via hematoxylin and eosin (H&E) staining to assess histological changes. Results: Untreated diabetic rats (Group B) exhibited significant weight loss and severe renal damage, characterized by interstitial edema, glomerular detachment, and inflammatory cell infiltration. In contrast, the treated groups (C-E) showed dose-dependent renal protection. Group E, receiving the highest dose, displayed the least damage with relatively intact glomeruli. While the herbal tea mitigated weight loss and kidney damage, it did not completely prevent nephropathy, as some tubular basement membrane disorganization persisted. Conclusion: This study confirms that untreated diabetes causes marked weight loss and significant kidney damage. The antidiabetic herbal tea provided a partial protective effect on the kidneys and moderated weight loss in diabetic rats. However, since complete prevention of nephropathy was not achieved, the herbal tea should be considered a beneficial complementary therapy rather than a standalone treatment for managing diabetic complications A concise and factual abstract is required (maximum length of 200 words). The abstract should state briefly the purpose of the research, the principal results, and major conclusions. An abstract is often presented separately from the article, so it must be able to stand alone. References should, therefore, be avoided, but if essential, they must be cited in full, without reference to the

reference list. Non-standard or uncommon abbreviations should be avoided, but if essential they must be defined at their first mention in the abstract itself.

Keywords**:** Alloxan, albino rats, antidiabetic tea, diabetes, herbal tea, kidney histology.

Acknowledgments: Special thanks to all respondents, their families, and colleagues for their unwavering support, ensuring the benefits of this research.

For citation:

Uwuigbe M., Ohiwerei W.O., Obeagu E.I., Uwuigbe A.A., Edebiri O.E. (2025). Histological Effect Of Antidiabetic Herbal Tea On The Kidney Of Albino Rats Induced With *Alloxan* (Diabetes). *Radinka Journal of Health Science, 3(1),* 446-457.

Introduction

In individuals with insulin-dependent diabetes mellitus (IDDM), the kidneys, which are vital for maintaining the body's balance, are prone to damage in their filtering units, the glomeruli. This condition, known as diabetic nephropathy, is the primary cause of end-stage renal disease (ESRD) ((Amann & Benz, 2007)The damage is characterized by specific lesions, including the hallmark thickening of the capillary basement membrane, which impairs the kidney's filtering ability and results in excess protein in the urine, a condition known as proteinuria (Cai & Yang, 2020). Other key changes include diffuse mesangial sclerosis, characterized by the enlargement of mesangial cells and their matrix (Rüster et al., 2008), and in advanced stages, nodular glomerulosclerosis or Kimmelstiel-Wilson lesions, which are unique to diabetes and signal severe kidney deterioration (Esposito et al., 2023). The progression of this damage is significantly worsened by chronic high blood sugar levels (hyperglycemia) (Kuczyński et al., 2024), along with other risk factors such as hypertension, dyslipidemia, and smoking (Rayne Gomes Amorim et al., 2019). Given the severe and specific renal complications outlined above, it is clear that diabetes mellitus (DM) represents not only a local organ risk but also a significant global health crisis.

Diabetes mellitus (DM) is a serious global health crisis, significantly impacting mortality and the economy. In 2017, about 5 million people aged 20 to 99 died from diabetes-related complications, a number greater than deaths from HIV/AIDS, tuberculosis, and malaria combined (Harding et al., 2024). The International Diabetes Federation (IDF) estimates that over 589 million adults currently have diabetes, a number projected to reach 853 million by 2050 without stronger preventive measures (Moța, 2017). The global prevalence has risen dramatically, with 463 million people affected in 2019, most of whom have type 2 diabetes(Ratre et al., 2024). A particularly alarming fact is that nearly half of all diabetes-related deaths occur in people under 60 (Harding et al., 2024). The economic costs are enormous, with global healthcare spending on diabetes reaching about $673 billion in 2015, largely due to the high costs of managing complications (Patil et al., 2023). In the UK, for example, an estimated 80% of all diabetes-related costs are spent on treating these complications (Gregg & Bracco, 2019). These alarming statistics on mortality and cost are primarily a consequence of the complex complications that arise from the disease's metabolic disorders.

Diabetes mellitus (DM) is a complex metabolic disorder characterized by chronic high blood sugar, stemming from issues with insulin secretion, insulin action, or a combination of both. This condition disrupts the body's metabolism of carbohydrates, fats, and proteins, causing widespread organ damage and complications. The two primary types of diabetes are Type 1, which accounts for about 10% of cases and results from the autoimmune destruction of pancreatic beta cells, leading to an absolute lack of insulin (Panja et al., 2006); and Type 2, which makes up about 90% of cases and is characterized by the body's resistance to insulin combined with insufficient insulin production (North Manchester General Hospital Department of Urology, Delaunays Road, Manchester, United

Kingdom & Grey Venyo, 2023). Metabolically, the condition impairs glucose uptake by muscles and fat, leading to high blood sugar levels, and forces the body to break down fats and proteins for energy, which can cause weight loss and muscle wasting(Banday et al., 2020) Diabetes can lead to both acute complications, such as hyperglycemic crises like ketoacidosis and hyperosmolar syndrome, and chronic complications, including long-term damage to the eyes (retinopathy), kidneys (nephropathy), and nerves (neuropathy), as well as increased cardiovascular disease risk (Toor et al., 2024). Given the widespread and severe complications of diabetes, there is growing interest in exploring alternative therapeutic strategies, including the use of natural substances.

Although the systemic damage caused by diabetes is well-documented, a number of natural substances are being studied for their therapeutic potential. Among them, herbal teas derived from plants such as Camellia sinensis are gaining increasing attention for their health benefits. Herbal teas from plants like Camellia sinensis, Moringa oleifera, and Zingiber officinale are gaining popularity for their potential to help manage diabetes. This is due to their high content of beneficial phytochemicals, such as catechins and flavonoids, which have hypoglycemic, antioxidant, and anti-inflammatory effects that improve glucose metabolism and reduce oxidative stress (Wei et al., 2024). Clinical studies suggest that regular consumption of these teas can lower the risk of developing diabetes and its complications, partly by enhancing the function of pancreatic β-cells (Mandal et al., 2024). Despite the documented benefits on blood sugar control, the specific long-term effects of these appear on the histological structure of vital organs like the kidneys remain underexplored, raising concerns about potential protective or harmful effects on kidney health(Singh et al., 2022).

Methodology

Study Area

This study was carried out in the experimental site at the histology laboratory college of medicine, Ambrose Alli University Ekpoma, Edo State. Edo state lies between longitude 06° 04^{I}E and 06° 43^{I}E and latitude 05° 44^{I}N and 07° 34^{I}N with a land mass of17, 450 sq.km located in the south south geopolitical zone of Nigeria with a population of 3.1 million people (World Gazzetter, 2007). his study investigated the histological effects of an antidiabetic tea on the kidneys of alloxan-induced diabetic Wistar rats. The findings indicate that diabetes itself caused significant kidney damage, which was evident in the negative control group (Group B) through substantial weight loss, consistent with uncontrolled metabolic breakdown (Banday et al., 2020). Histological analysis confirmed this, revealing signs of inflammation, edema, and glomerular harm(Kuczyński et al., 2024). The antidiabetic tea treatment, however, yielded varied effects. The low-dose group (Group C) showed only partial improvement, with residual inflammation and fibrosis. Surprisingly, the medium-dose group (Group D) exhibited an unexpected worsening of kidney damage, including severe tubular damage and glomerular shrinkage that surpassed even the negative control group. This suggests a potential toxic or paradoxical response at this concentration. While the highest dose (Group E) did not cause necrosis, it still showed tubular membrane disruption, indicating that the tea's benefits are not linear and further research is needed to understand its dose-dependent effects and potential risks

Study Duration

The preliminary studies, animal acclimatization, ingredients procurement, actual animal experiment and evaluation of results, lasted for a period of four weeks. However, the actual administration of substances to the test animals lasted for two (2) weeks.

Ethical Approval

Ethical Approval to utilize the animal intended for this work was sought from the Ethical Board of Ambrose Alli University, Ekpoma, Edo State and the ethical permission was given.

Animal Model and Handling

Fifty (50) Adult Albino Wistar rats having a mean weight of 265g were procured from the animal farm, college of medicine Ambrose Alli University Ekpoma and transferred to the experimental Laboratory at the histology laboratory college of medicine, Ambrose Alli University Ekpoma, Edo State, where they were allowed two (2) weeks of acclimatization. They were kept in wire mesh cages with tripod that separates the animal from its faeces to prevent contamination. During this period of acclimatization, the rats were fed with growers' mash and water provided *ad libitum*. The animals were maintained and utilized in accordance with the standard guide for the care and use of Laboratory animals.

Grouping of Animal Model

The experimental animals were separated into five groups (A – E). Each group contains ten rats each (n = 10) using 10 big cages to house them. Group A served as the positive control and Group B as negative control while groups C - E served as the test groups. Group C – E were induced with diabetes (alloxan) and administered with different concentrations of antidiabetic tea prepared accordingly and weighed to determine the quantity to be administered. Group A received only the normal feed (grower's mash) and water with no administration of alloxan or antidiabetic tea. Group B was induced with alloxan only.

Design of the Study

In all, fifty (50) adult Albino Wistar rats were used for this study. They were divided into five groups of ten rats each. Group A served as the positive control and the rats were given distilled water. Group B rats served as negative control and were given Alloxan only without antidiabetic herbal tea. Animals in the test groups (C, D and E) received each 3.4ml, 6.5ml and 13ml of antidiabetic tea respectively after alloxan was induced intra-peritoneally. Alloxan was induced 4 days before administration of antidiabetic tea for 10 days. After the administration, the rats were put under light chloroform anaesthesia and the kidney harvested for histological processing.

Substance of Study

Alloxan was purchased from a chemical store while antidiabetic tea was gotten from a supermarket.

Substance Administration

The rats were weighed before the administration of the substances (Alloxan and antidiabetic tea) and before they were sacrificed and similar weight measurements were done at the beginning and end of the experiment and the average weight recorded accordingly. The administration of the substance was via oral route for antidiabetic tea and intra-peritoneally for alloxan

- **Group A** (Control) received only normal feed (growers' mash) and distilled water daily for 28 days.
- **Group B** received Alloxan for 4 days
- **Group C** received Alloxan for 4 days and 3.4ml of antidiabetic tea for 10 days
- **Group D** received Alloxan for 4 days and 6.5ml of antidiabetic tea for 10days
- **Group E** received Alloxan for 4 days and 13ml of antidiabetic tea for 10days

Sample Collection and Analysis

The weights of the animals were measured before and after the experiment and recorded accordingly. The kidney of each rat was obtained at the end of the experiment under chloroform anaesthesia and fixed in 10% formol saline for histological processing.

Histological Processing

The tissues were processed using automatic tissue processor according to the processing schedule used in Irrua Specialist Teaching Hospital, Edo State, Nigeria. The fixed plastic cassette tissues in 10% formalin were automatically processed by passing them through different grades of alcohol as follows:

70% alcohol	2hrs
80% alcohol	2hrs
90% alcohol	2hrs
90% alcohol	2hrs
95% alcohol	2hrs
Absolute	2hrs
Xylene 1	2hrs
Xylene II	2hrs
Molten paraffin wax 1	2hrs
Molten paraffin Wax II	2hrs

After the last timing, the tissues were removed from their plastic cassettes and placed at the centre of the metallic tissue mould and then filled with molten paraffin wax. They were also left to solidify after which they were now placed in the refrigerator at 5^{o}C for 15 minutes. After the blocks were cool in the refrigerator for the time stated above (15 minutes), the blocks were then removed from the metallic case using a knife and after which the paraffin wax at the side of the blocks were removed.

The blocks were then trimmed at 10microns and cut serially at 5microns on a rotary microtome. The sections were floated in water bath at 55^{o}C and picked up by the use of a clean frosted end slides. The frosted end slides were now placed on the hot plate for 40 minutes for adequate attachment of the sections on the slides after which the sections were de-waxed, hydrated, air dried and stored in a slide box ready for staining process.

Staining Procedure

Sections for general tissue structure were stained by Haematoxylin and Eosin technique.

1. The sections were dewaxed in 3 changes of xylene 5 minutes
2. The sections were hydrated through descending grades of alcohol (absolute, 95%, 80% and 70%).
3. The sections were stained with Cole's haematoxylin 5 minutes
4. The sections were rinsed in running tap-water to remove excess stain
5. The sections were differentiated in 1% acid alcohol 15 seconds
6. The sections were blued in running tap water 10 minutes
7. The sections were counterstained with 1% eosin 2 minutes
8. Sections were finally rinsed in water, dehydrated in ascending grades of alcohol (70%, 80, 95% and absolute)
9. The sections were cleared in xylene, air-dried and mounted with dibuthylphthalate propylene xylene (DPX).

The slides were examined under a light microscope and photomicrographs were taken.

Data Analysis

The mean weight ± SD of the test animals and control before and after the administration of rohypnol were calculated using SPSS (version 20)

Results and Discussion

a. Figure. 4.1 shows the initial and final body weight of adult wistar induced with diabetes and administered anti-diabetic tea
   1. The mean initial body weight of Group A (Positive Control) was 209.26 g, which slightly increased to 212.54 g.
   2. Group B (Negative Control) had an initial mean body weight of 179.2 g, which decreased significantly to 144.62 g.
   3. In Group C, which received 3.4 ml of anti-diabetic tea, the initial mean body weight was 173.66 g, reducing to 152.34 g at the final measurement.
   4. Group D, administered 6.7 ml of anti-diabetic tea, had an initial mean body weight of 175.02 g, which later dropped to 145.6 g.
   5. For Group E, which received the highest dose of 13 ml of anti-diabetic tea, the mean body weight decreased from 180.24 g initially to 146.76 g at the final measurement. The body weight increase or decrease was statistically significant in Group B and Group C.

Figure 1: Initial And Final Body Weight Of Mice

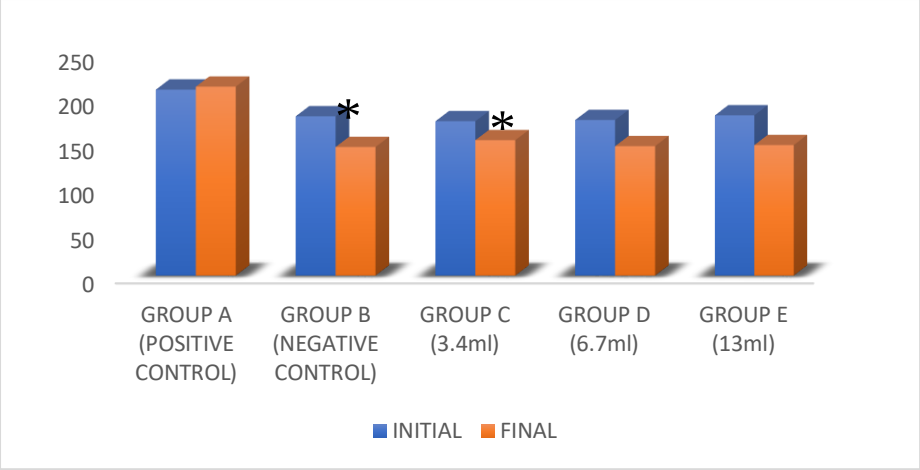

Figure 4.1: Initial and final body weight of adult wistar induced with diabetes and administered anti-diabetic tea

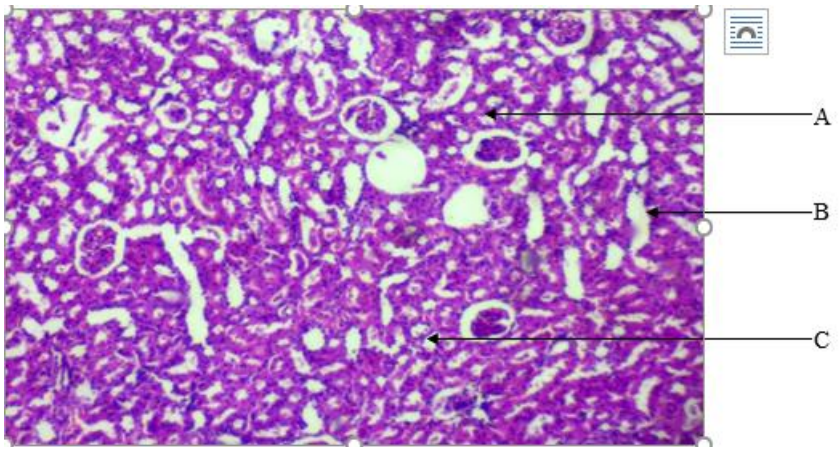

Plate 1a shows the Normal histological feature of the Kidney of the untreated (Positive control) Albino rat with presence of Glomerulus (A), Blood vessel (B) and Distal convoluted tubule (C) of the kidney. HandEX100

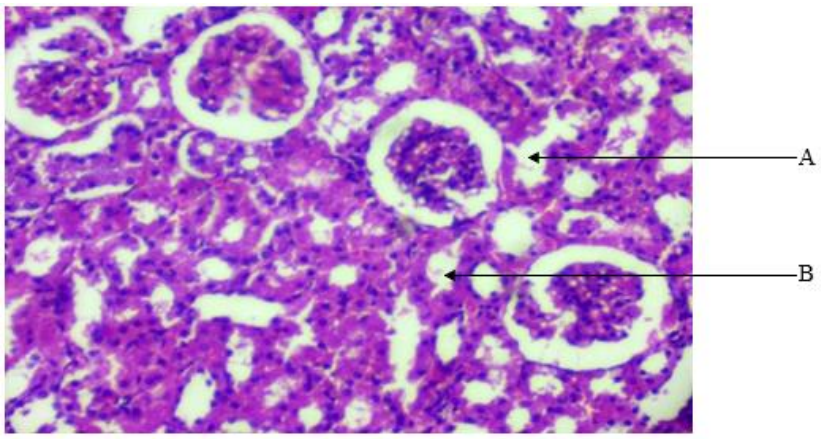

Plate 1b shows the Normal histological feature of the Kidney of the untreated (Positive control) Albino rat with presence of Glomerulus (A) and Distal convoluted tubule (B) of the ikidney. HandEX400

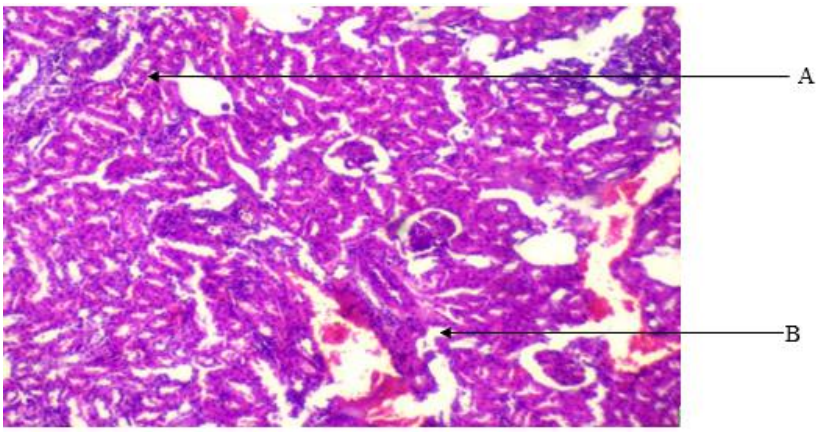

Plate 2a shows the histological feature of the Kidney of the treated (Group B – diabetic group on alloxan) Albino rat with presence of Inflammatory cells infitration (A) and Interstitial edema (B) of the kidney. HandEX100

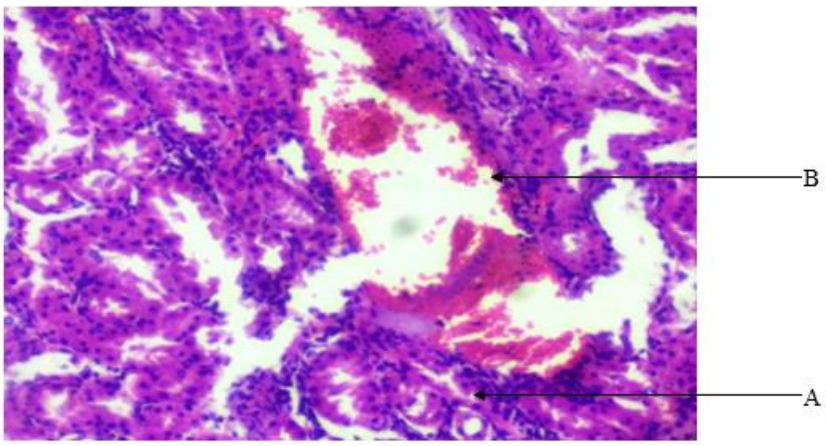

Plate 2b shows the histological feature of the Kidney of the treated (Group B – diabetic group on alloxan) Albino rat with presence of Glomerulus detachment (A) and Interstitial edema (B) of the kidney. HandEX400

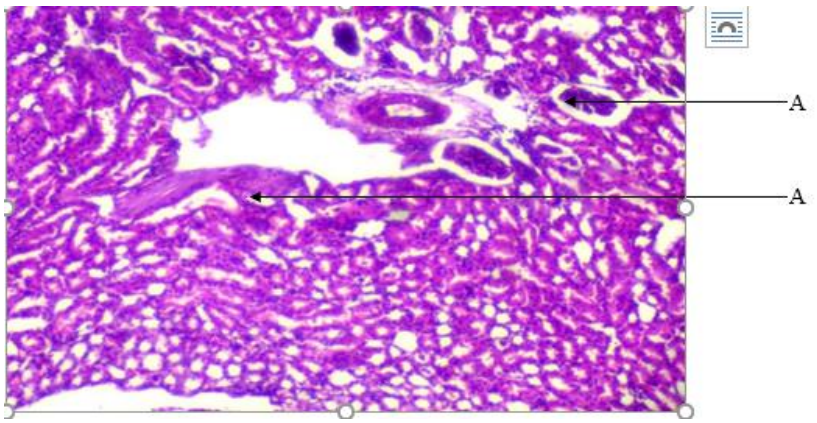

Plate 3a shows the histological feature of the Kidney of the treated (diabetic group C receiving 3.4ml of antidiabetic tea) Albino rat with presence of Inflammation occuring around Blood vessel (A) and Fibrosis (B) of the kidney. HandEX100

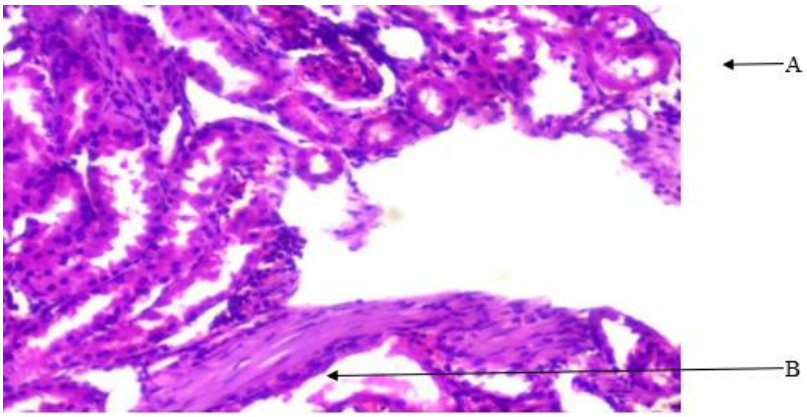

Plate 3b shows the histological feature of the Kidney of the treated (diabetic group C receiving 3.4ml of antidiabetic tea) Albino rat with presence of Slight tubular basement membrane distuction (A) and Fibrosis (B) of the kidney. HandEX400

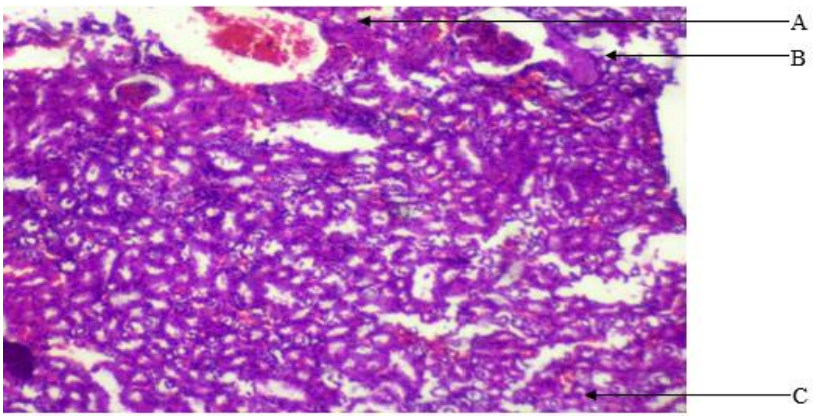

Plate 4a shows the histological feature of the Kidney of the treated (Group D diabetic group E receiving 6.5ml of antidiabetic tea) Albino rat with presence of Vascular occulasion (A), Glomerulus shrinkage (B) and Severe tubular membrane damage (C) suggestive of tubular atropy of the kidney. HandEX100

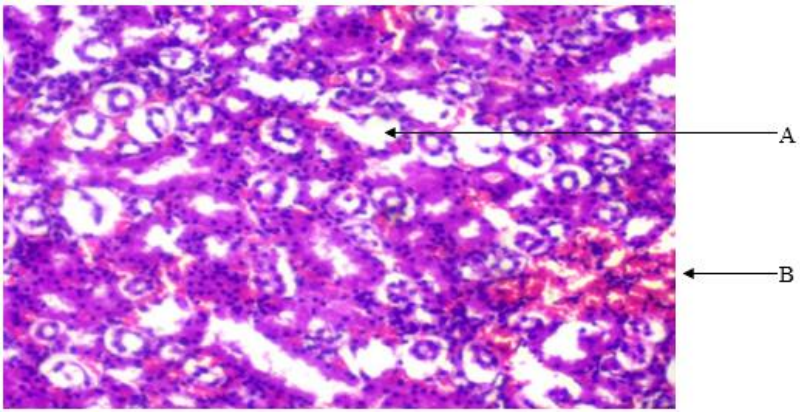

Plate 4b shows the histological feature of the Kidney of the treated (Group D diabetic group E receiving 6.5ml of antidiabetic tea) Albino rat with presence of Severe tubular membrane damage (A) suggestive of tubular atropy and Vascular congestion within enlarged urinary space (Glomerulus and Bowman's (A) of the kidney. HandEX400

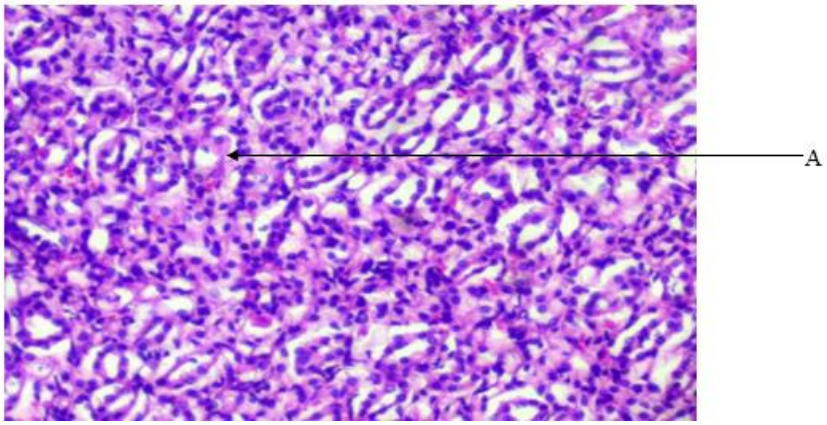

Plate 5a shows the histological feature of the Kidney of the treated (diabetic group E receiving 13ml of antidiabetic tea receiving 13 ml of antidiabetic tea) Albino rat with presence of No necrosis of renal cells, however, several areas show tubular basement membrane disarrangement (A) of the kidney. HandEX100

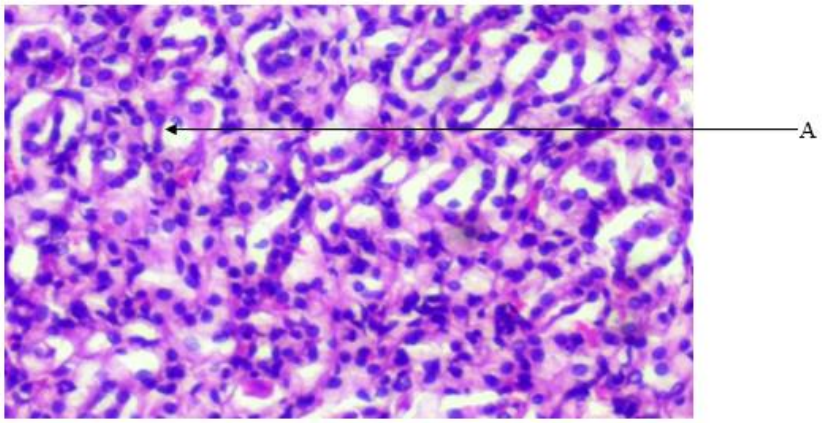

Plate 5b shows the histological feature of the Kidney of the treated (Group E diabetic group E receiving 13ml of antidiabetic tea) Albino rat with presence of No necrosis of renal cells, however, several areas show tubular basement membrane disarrangement (A) of the kidney. HandEX400

Discussion

His study investigated the histological effects of an antidiabetic tea on the kidneys of alloxan-induced diabetic Wistar rats. The findings indicate that diabetes itself caused significant kidney damage, which was evident in the negative control group (Group B) through substantial weight loss, consistent with uncontrolled metabolic breakdown (Banday et al., 2020). Histological analysis confirmed this, revealing signs of inflammation, edema, and glomerular harm(Kuczyński et al., 2024). The antidiabetic tea treatment, however, yielded varied effects. The low-dose group (Group C) showed only partial improvement, with residual inflammation and fibrosis. Surprisingly, the medium-dose group (Group D) exhibited an unexpected worsening of kidney damage, including severe tubular damage and glomerular shrinkage that surpassed even the negative control group. This suggests a potential toxic or paradoxical response at this concentration. While the highest dose (Group E) did not cause necrosis, it still showed tubular membrane disruption, indicating that the tea's benefits are not linear and further research is needed to understand its dose-dependent effects and potential risks.

Conclusion

This study demonstrates the complexity of using herbal remedies, such as antidiabetic tea, in medicine. While the tea is rich in phytochemicals with potential benefits (Wei et al., 2024), its effects on kidney health are not linear. The histological results show that a low dose provided partial protection, while a medium dose surprisingly exacerbated kidney damage. The highest dose had a more neutral effect. This finding underscores the importance of rigorous toxicity and dose-response studies for herbal products. A protective effect may only occur at a very specific dose or may depend on complex compound interactions that are not yet fully understood. Therefore, future research must focus on isolating the active compounds and systematically testing different doses, while also exploring the mechanisms behind these unexpected results. This proves that histological evaluation is crucialfor ensuring the safety and efficacy of these remedies.

Conflicts of Interest

The author declares that there is no conflict of interest

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