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Effect of *Aloe barbadensis* Root Extract on the Histology of Some Vital Organs of Male Rats Assessed for Aphrodisiac Activity

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Abstract
Histopathology is an essential tool in assessing the untoward effect of toxicants, synthetic drugs, and herbal medicines in toxicological animal studies. Therefore, the effect of the oral administration of graded doses of the ethanol extract of A. barbadensis root on the histology and organ-body weight ratios of some vital rat organs was evaluated. Seventy-five (75) healthy and matured male rats of 140-270 g body weights were used for this study. The rats were randomized entirely into five groups of 15 and orally given treatment using the orogastric tube. The animals were placed into Groups A, B, C, D, and E. Group A was administered the diluent (2 mL of distilled water) while groups B, C and D were given 100, 200 and 400 mg/kg of the extract for 14 days. Group E was given the standard drug-Sildenafil citrate (5 mg/kg). The vital organs (liver, kidney, heart, and spleen) were harvested, prepared, and stained after sacrificing the animals. The tissue sections were subjected to histopathological studies, and the photomicrographs of the tissues were captured appropriately. The organ-body weights were determined using an electronic weighing balance. The histopathological examination revealed no apparent abnormalities in the vital organs; instead, a vasoactive effect of active congestion was observed. The liver and spleen's histology showed a mild activation of the local immune system. The extract had no significant effect (P>0.05) on the organ-body weight ratios. The study showed that the extract had no untoward effect on the selected essential organs within the dose range explored.

Keywords: **Histology, Male rat organs, Medicinal plant, *Aloe barbadensis*, Ethanol extract**

INTRODUCTION
Over the years, plants have been used to manage various diseases/ailments, and a significant proportion of the world's population still relies on local medicines and folk treatments principally from plant extract (Prasathkumar *et al*., 2021). Medicinal plants are nature's gift to

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humankind, and many rural folks depend on medicinal plants for health care. Modern medicine practitioners often overlook the wealth of indigenous knowledge, gradually eroding precious lifesaving information (Gill *et al*., 1997). The knowledge gained from indigenous people can be vital for human health development programmes and the local population. The resources can be harnessed for pharmacological investigations in modern medicine (Idu *et al*., 2006a; Ezekwesili-Ofili and Okaka, 2019). A pivotal setback of this medicinal resource is linked to their little or lack of dose regimen and inadequate data on toxicity to ascertain their safety.

Aloe barbadensis, also called *Aloe vera*, is a common garden medicinal plant in Nigeria with a cocktail of medicinal uses. *A. barbadensis* (Syn: *Aloe vera* L. (Webb). belong to the xanthorrhoeaceae family. It is a tropical, succulent, drought-resistant plant (Hęś *et al*., 2019). The plant is popularly used for cosmetic purposes, wound healing, diabetes, tissue burn, microbial infections, and boosting immunity. Other uses include as laxative, anti-inflammatory, hepatoprotective, aphrodisiac, low sperm count, weak erection, general infertility, and to treat frostbite and psoriasis (Erhabor *et al*., 2013; Hashem and Kaviani, 2010; Sánchez-Machado *et al*., 2017). Other uses include.

However, traditional medicine practitioners and most users of medicinal plants believe that herbs are safe because they are of natural origin, cheap and effective (Kubkomawa *et al*., 2020; Romero *et al*., 2022). These herbs have chemical substances capable of producing harmful effects on their consumers. These effects could be on various organisms such as man, animals, plants, or microorganisms. This detrimental effect can be on the sub-structure of the organism, such as the cell (cytotoxicity) or liver (hepatotoxicity) (Erhabor and Idu, 2022a). Nevertheless, the occurrences of these injuries depend on the quantity of chemicals absorbed (Plaa, 1998, ILO, 2004). It has been observed that most of these herbs have been limited by a lack of a standard dose regimen and adequate toxicity data to test their safety (Ekor, 2014). It is pertinent that proper scientific investigation of both the beneficial and harmful effects of any medicinal plant be done (Idu *et al*., 2006b). The toxic effect of a drug in man has been observed and reported as similar to that of some animals. This is a premise for why animal models are used in toxicological studies (Range *et al*., 1995). Validating a drug's toxicity has helped determine the upper limits of administering effective therapy (Steinmetz and Spack, 2009).
It is important to explicitly state from the available literature that in an aphrodisiac study of *A. babrdensis* root, the toxic effect of ethanol extract on some vital organs is yet to be accomplished. Therefore, the histology of some vital organs as a toxicological tool following the aphrodisiac assessment of *Aloe barbadensis*, root extract was explored.

METHODOLOGY

Plant Collection and Authentication

The roots of *Aloe barbadensis* were collected from Okene town in Okene local government area of Kogi State, Nigeria. It was identified by Prof. MacDonald Idu of the Department of Plant Biology and Biotechnology, University of Benin, Benin City, and further authenticated by Mr. G. Ibhanesebhor, of the Herbarium Unit of the Obafemi Awolowo University, Ile-Ife, Nigeria, with voucher number IFE17004 where the plant was deposited.

Preparation of Ethanolic Extract

The extract was prepared following the procedure described by Erhabor and Idu (2017). The fresh roots of *Aloe barbadensis* were detached from the whole uprooted plant, rinsed in water

and spread on laboratory tables for drying at room temperature. The plant material was further dried in an oven set at 40 ^{o}C for 10 minutes before being reduced to a fine powder with a mechanical grinder. Two thousand grams (2000 g) of the powdered plant material was extracted with 5000 mL of ethanol using a soxhlet extractor. The extract was then concentrated to dryness using a water bath (HH-S Water Bath; Searchtech Instruments) set at an average temperature of 50 ^{o}C. The percentage yield of the ethanolic extract was determined using the formula (% Yield= weight of extract/weight of powder sample x 100/1).

Animal handling and grouping

Seventy-five (75) healthy and matured male rats and thirty (30) non-oestrus female rats of 140-270 g and 145- 260 g body weights were used for this study. The animals were obtained from the animal house of the Department of Anatomy, Faculty of Basic Medical Sciences, University of Benin. The animals were housed in clean wooden cages and placed in a well-ventilated animal house domiciled at the Department of Animal and Environmental Biology, Faculty of Life Sciences, for acclimatization with optimum conditions (temperature, 25^{o}C; photoperiod, 12 hours of natural light and 12 hours of dark). The animals were allowed free water access and fed with standard commercial pellets. The cages were cleaned daily throughout the study. The 75 male rats were randomized entirely into five groups of 15 and orally given treatment.

Administration of Extract

The animals were placed into Groups A, B, C, D, and E. Group A (negative control) was administered with the diluent (2 mL of distilled water). At the same time, groups B, C and D were given 100, 200 and 400 mg/kg body weight of *Aloe barbadensis* root extract for fourteen (14) days. Group E (positive control) was given the standard drug-Sildenafil citrate (5 mg/kg). The oral administration was carried out using an orogastric tube. All animals used in this study were handled following the international guiding principles for biomedical research involving animals as outlined by the Council for International Organization of Medical Sciences and the International Council for Laboratory Animal Science (CIOMS and ICLAS) (2012). The ethical committee on experimental animal use and care of the Faculty of Life Sciences, University of Benin, Nigeria, reviewed and approved the protocol (LSC15101).

Histopathological study

The animals were sacrificed for histopathological studies after a 14-day administration of the ethanol extract of *A. barbadensis* root and its assessment for aphrodisiac potential. The organs (liver, kidney, heart, and spleen) were harvested and fixed in 10% (vol/vol) formaldehyde and the testis fixed in Buoin's fluid. The organs were dehydrated through ascending grades of ethanol (70%, 90% and 95% vol/vol), cleaned in xylene and embedded in paraffin wax (melting point at 56 ^{o}C) (Krause, 2001). Tissue sections were prepared according to the method of Drury and Wallington (1973) and stained with hematoxylin/eosin. The photomicrographs were taken at x 400 with a digital camera.

Determination of organ weight

The organ-body weights were determined on the first, seventh and fourteenth day of assessing the aphrodisiac effect of the extract on the rats. Five male rats were sacrificed from the designated groups for the aphrodisiac assessment on the first, seventh, and fourteenth days. The excised organs (liver, kidney, heart, spleen and testis) were weighed using an electronic weighing balance before they were preserved in their respective solutions. The organs were weighed to ascertain the organ-body weight ratio or swelling of the organs, or hypertrophy

Analysis of Data

Data obtained were presented as mean ± SEM of five replicates. The data were subjected to one-way analysis of variance to compare the means of the different groups. The Duncan multiple range test was utilized to determine the differences among the various means and the interaction between the variables. The computer software package SPSS 15.0 was used in analyzing the data. Differences at $p<0.05$ were considered statistically significant.

RESULTS

Effect of administration of *A. barbadensis* root extract on the spleen

The administration of graded doses of the ethanol extract of *Aloe barbadensis* and Viagra (5 mg/kg) induced mild follicular activation, as shown in Plate 1.

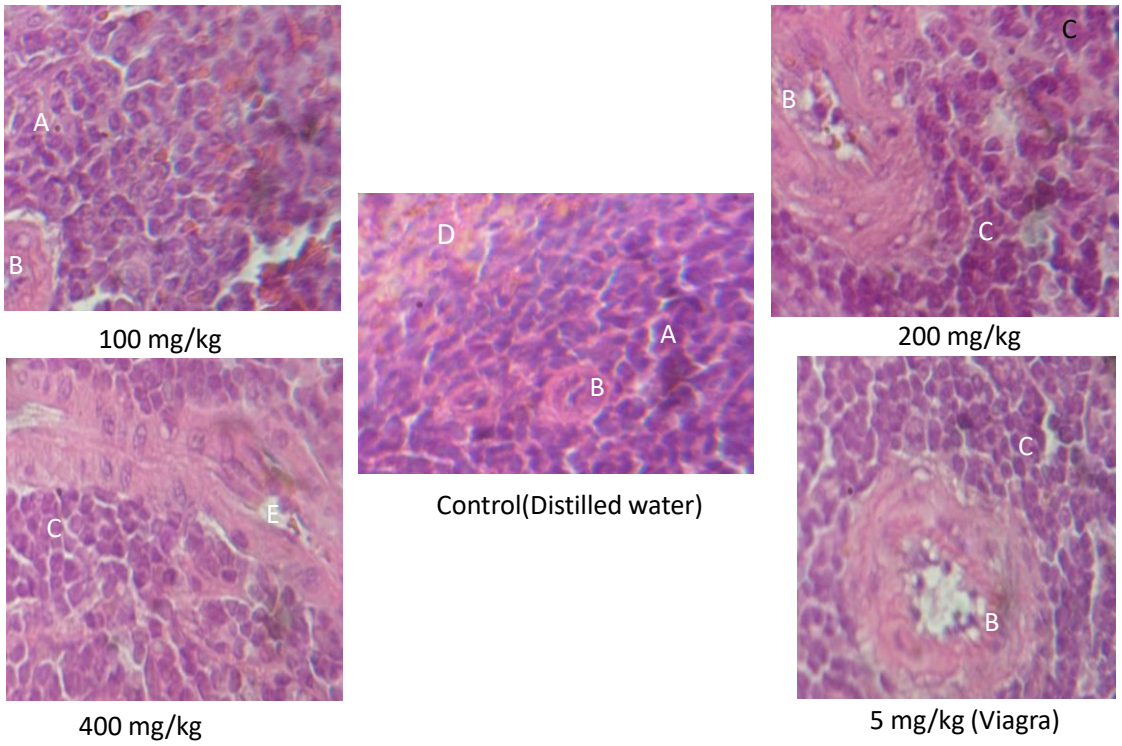

Plate 1: Photomicrograph of a section of the spleen of a male rat treated with ethanol extract of *A. barbadensis* root for 14 days. unremarkable follicle A; arteriole B; mild follicular activation C; red pulp D; arteriolar hypertrophy E (H&E x 400)

Effect of Administration of *A. barbadensis* root extract on Liver

Plate 2 shows that the extract induced active congestion and mild activation of the periportal lymphocytes. The 400 mg/kg group caused a moderate activation of the lymphocytes of the liver of the male rats.

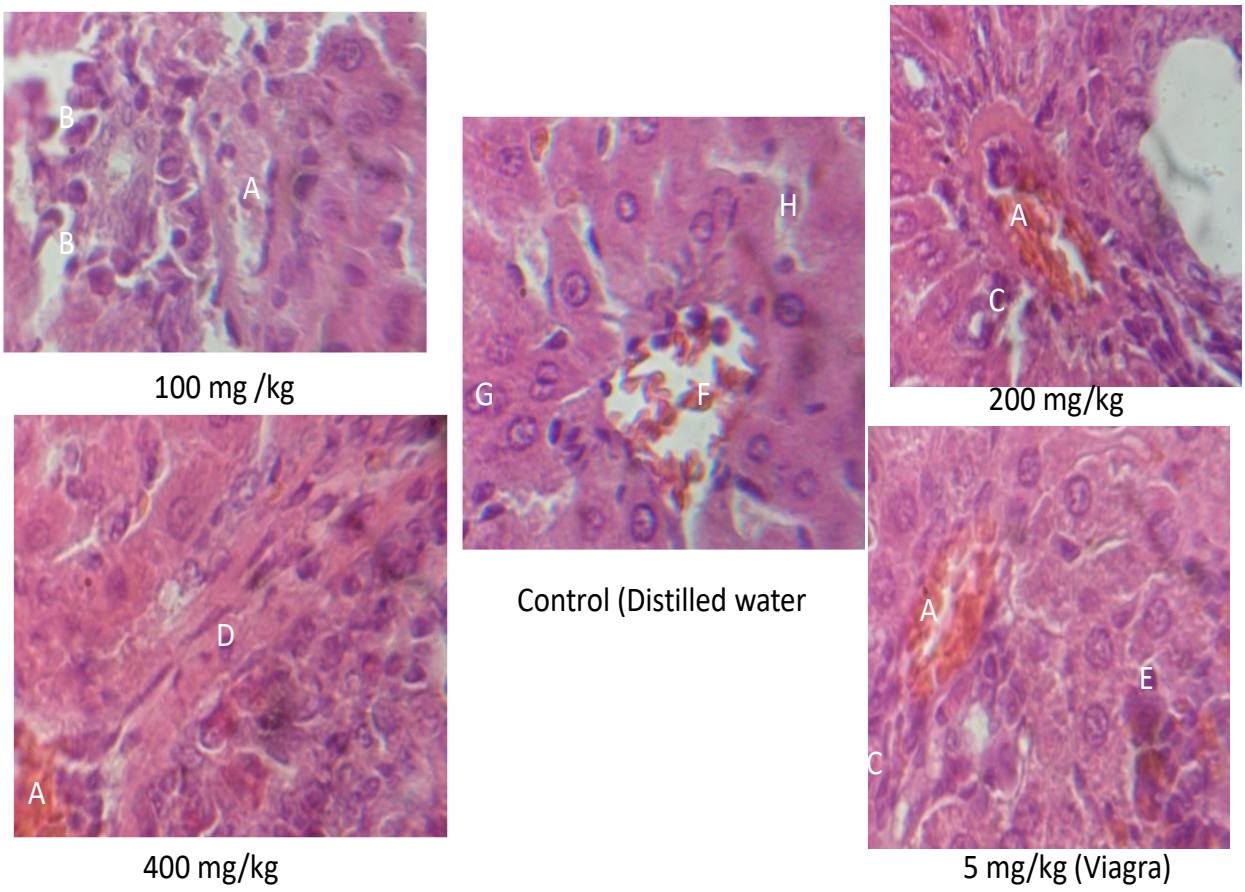

Plate 2: Photomicrograph of a section of the liver of male rat treated with ethanolic extract of *A. barbadensis* root for 14 days. mild congestion A, periportal inflammation B, mild periportal inflammation C; moderate periportal inflammatio D; kupffer cell activation E; central vein F; hepatocytes G; sinusoids H(H&E x 400)

Effect of administration of *A. barbadensis* root extract on Heart

The effect of the extract, as revealed in Plate 3, showed that mild coronary congestion and hypertrophy were induced. A focal intimal erosion was noticed in the group administered 5 mg/kg of Viagra.

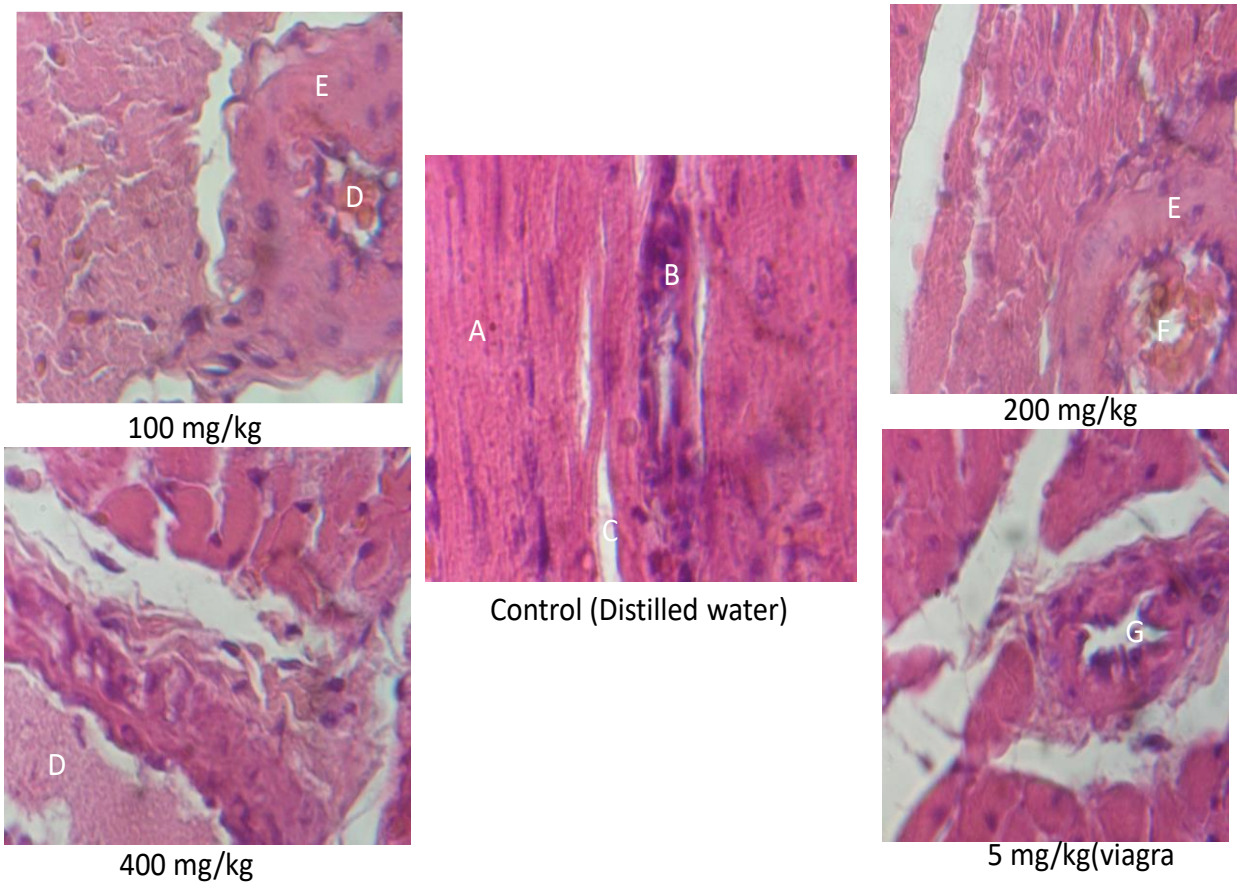

Plate 3:Photomicrograph of heart of male rat treated with ethanol extract of *A. barbadensis* for 14 days. Mycardiac muscle bundles A; coronary vessel B ;interstitial space C; mild coronary congestion D, hypertrophy E, mild congestion F, focal intimal erosion G(H&E x 400)

Effect of administration of *A. barbadensis* root extract on Kidney

Administration of the extract to the male rats, as shown in Plate 4, induced mild active vascular congestion and hypertrophy.

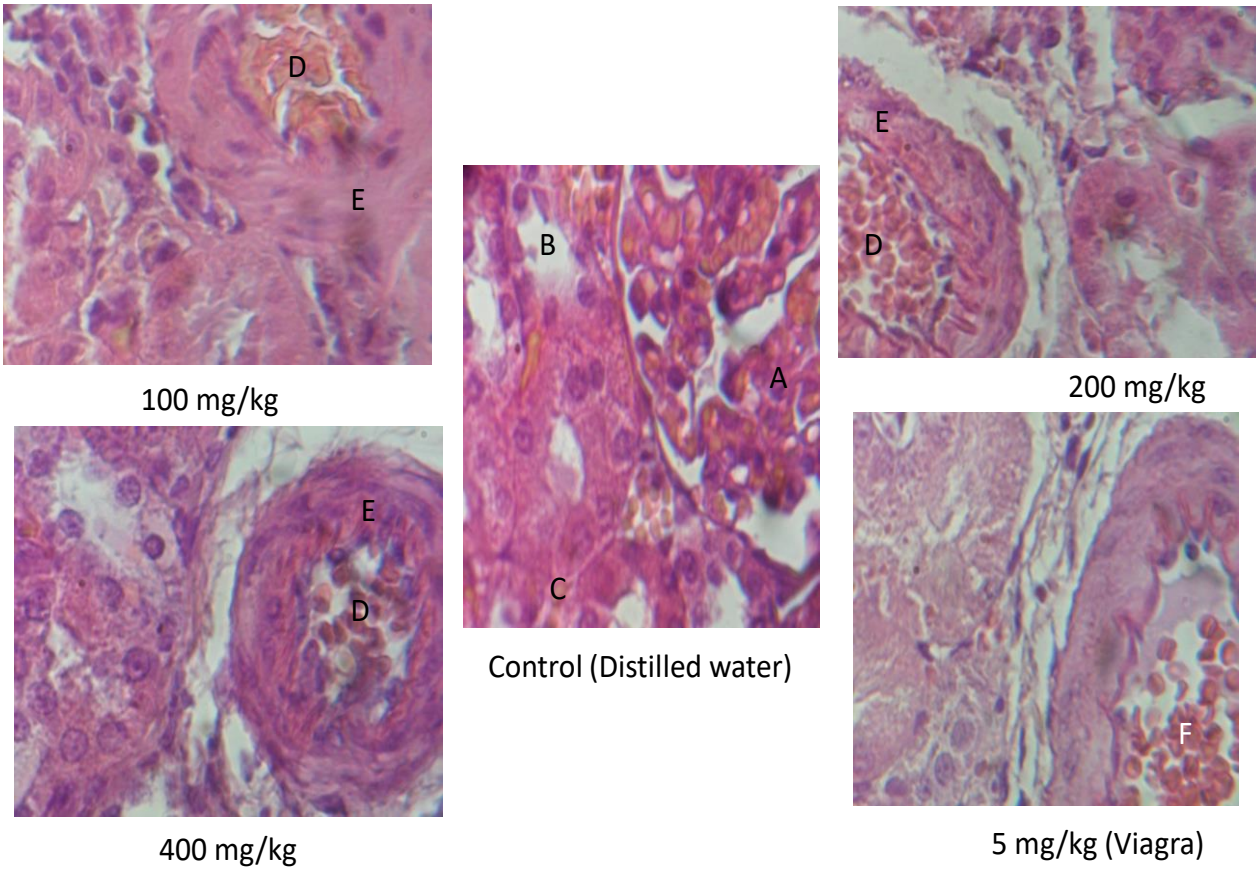

Plate 4: Photomicrograph of kidney of male rat treated with ethanol extract of *A. barbadensis* for 14 days. glomerulus A, tubules B, interstitial space C, mild congestion D, hypertrophy E, mild vascular congestion A, (H&E x 400)

Effect of administration of *A. barbadensis* root extract on testis

In Plate 5, the effect of the extract on the testis revealed that mild Leydig cell hyperplasia was induced.

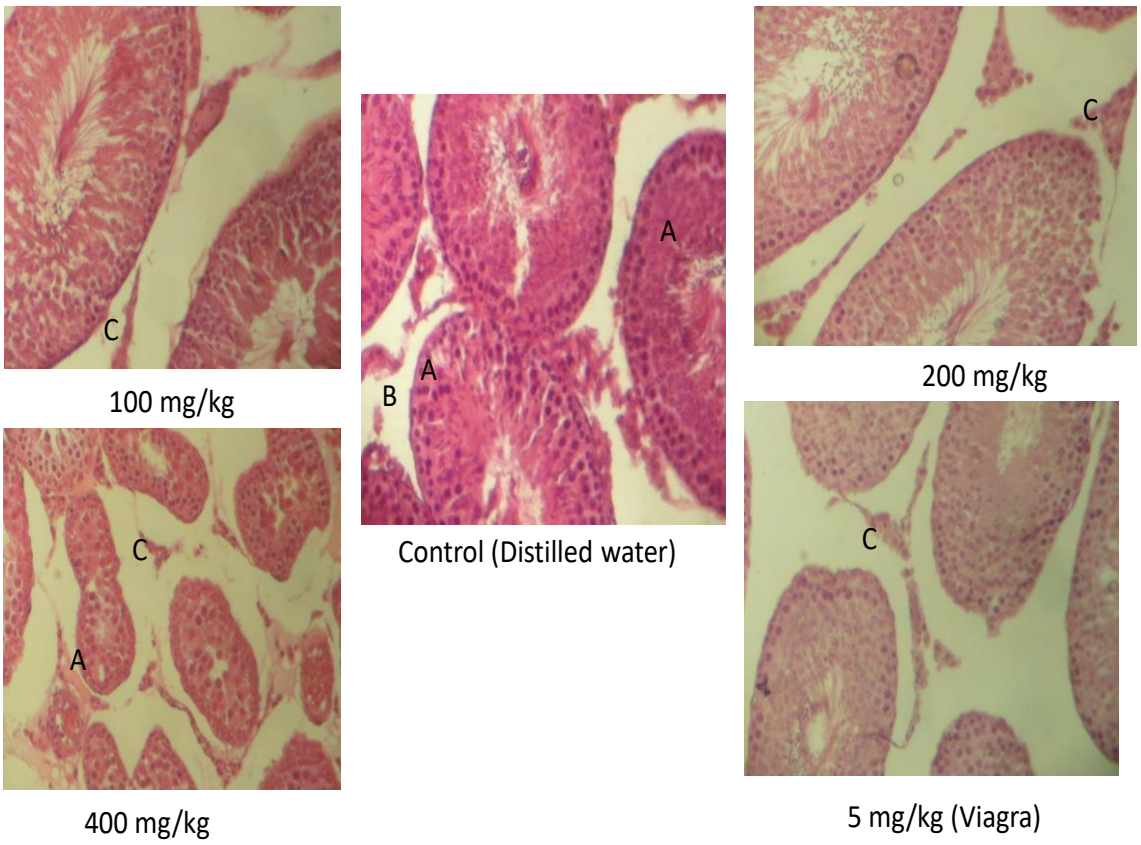

Plate 5: Photomicrograph of a section of the testis of male rats treated with ethanol extract of *A.barbadensis* for 14 days. seminiferous tubules A, interstitial space B, mild leydig cell hyperplasia C (H&E x 100)

Effect of *A. barbadensis* root extract on the organ-body weight ratio of Testis

The oral administration of the ethanol extract of *A. barbadensis* at all tested doses on the first, seventh and fourteenth day did not show any significant change ($p>0.05$) in the size of the Testis in relation to the whole body of the male rat when compared to the control.

Table 1: Effect of oral treatment of ethanol extract of *A. barbadensis* roots on testis-body weight ratio.

Groups	Day 1	Day 7	Day 14	P-Value
Control (DW)	0.52±0.14	0.51±0.06	0.46±0.08	NS
Viagra (5mg/kg)	0.56±0.10	0.49±0.08	0.51±0.05	NS
100 mg/kg	0.59±0.04	0.43±0.07	0.48±0.07	NS
200 mg/kg	0.49±0.06	0.46±0.02	0.58±0.05	NS
400 mg/kg	0.37±0.09	0.52±0.06	0.32±0.06	NS
	p>0.05	p>0.05	p>0.05	

DW-Distilled water; All values are expressed as Means±SEM of five rats in each group
NOTE: p>0.05= Not Significant; NS =No Significant difference in days across the rows.

Effect of *A. barbadensis* root extract on the organ-body weight ratio of Liver

Results depicted in Table 2 revealed that the effect of *A. barbadensis* extract on liver-body weight index at all doses within and across each of the examination days was insignificant (p>0.05) compared to the control.

Table 2: Effect of oral treatment of ethanol extract of *A. barbadensis* roots on liver-body weight ratio.

Groups	Day 1	Day 7	Day 14	P-Value
Control (DW)	3.05±0.28	2.86±0.2	2.75±0.29	NS
Viagra (5mg/kg)	3.4±0.32	2.82±0.08	2.96±0.11	NS
100 mg/kg	3.34±0.36	2.89±0.18	2.91±0.16	NS
200 mg/kg	3.58±0.08	8.31±5.56	3.07±0.13	NS
400 mg/kg	2.47±0.66	3.04±0.27	3.01±0.2	NS
	p>0.05	p>0.05	p>0.05	

DW=Distilled water; All values are expressed as Means±SEM of five rats in each group
NOTE: p>0.05= Not Significant; NS=No Significant difference in days across the rows.

Effect of *A. barbadensis* root extract on the organ-body weight ratio of Kidney

The ethanol extract of *A. barbadensis* after oral administration at all tested doses on the first, seventh and fourteenth day did not yield any significant change (p>0.05) in the size of the kidney in relation to the whole body of the male rat when compared to the control groups.

Table 3: Effect of oral treatment of ethanol extract of *A. barbadensis* roots on kidney-body weight ratio.

Groups	Day 1	Day 7	Day 14	P-Value
Control (DW)	0.29±0.02	0.25±0.04	0.29±0.03	NS
Viagra (5mg/kg)	0.3±0.03	0.23±0.02	0.3±0.02	NS
100 mg/kg	0.26±0.02	0.29±0.01	0.28±0.01	NS
200 mg/kg	0.26±0.01	0.29±0.01	0.31±0.01	NS
400 mg/kg	0.25±0.02	0.27±0.03	0.33±0.03	NS
	p>0.05	p>0.05	p>0.05	

DW=Distilled water; All values are expressed as Means±SEM of five rats in each group
NOTE: P>0.05= Not Significant; NS =No Significant difference in days across the rows.

Effect of *A. barbadensis* root extract on the organ-body weight ratio of Heart.

At all doses of the extract administration, as shown in Table 4, there was no significant change ($p>0.05$) in the size of the Heart relative to the entire body of the rat on days 1, 7 and 14.

Table 4: Effect of oral treatment of ethanol extract of *A. barbadensis* roots on heart-body weight ratio.

Groups	Day 1	Day 7	Day 14	P-Value
Control(DW)	0.34±0.02	0.33±0.02	0.36±0.04	NS
Viagra (5mg/kg)	0.34±0.04	0.32±0.05	0.31±0.02	NS
100 mg/kg	0.31±0.01	0.25±0.04	0.33±0.02	NS
200 mg/kg	0.34±0.02	0.31±0.02	0.4±0.01	NS
400 mg/kg	0.3±0.03	0.29±0.02	0.36±0.03	NS
	p>0.05	p>0.05	p>0.05	

DW=Distilled water; All values are expressed as Means±SEM of five rats in each group
NOTE: P>0.05= Not Significant; NS =No Significant difference in days across the rows.

Effect of *A. barbadensis* root extract on the organ-body weight ratio of spleen

The administration of the ethanol extract of *A. barbadensis* root at all tested doses on the first day did not show any significant change ($p>0.05$) in the size of the spleen in relation to the whole body of the male rat when compared to the control (Table 5). Also, repeated administration of the extract at all doses tested for the seventh and fourteenth day did not produce any significant change ($p>0.05$) in the size of the spleen.

Table 5: **Effect of oral treatment of ethanol extract of *A. barbadensis* roots on spleen-body weight ratio.**

Groups	Day 1	Day 7	Day 14	P-Value
Control(DW)	0.54±0.07	0.4±0.05	0.52±0.09	NS
Viagra (5mg/kg)	0.49±0.07	0.42±0.08	0.52±0.05	NS
100 mg/kg	0.36±0.01	0.37±0.06	0.43±0.04	NS
200 mg/kg	0.4±0.04	0.38±0.07	0.5±0.05	NS
400 mg/kg	0.4±0.05	0.4±0.06	0.54±0.07	NS
	p>0.05	p>0.05	p>0.05	

DW=Distilled water; All values are expressed as Means±SEM of five rats in each group
NOTE: p>0.05= Not Significant; NS =No Significant difference in days across the rows.

DISCUSSION

In developing plant-based drugs, regulatory authorities do seek data on the toxicity of the drugs. Histology and organ-body weight ratios are among the data in assessing the safety of the drugs. The histological study of the selected organs after examination on the fourteenth day following administration of graded doses of the ethanolic extract of *A.barbadensis* in adult Wistar rats when compared to the controls induced vasoactive effects of active congestion (increased blood flow) and vascular hypertrophy in the assessed vital organs (spleen, liver, heart, kidney and testis). In the liver (Plate 2) and kidney (Plate 4), there was mild activation of the local immune system (follicular activation and lymphocytosis) after the administration of the extract for 14 days. It was also noted that in the heart's blood vessels (Plate 3), viagra induced intimal erosion (injury). These effects can be comparable at all doses, including the

controls. However, the extract's immune-boosting property and its inducement of Leydig cell hypertrophy were optimal at the 400 mg/kg dose group (Plate 5). This implies that the extract increases testosterone production by activating the Leydig cells, which can stimulate spermatogenesis. The findings of the histological assessment of these organs agree with reports in another study of the biochemical indices assessed against the ethanol extract, which indicated the non–toxic effect of the extract at the tested doses (Erhabor and Idu, 2022b).

Alterations in the weight of the internal organs of an organism are a simple and sensitive index of toxicity after exposure to a toxic substance (Tofovic and Jackson, 1999; Teo *et al*., 2002). An earlier report by Moore and Dalley (1999) showed that an increase or decrease in the organ body-weight ratio indicates inflammation or cellular constriction of the respective organ. Following the administration of the extract, there was no significant ($p>0.05$) change in the organ body weight index or ratio of the assessed organs (testis, liver, kidney, heart and spleen) in both treatment and control groups (Tables 1- 5). This implies that the extract did not cause inflammation or cellular constriction in the selected organs. Additionally, there was no swelling or constriction of the hepatocyte of the liver and no inflammation of the kidney's nephrons (Ashafa *et al*., 2009). This finding corroborates earlier published research by Erhabor and Idu (2022b) on the non-toxic effect of the extract on the liver and kidney following the non-significant effect of the extract on the biochemical indices of the said organs. These findings agree with previous studies by Amresh *et al*. (2008) in which the administration of *Cissampelos pareira* produced no effect on the organ-body weight ratio investigated.

CONCLUSION

Exploring histological parameters has widened the toxicological scope of assessing the pre-clinical safety of herbal drugs. In the present study, the histopathological examination revealed the extract was safe at the administered doses. The extract had no significant harmful effect on the spleen, liver, heart, kidney, or testis' functional capacity. It is recommended that the immune-boosting property of the root be further elucidated.

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