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# Histological Effect of Antidiabetic Herbal Tea on The Spleen of Albino Rats Induced with *Alloxan* (Diabetes)

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Abstract

This study investigated the effects of an anti-diabetic herbal tea on splenic histology and body weight in alloxan-induced diabetic Wistar rats. Thirty rats were divided into five groups (n=6): a positive control (Group A), a negative diabetic control (Group B), and three experimental groups (Groups C, D, and E) that received 3.4 ml, 6.7 ml, and 13 ml of the herbal tea, respectively, for 14 days. Body weight was recorded before and after treatment, and spleen tissues were analyzed histologically with H&E staining. The results showed that the diabetic control group (Group B) had a significant decrease in body weight (p=0.01), while the positive control group (Group A) showed no significant change (p=0.85). In the treatment groups, a low dose (Group C) resulted in a significant weight reduction (p=0.03), whereas higher doses (Groups D and E) showed non-significant trends toward weight loss (p=0.07 and p=0.08, respectively). Histological analysis revealed splenic infarcts and coagulative necrosis in the diabetic control group. The low-dose group (Group C) exhibited inflammatory changes and congestive splenomegaly. In contrast, the moderate-dose group (Group D) showed a near-normal splenic architecture, and the high-dose group (Group E) displayed a preserved fibroelastic capsule. These findings indicate that the anti-diabetic herbal tea has dose-dependent effects on body weight and spleen histology. Specifically, a moderate dose (6.7 ml) restored splenic structure, while a higher dose (13 ml) provided structural protection, suggesting its potential as a therapeutic agent for managing diabetes. The study underscores the importance of proper dosing to optimize therapeutic outcomes.

Keywords: Albino rats, Alloxan, Anti-diabetic herbal tea, Diabetes mellitus, Spleen Histology.

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## Introduction

The spleen, as the body's largest secondary lymphoid organ, is vital for regulating immune and inflammatory responses. Its location in the circulatory system allows it to filter blood, remove pathogens, and facilitate essential interactions between antigen-presenting cells (APCs) and lymphocytes, which are critical for initiating adaptive immunity. The spleen captures and processes antigens from the bloodstream for presentation to lymphocytes, a process crucial for host defense against blood-borne threats. In addition to its immune functions, the spleen is important for blood quality, as it removes old or abnormal red blood cells and recycles iron (Dailey, 2002). While other organs like the liver and lymph nodes also clear antigens and initiate immune responses, the spleen's direct connection to the bloodstream enables a faster and more thorough defense (Pernar & Tavakkoli, 2019). This widespread damage to the pancreas, however, can trigger systemic effects that also impact other vital organs involved in immunity and homeostasis, such as the spleen.

Alloxan induces hyperglycemia by damaging pancreatic beta cells, which are crucial for insulin production. The primary mechanism of this damage is the formation of highly cytotoxic reactive oxygen species (ROS). Alloxan specifically targets and accumulates in pancreatic beta cells through the GLUT2 glucose transporter (Lenzen, 2008). Inside the cell, alloxan participates in a redox cycle with its reducing product, dialuric acid, generating superoxide radicals. These radicals are then converted into hydrogen peroxide and hydroxyl radicals via the Fenton reaction, which accelerates cell damage (Araujo et al., 2022) This increase in ROS significantly increases cytosolic calcium concentrations, contributing to rapid beta cell destruction. Furthermore, alloxan anion radicals can directly damage DNA, which also plays a role in the observed cytotoxicity in beta cells (Vasu et al., 2016). This type of cellular damage underscores the need for effective and affordable diabetes treatments, which has led to a growing interest in plant-based alternatives like herbal teas.

The rising cost and side effects of conventional antidiabetic medications have fueled growing interest in plant-based alternatives, particularly herbal teas. These herbal remedies are increasingly recognized for their effectiveness in managing diabetes with fewer side effects. Various medicinal plants, such as Momordica charantia (Stingray), Trigonella foenum-graecum (Valley fenugreek), and Panax ginseng, have demonstrated significant antidiabetic effects by improving insulin sensitivity and reducing glucose absorption (Khan et al., 2012). These plants contain bioactive compounds such as flavonoids and alkaloids that improve pancreatic function and help lower blood sugar levels (Kolay et al., 2023). Because they are easy to brew and integrate into daily routines, these herbal teas have become a convenient treatment option. Their popularity continues to grow, especially in regions with rich herbal traditions (Gurjar et al., 2016).

## 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 $06^{o}$ $04^{I}$E and $06^{o}$ $43^{I}$E and latitude $05^{o}$ $44^{I}$N and $07^{o}$ $34^{I}$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).

## 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 spleen 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 spleen 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)

**Results and Discussion**

Table 4.1 shows the mean and standard deviation of initial and final body weight of adult wistar rats induced with diabetes and administered anti-diabetic tea. In Group A (Positive Control), the initial mean body weight was 209.26 ± 25.08 grams, and the final mean body weight was 212.54 ± 26.22 grams. Despite the slight increase in body weight, the t-value of -0.02 and the p-value of 0.85 indicate that the change was not statistically significant.

For Group B (Negative Control), the initial body weight was 179.20 ± 13.33 grams, and the final body weight was 144.62 ± 9.66 grams. The t-value of 4.69 and p-value of 0.01 reveal a significant decrease in body weight.

Group C (3.4ml) began with an initial mean body weight of 173.66 ± 8.91 grams and concluded with a final mean body weight of 152.34 ± 15.41 grams. The t-value of 2.68 and p-value of 0.03 suggest that the administration of 3.4ml of anti-diabetic tea led to a statistically significant reduction in body weight.

In Group D (6.7ml), the initial mean body weight was 175.02 ± 26.83 grams, and the final mean body weight was 145.60 ± 8.74 grams. The t-value of 2.33 and p-value of 0.07 indicate a trend toward a decrease in body weight, but the result was not statistically significant at the conventional 0.05 level.

Group E (13ml) exhibited an initial mean body weight of 180.24 ± 22.50 grams and a final mean body weight of 146.76 ± 28.59 grams. The t-value of 2.06 and p-value of 0.08 suggest a reduction in body weight, though it was not statistically significant at the 0.05 threshold.

**Table 1.** Mean± Standard Deviation (S.D) of initial and final body weight of adult wistar induced with diabetes and administered anti-diabetic tea

| | **Initial (Mean±S.D)** | **FinaL (Mean±S.D)** | **t-value** | **p-value** |
|---|---|---|---|---|
| **Group a (positive control)** | 209.26±25.08 | 212.54±26.22 | -0.02 | 0.85 |
| **Group b (negative control)** | 179.20±13.33 | 144.62±9.66 | 4.69 | 0.01 |
| **GROUP C (3.4ml)** | 173.66±8.91 | 152.34±15.41 | 2.68 | 0.03 |
| **GROUP D (6.7ml)** | 175.02±26.83 | 145.60±8.74 | 2.33 | 0.07 |

| | | | | |
|---|---|---|---|---|
| **GROUP E (13ml)** | 180.24±22.50 | 146.76±28.59 | 2.06 | 0.08 |

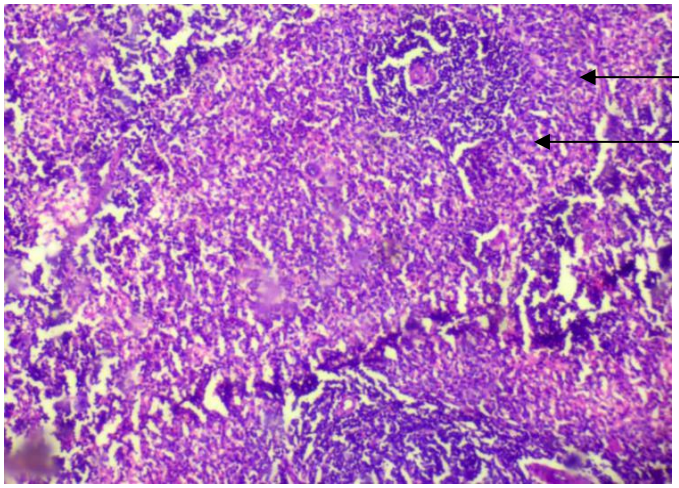


Plate 1a shows the Normal histological feature of the Spleen of the untreated (Positive control) Albino rat with presence of Germinal center (A) and Central artery (B) of the spleen. HandEX100

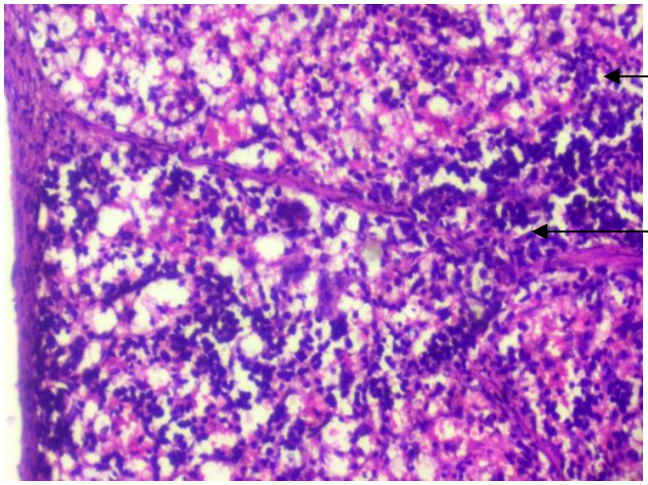


Plate 1b shows the Normal histological feature of the Spleen of the untreated (Positive control) Albino rat with presence of Germinal center (A) and Central artery (B) of the spleen. HandEX400

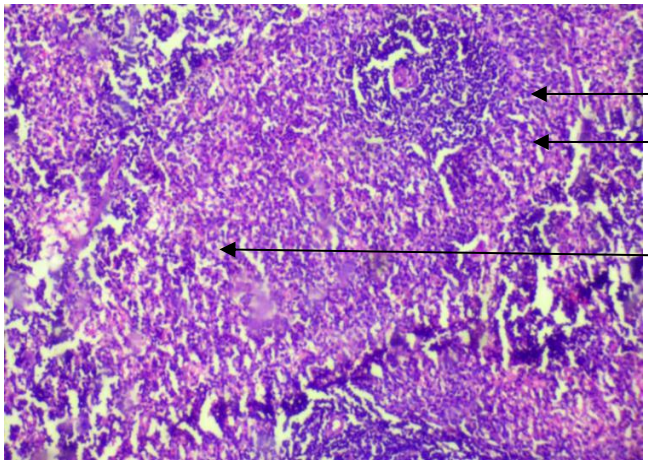

Plate 2a shows the histological feature of the Spleen of the treated (Group B – diabetic group on alloxan) Albino rat with presence of Germinal center (A), Central artery (B) and cogulative necrosis with ghost outlines of cells suggestive of splenic infarcts (C) of the Spleen. HandEX100

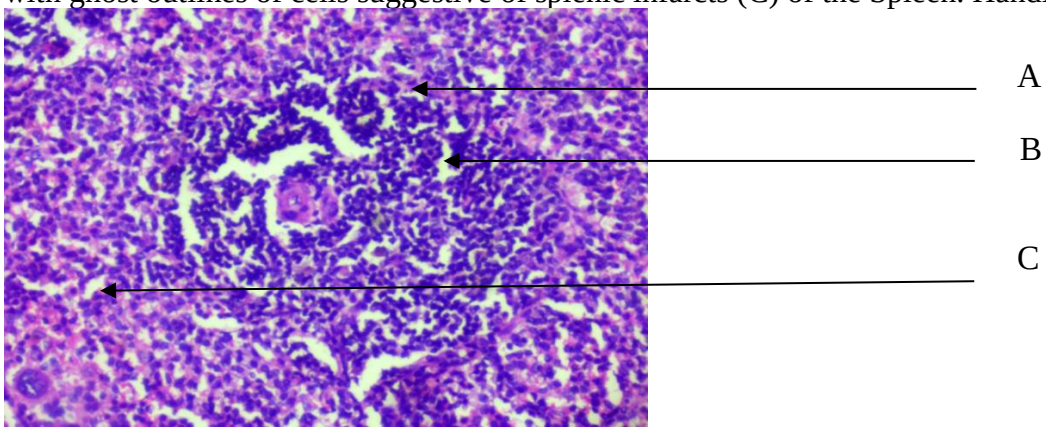


Plate 2b shows the histological feature of the Spleen of the treated (Group B – diabetic group on alloxan) Albino rat with presence of Germinal center (A), Centrajl artery (B) and cogulative necrosis with ghost outlines of cells suggestive of splenic infarcts (C) of the Spleen HandEX400

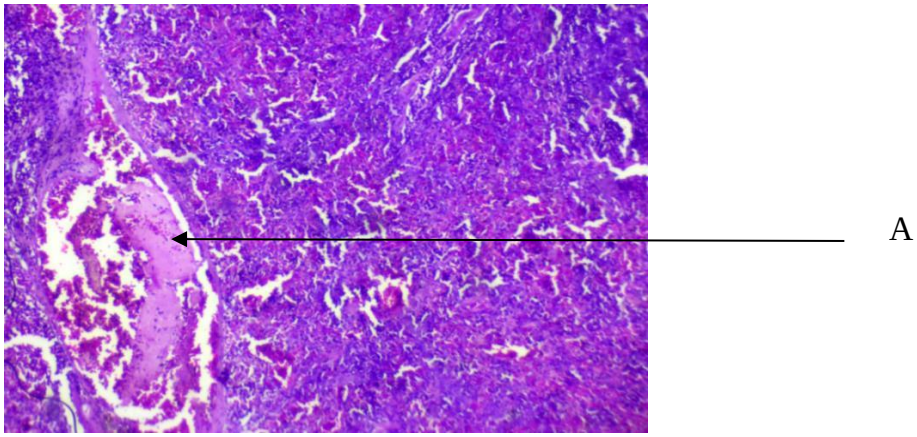


Plate 3a shows the histological feature of the Spleen of the treated (diabetic group C receiving 3.4ml of antidiabetic tea) Albino rat with presence of Inflammation and necrosis seen around the germinal center region suggestive of congesture splenomegaly (A) of the Spleen. HandEX100

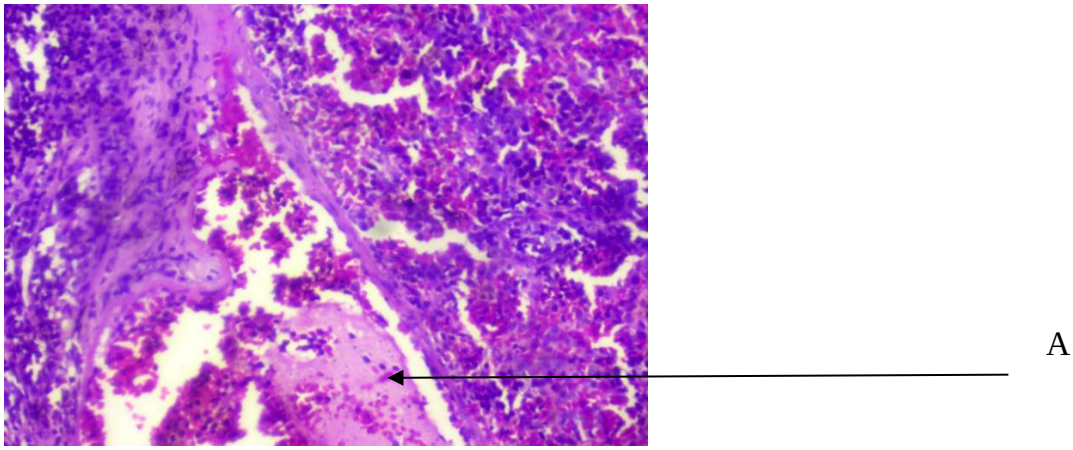

Plate 3b shows the histological feature of the Spleen of the treated (diabetic group C receiving 3.4ml of antidiabetic tea) Albino rat with presence of Inflammation and necrosis seen around the germinal center region suggestive of congesture splenomegaly (A) of the Spleen. HandEX400

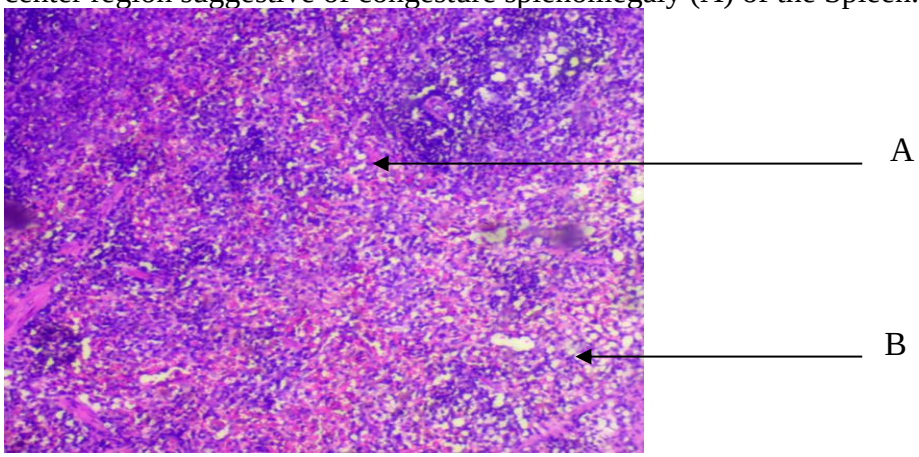


Plate 4a shows the histological feature of the Spleen of the treated (diabetic group D receiving 6.7ml of antidiabetic tea) Albino rat with presence of Red pulp (A) and White pulp (B) of the Spleen with normal architecture . HandEX100

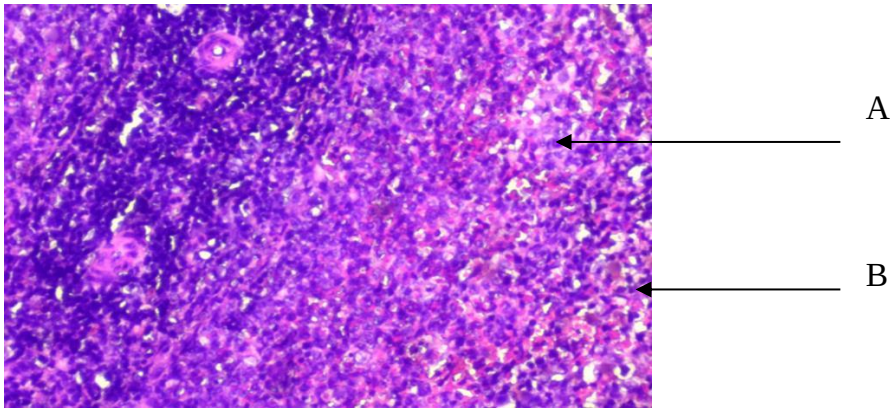


Plate 4b shows the histological feature of the Spleen of the treated (diabetic group D receiving 6.7ml of antidiabetic tea) Albino rat with presence of Red pulp (A) and White pulp (B) of the Spleen with normal architecture. HandEX400

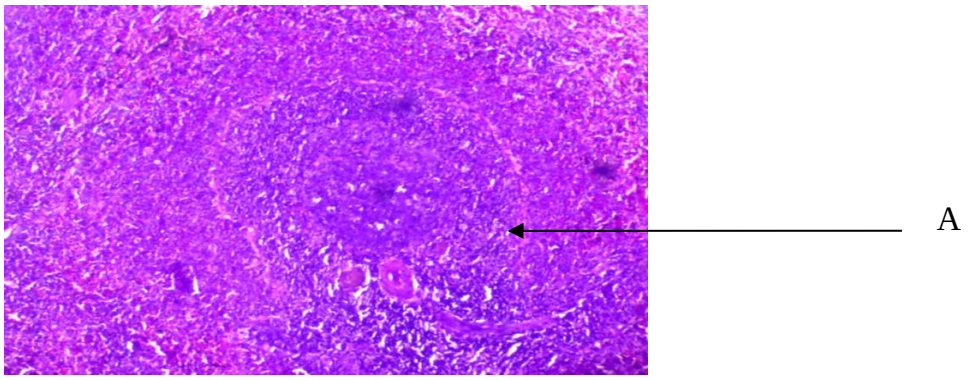


Plate 5a shows the histological feature of the Spleen of the treated (diabetic group E receiving 13ml of antidiabetic tea) Albino rat with presence of Fibroelastic capsule consisting of well arranged cells and tissue (A) of the Spleen. HandEX100

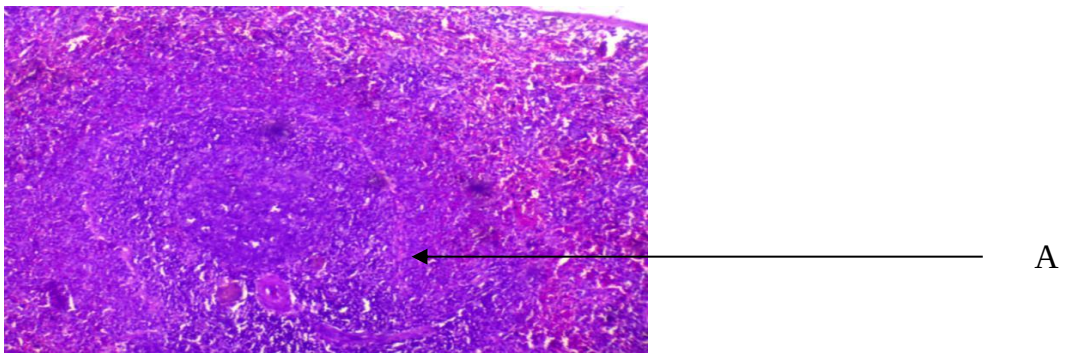


Plate 5b shows the histological feature of the Spleen of the treated (diabetic group E receiving 13ml of antidiabetic tea) Albino rat with presence of Fibroelastic capsule consisting of well-arranged cells and tissue (A) of the Spleen. HandEX400.

The current study evaluated the effects of anti-diabetic tea on body weight changes in adult Wistar rats induced with diabetes. The results from various groups, including positive and negative controls as well as different doses of anti-diabetic tea, provide insight into the potential therapeutic effects of the tea on body weight regulation in a diabetic model.

Group A (Positive Control), which was not treated with the anti-diabetic tea but exposed to diabetes induction, showed a marginal increase in body weight from 209.26 ± 25.08 grams to 212.54 ± 26.22 grams. However, the t-value of -0.02 and the p-value of 0.85 indicated that the observed change in body weight was not statistically significant. This lack of significant change could be attributed to the nature of the control group, where the absence of a treatment led to no meaningful effects on weight, despite slight fluctuations. These results align with findings by (Scridon et al., 2015), who observed no significant weight changes in untreated diabetic rats over a short duration. It is possible that the duration or conditions under which the rats were observed may not have been sufficient to induce major changes in body weight, which could explain the minimal and statistically insignificant increase observed in this group.

In Group B (Negative Control), there was a marked reduction in body weight from 179.20 ± 13.33 grams to 144.62 ± 9.66 grams, with a t-value of 4.69 and a p-value of 0.01, indicating a significant decrease in body weight. This finding suggests that the diabetic condition had a substantial impact on body weight, possibly due to the effects of hyperglycemia and insulin resistance. Weight loss in diabetic animals is commonly attributed to the body's inability to efficiently utilize glucose, leading to catabolic processes that deplete body fat and muscle mass (Haruna et al., 2024). Similar results have been reported in studies where diabetic rats showed significant weight loss when untreated, underscoring the metabolic disturbances caused by diabetes.

Group C (3.4ml) showed a statistically significant reduction in body weight from 173.66 ± 8.91 grams to 152.34 ± 15.41 grams, with a t-value of 2.68 and a p-value of 0.03. This reduction in body weight indicates that the 3.4ml dose of anti-diabetic tea had a beneficial effect on weight management in diabetic rats. The anti-diabetic properties of the tea may have contributed to improved glucose metabolism, thereby reducing excess fat accumulation or promoting fat utilization. Several studies have pointed to the potential of plant-based anti-diabetic therapies in regulating body weight, as they often enhance insulin sensitivity and help in the management of blood glucose levels (Haruna et al., 2024). This finding supports the hypothesis that moderate doses of anti-diabetic tea may assist in restoring metabolic balance and preventing diabetic-associated weight loss.

For Group D (6.7ml), the initial mean body weight was 175.02 ± 26.83 grams, and the final mean was 145.60 ± 8.74 grams, indicating a reduction in body weight. However, the t-value of 2.33 and the p-value of 0.07 suggest a trend towards weight loss, but it was not statistically significant at the conventional 0.05 level. The lack of significance could be due to several factors, such as the possibility of diminishing returns at higher doses of the tea, or the variability in individual responses

to the treatment. Higher doses of herbal extracts are sometimes associated with reduced bioavailability or altered pharmacokinetics, leading to less predictable outcomes (Zhong et al., 2015). Therefore, while the weight reduction trend is suggestive of an effect, it may not be strong enough at this dosage to reach statistical significance.

In Group E (13ml), the reduction in body weight from 180.24 ± 22.50 grams to 146.76 ± 28.59 grams was also not statistically significant (t-value of 2.06, p-value of 0.08). This result suggests that higher doses of the anti-diabetic tea may not necessarily lead to greater or more significant changes in body weight. It is possible that at such a high dose, the tea's active compounds might not be absorbed efficiently, or that there is a limit to the beneficial effects on body weight. Research by (Lee et al., 2024) has demonstrated that while certain herbal treatments show promise at moderate doses, higher doses sometimes fail to produce enhanced therapeutic effects, possibly due to saturation or altered physiological responses. This could explain why a larger dose did not yield more pronounced or statistically significant results.

The histological examination of spleen tissue in the albino rat model used to investigate the effects of various treatments on diabetes reveals important insights into the structural changes induced by both diabetes and subsequent interventions. The untreated positive control group (Plate 1a and 1b) displayed normal histological features, including the presence of the germinal center, central artery, and other typical splenic structures. This is consistent with standard observations of a healthy spleen in rodents, which exhibits a well-organized structure, facilitating immune response (Sharkey *et al*., 2019). However, as we move into the diabetic groups, more complex alterations were observed, reflecting the impact of diabetes and the treatments administered.

In Group B, consisting of diabetic rats induced by alloxan (Plate 2a and 2b), there was noticeable cogulative necrosis, with ghost outlines of cells, which is indicative of splenic infarcts. Splenic infarcts are often caused by ischemic damage and have been linked to the altered vascular and immune responses in diabetic conditions (Moscow State Academy of Veterinary Medicine and Biotechnology – MVA by K. I. Skryabin", Moscow, Russia & Gildikov, 2024). The presence of these necrotic areas suggests that alloxan-induced diabetes leads to splenic dysfunction, possibly due to compromised blood supply or the effects of hyperglycemia on cellular metabolism (Rashid et al., 2012)

In Group C, treated with 3.4 ml of antidiabetic tea (Plate 3a and 3b), the spleen exhibited inflammation and necrosis around the germinal center, suggestive of congestive splenomegaly. Splenomegaly, or enlargement of the spleen, is often associated with inflammatory conditions, and its development in this group could be attributed to the body's immune response to diabetic stress and potential therapeutic intervention (Soares et al., 2024). The findings in this group are in line with studies that show that the spleen often enlarges in the presence of systemic inflammation, which is common in diabetic animals (National Cancer Institute, 2020). However, the inflammation observed here, particularly around the germinal center, contrasts with the lack of such changes in other treatments or controls, indicating that the tea's impact may involve immune modulation, which warrants further investigation.

Group D, which was treated with 6.7 ml of antidiabetic tea (Plate 4a and 4b), showed a return to normal splenic architecture, with clear separation of red and white pulp. This result suggests that the moderate dose of the antidiabetic tea may have a beneficial effect on spleen morphology, potentially mitigating the damage caused by diabetes. This finding supports the idea that herbal interventions, such as antidiabetic teas, could have restorative properties in diabetic animals, an effect that has been documented in other studies on herbal medicine in diabetes management (Sarma et al., 2024). The normal architecture observed in this group may indicate that the treatment alleviated the systemic inflammation that typically accompanies diabetes, helping restore spleen structure and function.

Finally, Group E, treated with 13 ml of antidiabetic tea (Plate 5a and 5b), revealed a well-preserved fibroelastic capsule consisting of well-arranged cells and tissues. This outcome suggests that a higher dose of the tea may contribute to the structural integrity of the spleen, possibly by

enhancing tissue remodeling and collagen deposition (Özerkan et al., 2021). The well-structured capsule observed in this group contrasts with the inflammation and necrosis seen in Group C, implying that larger doses of the antidiabetic tea could provide more substantial protective or reparative effects on splenic tissue. This finding aligns with reports from (Hanchang et al., 2022), who demonstrated that higher doses of certain herbal treatments could induce tissue regeneration and fibrosis, helping to prevent organ damage in diabetic rats.

These findings can be explained by several factors. First, the differential effects of the antidiabetic tea may be due to the dosage and the concentration of active compounds in the tea, which might influence the degree of tissue protection or damage. At lower doses, the tea may promote mild immune modulation, leading to inflammation and congestion, whereas higher doses could enhance reparative processes, reducing necrosis and maintaining structural integrity. Second, the impact of diabetes on the spleen could vary based on the severity of hyperglycemia and the duration of the condition, suggesting that longer-term diabetes may lead to more pronounced organ dysfunction. Lastly, individual rat variability in response to diabetes and treatment may also contribute to the differing histological outcomes.

## Conclusion

This study revealed varying degrees of injury, inflammation, and repair. While the untreated diabetic rats displayed significant necrotic changes indicative of splenic infarcts, moderate doses of antidiabetic tea appeared to reduce inflammation and restore normal splenic architecture. Higher doses of the tea demonstrated potential benefits in maintaining structural integrity, particularly through fibroelastic capsule formation. These findings suggest that antidiabetic tea could be beneficial in mitigating spleen damage in diabetic rats, but the dosage plays a crucial role in determining the extent of protection or damage.

## Conflicts of Interest

The author declares that there is no conflict of interest.

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