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Effects of aqueous methanolic extract of *Bridella ferruginea* stem bark on the spermatogenesis, accessory reproductive organs, and organosomatic indices in pubertal male *Rattus norvegicus*

Mlumun L. Engel, Victor Masekaven Ahur, Solomon Tsekohol Agu *, Veronica Inyamu Ochigbo and Adakole Hyacinth Abu

Department of Veterinary Physiology and Biochemistry, College of Veterinary Medicine, Federal University of Agriculture, Makurdi, Benue State, Nigeria.

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Abstract

There is an increasing trend in the usage of medicinal plants in livestock production with some significantly toxic effects especially to vital organs such as reproductive tissues which necessitates this search. Twenty (20) matured male rats weighing 150-239g randomly assigned into 4 groups (n=5) were used for the sub-acute toxicity. Group 1 rats were treated with distilled water while groups 2-4 were treated with 100, 200, and 400mg/kg respectively of the aqueous methanolic extract of *Bridelia ferruginea* stem bark (AMEBFSB) for 30 days. Animals were terminated on day 31 and some of the reproductive organs were collected and weighed. The Cauda epididymis served as a semen source for sperm analysis and testes were collected for histological examination.

There was a significant decrease ($p<0.05$) in the final body weights, average weights of testes, epididymis, vas deferens, seminal vesicles, and prostate gland of the treated groups as compared to the control. The semen evaluated showed a significant decrease ($p<0.05$) in sperm motility, sperm count, and percent normal sperm cell morphology in the 400mg/kg group, no significant difference ($p<0.05$) in the sperm viability and acrosome integrity as compared to control. Photomicrograph revealed loss and thinning of germinal epithelium and widened seminiferous tubule lumen. In conclusion, the oral administration of AMEBFSB for 30 days in male rats showed that the plant has antispermatogenic effects on reproductive functions.

Keywords: *Bridelia ferruginea*; Spermatogenesis; Reproductive organs; Rats

1. Introduction

Plants have been used globally and from generation to generation as a valuable and safe source of medicines. Humans have not only relied on plants for healing ailments but also on meeting their necessities such as food, shelter, and fuel. Traditional medicine has been and continues to be the main source and method of health care in most developing countries due to the extremely high cost of imported drugs, inadequate modern health care infrastructure, and qualified personnel [1]. As a result, a high percentage of people in developing countries use herbal medicines [2] and this is consistent with the estimation of the World Health Organization (WHO) that about 80% of the world's population relies on medicinal plants for their primary healthcare [3]. In Nigeria, it is part of the culture of the people and it is practiced widely [4].

* Corresponding author: Solomon Tsekohol Agu

Bridelia ferruginea is a shrub or straggly tree of about 15m high with a crooked bole and an up to I.80m girth. It is the commonest *Bridelia* species of the savannah woodland widely distributed in West Africa including Nigeria and throughout the wooded Savannah regions of Africa [5]. *Bridelia ferruginea (Euphorbiaceae)* is extensively employed in Traditional African Medicine in Nigeria. [6] report that other species of *Bridelia* have been studied for their effects as anthelmintic, antiamebic, antianemic, antibacterial, anticonvulsant, antidiabetic, antidiarrhoeal, anti-inflammatory, antimalarial, antinociceptive, antiviral, and abdominal pain, cardiovascular, gynecological related diseases, topical anti-inflammatory, antiarthritic, and antipyretic properties. Decoction of the bark, leaves, and roots are all ingredients in the infusions given to children as a mouth wash and remedy against thrush, and also for dysentery, diarrhea, or as laxatives [7], used for rheumatic pains, anti-inflammatory activity and used for sedimentation of solids in water purification [8]. The stem bark extract is used in the treatment of epilepsy and skin [9], roundworms, and cystitis [10]. Ethnobotanical literature and folklore use describe ulcer-protective and anti-diarrheal effects of the aqueous stem bark of *Bridelia ferruginea* [7], prevention of metabolic syndrome in type 2 diabetes [11]. Several studies have revealed the plant to have astringent, diuretic, febrifuge, laxative, and purgative properties [12].

An experimental study showed that crude extracts of *Bridelia ferruginea* had blood glucose-lowering effects in rats [13]. The efficacy of *Bridelia ferruginea* has been attributed to bioactive compounds such as quinones, gallic, catechin tannins, alkaloids, sterols, polyterpenes, polyphenols, reducing compounds, flavonoids, and saponosides [14]. Other phytochemicals such as alkaloids, anthraquinones, flavonoids, oxalate, phenolic compounds, saponins, sterols, tannins, terpenoids as well as several minerals have been isolated from the bark, fruits, leaves, and roots of *Bridelia* species [15]. Gallocatechin-(4'-O-7)-epigallocatechin, quercetin, myricetin glycosides, bridelone, bridelonine, isoflavone found in some *Bridelia* species may justify their uses against pains in African and Asian traditional medicines [6].

Although many reported therapeutic efficacies of *Bridelia species*, no scientific information exists on its effects on reproductive functions. The objective of this study: effects of aqueous methanolic extract of *Bridella ferruginea* stem bark on the spermatogenesis, accessory reproductive organs, and organosomatic indices in pubertal male *Rattus Norvegicus* were investigated.

2. Material and methods

2.1. Chemicals and Reagents

Formol-saline,10% Buffered formalin, 5% Eosin Y stain, 10% Nigrosin stain, Giemsa stain, Hancock's fixative, Physiological saline, Distilled water, Ketamine, and Absolute alcohol.

2.2. Collection of plant material and crude extract preparation

Fresh stem barks of *Bridelia ferruginea* were collected from the university farm, washed, air-dried, powdered, and stored in an air-tight container. 200 g of the powdered stem bark was soaked in 2000 ml of aqueous methanolic solvent (80% methanol) for 48 hours. This was filtered after 48 hours using a clean Muslin cloth and then with a Whatman number one filter paper. The filtrate was concentrated in a water bath at 45^0C, giving a dark brown coloured extract. The extract was weighed and kept at a temperature of 4 ℃

2.3. Experimental design

Twenty (20) male Wistar rats were purchased from the Animal House, College of Medicine Benue State University, Makurdi, and kept under standard environmental conditions (24–25 °C, 12 h/12 h light/dark cycle) and fed on a pellet diet. Water was given *ad libitum*. They were acclimatized for two (2) weeks before the experiment. The experimental protocol was by the guidelines on the care and well-being of research animals [16] and was approved by the Ethics Committee of the Department of Veterinary Physiology and Biochemistry, University of Agriculture, Makurdi, Benue State, Nigeria. The rats were handled according to the standard protocols for the use of laboratory animals for experiments. The rats were randomly grouped into four groups of five rats post-adaptation and weighed before the start of extract administration.

- Group I was treated with distilled water and served as the control group
- Group II was treated with the AMEBFSB at 100 mg/kg body weight (bwt.)
- Group III was treated with the AMEBFSB at 200 mg/kg bwt.
- Group IV was treated with the AMEBFSB at 400 mg/kg bwt.
- The animals were given the AMEBFSB for 30 days, rats were weighed weekly, and finally on day 31 at the termination of the study.

2.4. Collection and preparation of tissues

About 24 hours after the last treatments, individual rats were euthanized with ketamine at the dosage of 90 mg/kg body weight. Laparotomy extending to the scrotal sac was performed to expose the reproductive tract and excise the accessory reproductive organ and harvest the testes. These organs were exteriorized, blotted free of blood, connective tissues removed, and the organs were weighed using an electric balance scale (C282001, CHINA). Epidydimal sperm was collected by mincing one epididymis into small pieces in 5 ml of physiological saline at room temperature.

For the histopathological studies, the testicular tissues were fixed in Bouin's fluid for 24 h and fixed tissues proceeded for paraffin embedding, staining with hematoxylin–eosin (H&E), and sectioned at 5µ mM thick sections. Microscopic observations were made at 200× magnifications with proper resolution and subsequently made microphotography [17 & 18].

2.5. Organ weights

The testes, epididymides, and accessory sex organs (seminal vesicles, vas deference, and prostate glands) were removed and dissected, and the index weight (I.W.) of the excised organs was computed as follows: I.W. = organ weight (g) /body weight (g) ×100 %, and the average value obtained for each paired organ was considered one observation, with values, reported as g/100 g body weight. Each animal's testis was fixed in Bouin's fluid for histological examination.

2.6. Testis index, testicular coefficient, and gonadosomatic index

The testis index was calculated by dividing the left testis weight by the total b.w. and multiplying it by 100 [19]. The testicular coefficient was calculated by dividing the total organ weight by b.w. and then multiplying it by 100 [20]. Gonadosomatic index (GSI) was calculated by dividing gonadal weight with total b.w. and then multiplying it by 100, where gonadal weight = (weight of the right testis + weight of the left testis)/2 [21]

2.7. Semen analysis

2.7.1. Sperm motility

Determination of cauda epididymal sperm motility was done using the method described by [22]. The individual motility was determined by the formula;

$$\text{Motility (individual) (\%)} = \frac{\text{Number of motile sperm}}{\text{Total number . of sperm (motile+ immotile)}} \times 100$$

2.7.2. Sperm concentration

Sperm count was determined using an improved Neubauer hemocytometer by the method described by [23 & 24]. Epididymal spermatozoa were obtained by the invasive opening of the cauda epididymis and released into a sterile universal specimen bottle, containing 1 ml of normal saline. Briefly, five µl of epididymal fluid was delivered onto a glass slide covered with a 22×22 mm coverslip and examined under the light microscope at a magnification of ×400. The microscopic field was scanned systematically and each spermatozoon encountered was assessed.

2.7.3. Sperm viability test

The viability (percentage of live spermatozoa was determined using eosin nigrosin stain as described by [23 & 25].

$$\text{Viability (\%)} = \frac{\text{Number of viable sperm}}{\text{Total number of sperm (viable + non - viable)}} \times 100$$

2.7.4. Determination of acrosome integrity

The sperm acrosome integrity was determined by the method described by [26]. Acrosome integrity was determined by placing a drop (100 µL) of sperm sample on a clean, grease-free slide and mixed with a single drop of Giemsa stain. The spermatozoa were allowed to interact with the stain for at least 2 min and then a smear was prepared. The prepared smear was air-dried and examined under an oil immersion objective (100× magnification) to determine the percentage of spermatozoa with intact acrosomes.

The spermatozoa that pick the Eosin-Nigrosine stain means Acrosome integrity is compromised or dead. The spermatozoa with intact Acrosome integrity do not pick the stain. The mean results were expressed as percent intact acrosomes.

2.7.5. Sperm morphologies

Sperm morphology was determined by examining air-dried slides under oil immersion as described by [23]. The sperm cells were scored as follows:

Normal morphology: sperms with normal head and tail. Abnormal morphology: sperm cells with isolated heads – misshapen head or not; head misshapen head with abnormal tail and fused sperm. The percentage of abnormal forms was evaluated; Normal semen has fewer than 30 % of abnormal forms [27].

2.8. Statistical Analysis

Results are expressed as mean ± standard deviation (Mean ± SD). The data were analyzed by one-way analysis of variance (ANOVA). The mean was considered to be significant at $P < 0.05$ and was separated using Duncan Multiple Range Test. All statistical analysis was carried out using the Graph Pad Prism version 8.01 for Windows**.**

3. Results

3.1. Effects of AMEBFSB on the body and relative organ weights of male albino rats.

The results of the effect of oral administration of AMEBFSB on initial and final body weights, average weights of testes, epididymis, vas deferens, seminal vesicles, and prostate glands of male albino rats after 30 days of treatment are presented in Table 4. There was a significant decrease in the final body weights of the treated groups relative to the control. There was a significant decrease ($p<0.05$) in the average weights of testes, epididymis, vas deferens, seminal vesicles, and prostate gland in the treated groups when compared with the control, treated groups especially group 1 and group 2 did not differ much between themselves.

Table 1 Effect of AMEBFSB on body and relative organ weights of male Wistar rats

Dose (mg/kg)	Weights (g)										
	Bodyweight		Testes		Epid*.	Vas d**	S. V***.	Pros****.	Test Ind	Test Coef	Gonad Ind
	Initial	Final	Left	Right							
Control	175± 11.5	221±16.2	1.82±0.7	1.57±0.7	0.50±0.6^{a}	0.12± 0.03^{a}	1.17 ±0.1^{a}	0.12± 0.01^{a}	0.82±0.10	1.53±0.01	1.70±0.12
100	183± 14.1	164± 8.72^{a}	1.5±0.04^{a}	1.4± 0.02^{a}	0.40±0.03^{b}	0.05±0.01^{b}	0.49±0.11^{b}	0.05± 0.01^{b}	0.89±1.10^{a}	1.77±0.03^{a}	1.45±0.03^{a}
200	173± 11.9	180± 12.0^{b}	1.3± 0.06^{a}	1.29± 0.06^{b}	0.40±0.01^{b}	0.07± 0.01^{b}	0.65±0.11^{b}	0.06± 0.01^{b}	0.72±0.10^{b}	1.44±0.02^{b}	1.30±0.01^{b}
400	189± 17.9	131± 16.6^{c}	1.25±0.1^{a}	1.19±0.0^{b}	0.27±0.0^{b}	0.04± 0.01^{c}	0.26±0.05^{c}	0.07± 0.00^{b}	0.95±0.70^{c}	1.86±0.01^{c}	1.22±0.02^{b}

Values are mean ± SEM. n=5. Values with different alphabet superscripts on the same column are significantly ($p< 0.05$) different

Key: Epid* = Epididymis, Vas d** =Vas deferens, S.V***= Seminal Vesicles, Pros****=Prostate, Test Ind= Testes index, Test Coef= Testicular Coefficient, Gonad Ind= Gonadosomatic index

3.2. Effects of AMEBFSB on sperm characteristics of the male albino rats.

The results of sperm motility, viability, sperm concentration, sperm morphology, and acrosome integrity after 30 days of treatment with the AMEBFSB are shown in Table 1. The extract significantly ($p<0.05$) decreased sperm motility in a dose-dependent manner in all the treated groups and sperm morphology only in the 400 mg/kg group as compared with the control. There was no significant difference ($p<0.05$) in viability and acrosome integrity of the treated groups as compared with the control, treated groups also did not differ between themselves.

Table 2 Effect of AMEBFSB on sperm motility, viability, concentration, morphology, and acrosome integrity

Dose (mg/kg)	Sperm Motility (%)	Viability (%)		Sperm Conc*. ($x10^6$/ml)	Morphology (%)		Acrosome Integrity (%)	
		Live Sperm	Dead Sperm		Normal	Abnormal	Intact	Not Intact
Cont.	82.0±4.01[a]	125±6.12	74.8±6.12	71.4±8.72[a]	111±3.57[a]	89.3±3.58[a]	121±2.11	78.8±2.11
100	38.4±4.06[b]	132±12.2	68.4±12.2	50.8±11.11[b]	123.2±2.95	76.8±2.95	124±2.16	75.6±3.56
200	27.5±5.95[c]	127±5.43	73.0±5.43	49.3±5.83[b]	122±13.2	65.7±5.47	122±4.68	78.7±4.68
400	22.5±4.31[c]	122±6.10	78.3±6.10	46.3±6.11[b]	90±7.2[b]	123±12.7[b]	119±4.41	71.3±4.11

Values are mean ± SEM. n=5. Values with different alphabet superscripts on the same column are significantly ($p<0.05$) different.

Key: Conc = Concentration, % = Percentage

3.3. The photomicrograph of testes of male albino rats that were administered AMEBFSB orally for 30 days in treatment groups and control

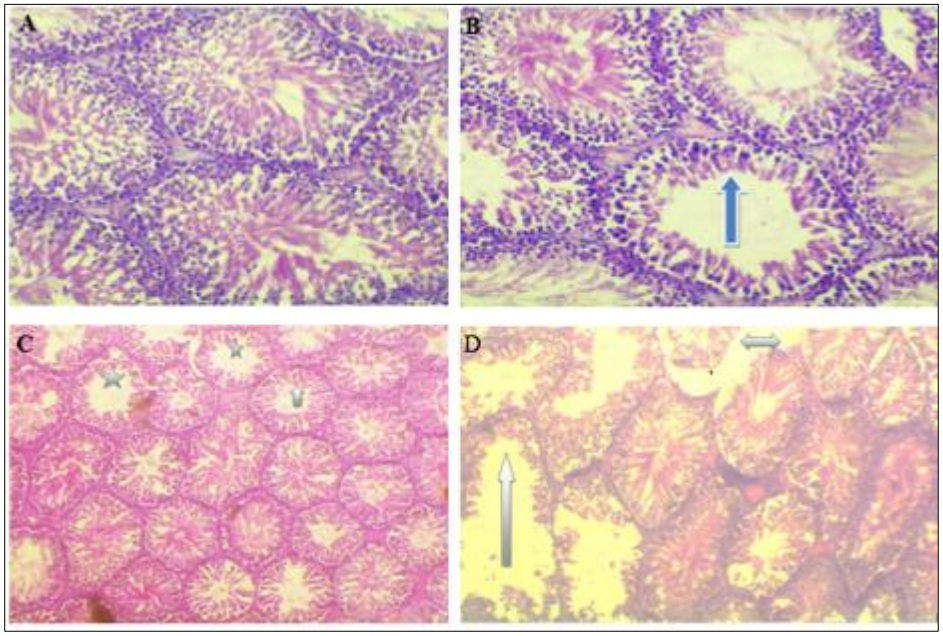

Figure 1 (A): Section through the Testis of control rat showing normal seminiferous tubules with copious spermatozoa with the normal lumens of seminiferous tubules & germinal layers, and normal Interstitium H&E, 100X. (B) Showed seminiferous tubules with a level of degeneration in the stratified epithelium and scanty spermatogenic cells (arrowed). (c) shows seminiferous tubules with less dense lumen, lumen with no sperm cells seen and thinning basal lamina membrane, (D) showed some seminiferous tubules with basal lamina separated from the luminal cellular compartments (double arrow) and some with degenerated germinal epithelium with no sperm cells seen in the lumen (white arrowed)

4. Discussion

Following AMEBFSB administration, there was a significant decrease ($p<0.05$) in the final body weights of the treated animals as compared to the control, especially in group 4. In the present study, lower dosages reduced the body weights slightly as seen in the 100 and 200 mg/kg body weight dosage but at a higher dosage of 400mg/kg body weight, the weight drop was so remarkable. This finding is in contrast with [10], who reported that the aqueous extract of *Bridelia ferruginea* increased body weights in all the treated groups as compared to the control group, smaller weight increase at lower doses, and increased weights at higher doses were observed. The works of [28] on *Ficus deltoidea* leaf extract disagree with this finding. These variations may be due to season, nutrition, etc. Further studies need to be done on this activity to explore and also to determine the influence of dosage on weight.

A sensitive parameter for the detection of any adverse effects of a substance on the male reproductive system is the weight of reproductive organs. This also provides information on the general health of the animal [28]. In this study, and there was a decrease in the weights of the right and left testes as compared to the control, treated groups did not differ with themselves, there was no significant decrease seen in the weights of the epididymis in the treated except at 400mg/kg as compared to control. The testicular is an indication of a possible shift in androgen status. The GSI, which represents the percentage of body mass that corresponds to the testis and it is a variable used to measure the seminiferous tubules in conjunction with body mass [30] showed significant decrease values in dose-dependent manner. This is probably due to the decrease of body weight in the treated groups relative to the control. A reduced testicular weight and GSI (a better way to assess the damage to the testes in relation to the body) in experimental rats is most likely due to decreased levels of male sex hormones and androgen especially plays a pivotal role in sex organs[31]. GSI is inversely proportional to the reproductive efficiency of the rats [32] and a pertinent value to be accessed in reproductive studies. When compared to the treated groups, the GSI was higher in the control group, suggesting a reduction in the animals' commitment to spermatic production after receiving the concentrated extract. Testis organ coefficient (TC) is a ratio of the weight of the testis per 100 g of body weight [33], which is a frequent observation in sub-acute and sub-chronic toxicity studies. Following the administration of AMEBFSB, it was found that the organ coefficient in treated groups was significantly higher than that in the control. A cause of the drop in the weight of the testes, TC, and GSI that needs to be explored is suppressed spermatogenesis. It has been shown that a strong correlation exists between testis weight, TC, and function (spermatogenesis) in mammals. In the absence of any known pathology, testis weight is highly related to daily sperm production [34].

The weights of the vas deferens were significantly different ($p<0.05$) to the control only in group 4, weight of seminal vesicles significantly decreased only in group 4 as compared to the control. There was no significant difference between the treated groups and control for the prostrate [35] reported findings on weights of reproductive organs treated with extracts of *Garcinia kola* in male rats which are similar to the results in this study except that in his findings the reduction in weights was in a dose-dependent manner. Extracts of *Polygala rosmarinifolia* were reported in male albino rats to relatively decrease testes' weight and epididymis weight which agrees with this study [36]. Previous investigations have demonstrated that AMEBFSB was found to cause a non-significant increase in the weights of organs which disagrees with this study [10]. Additionally, the report of [28] in a similar study on *Ficus deltoidea leaf* extract is also in conflict with this current study.

The epididymis is recognized to be in charge of the transportation, concentration, storage, maturation, and protection of the spermatozoa produced by the testes [37].

Total sperm count, motile sperm count, and normal sperm morphologic features have been reported as indices of fertility in males [38]. This study demonstrates that AMEBFSB administration induced epididymal and testicular dysfunction. The marked decrease in functional sperm parameters specifically: decreases in sperm motility and count signify the adverse effect of AMEBFSB on the spermatozoa and epididymal milieu in a dose-dependent manner when compared to the control. Male rats treated with the hydro-alcoholic extract of *Astragalu ovinus* also showed a similar significant diminution in epididymal sperm motility which agrees with this finding [39]. For sperm viability, there was no statistical difference between the treated groups and the control. There was a significant decrease ($p<0.05$) in the sperm concentration of the treated groups as compared to the control, and this aligns with the works of [40] on this same plant. The findings here also agree with the work of [15] on this plant with respect to sperm count and varied slightly as they reported that changes in motility and morphological aberration were insignificant. [41] Corroborate this finding using the same plant with respect to sperm motility, sperm count, and sperm cell morphology. *Garcinia kola* extracts were found to markedly reduced sperm concentration, motility, and normal spermatozoa when used in male rats which is a similar trend in this study [35]. Comparing the normal morphology to the control in the present study, there was a significant decrease ($p<0.05$) in the 400 mg/kg dosage group (group 4) as seen in Table 2. [42] Worked on *Jathropha* spp and reported an increase in abnormal sperm morphology only at 400 mg/kg not lower dosages which

also agrees with this finding. There was no statistical difference ($p<0.05$) in the acrosome integrity of sperm cells in all the groups.

The reduced weights of testes, epididymis, prostate, and seminal vesicles may have led to the observed reduced epididymal sperm functions and suggest destruction or deleterious effects of the extract on the gonads [35]. Decreased Sperm motility may be attributed to the production of Reactive Oxygen species (ROS) and lipid peroxidation caused by the extract [10 & 43]. The production of highly reactive oxygen species (ROS) induces oxidative stress, which plays a fundamental role in the pathogenesis of many physiological disorders, including cell injury. Decreased sperm count may be due to dilute sperm entering the epididymis caused by low estrogen levels [41] which directly suppress gonadal testosterone levels [10] and the cytotoxicity caused by the natural compounds extracted from *Bridelia ferruginea*. Decreased normal sperm morphology at 400 mg/kg may be due to high lipid peroxidation which leads to the destruction of cell membrane structure [10].

There was no significant decrease ($p<0.05$) in the viability and acrosome integrity across the groups when compared to the control. Works did on *croton spp* (euphorbiaceace) by [44], a plant from the same family as the present plant, revealed the pro fertility effects of flavonoids, this phytochemical is also identified in this plant, this may be responsible for the no significant difference in all the groups for sperm viability as an index of fertility. [45 & 46] reported that alkaloids, flavonoids, and phenols have an androgenic property while steroids, glycosides, and tannins have antifertility effects, all these phytochemicals were identified in the present plant, and these may have a synergistic additive effect seen on the viability and acrosome integrity of the sperm. [47] inferred that the synergistic effect of phytochemicals resides in medicinal plants. The adverse effects on sperm indices observed in the present study on some sperm indices as well as that reported earlier [10] suggest a possible antispermatogenic property of this extract.

Photomicrographs from the testes from group 1 (control) showed normal and intact germinal epithelium with about four layers of spermatogenic cells; spermatogonia, spermatocyte, and spermatids, the basal lamina is intact and the lumen is seen with sperm cells. The testes of rats in group 2 (100 mg/kg AMEBFSB) showed seminiferous tubules with a level of degeneration in the stratified epithelium and scanty spermatogenic cells (arrowed) as compared to the control. It can be inferred that the changes that were seen in the lumen and basal membrane of some seminiferous tubules are caused by the extract. Similarly, rat testes administered AMEBFSB 200 mg/kg (group 3) shows seminiferous tubules with less dense lumen, lumen with no sperm cells seen and thinning basal lamina membrane, and the group 4 rats administered AMEBFSB 400 mg/kg (group 4) showed some seminiferous tubules with basal lamina separated from the luminal cellular compartments (double arrow) and some with degenerated germinal epithelium with no sperm cells seen in the lumen (white arrowed). From these histological observations, it can be said that this plant extract can cause damage to the histo-architecture of the testes. [10] been the only author that has worked on this plant on semen analysis so far also reported that the plant extract has the potential to cause lipid peroxidation, and by extrapolation, damage the architecture of the tubules of the seminiferous tubules thereby agreeing with the findings of this study. This is the first work that is recording histological changes in the testes of rats administered this plant. The works by [48] on rat testes administered with seeds of *Momodora myristica* corroborate this finding. Extracts of the bark of *Carica papaya* on the rat testes caused histological changes ranging from seminiferous tubular distortion to destruction/ degeneration of the seminiferous tubules which is a similar finding in the present study [49].

5. Conclusion

This study has established that the oral administration of aqueous methanolic extract of *Bridelia ferruginea* stem bark for 30 days shows the antispermatogenic properties of the plant in male rats. There is a need for further study on the quantitative screening of *Bridelia ferruginea* stem bark to identify the active principles responsible for the antispermatogenic property.

Compliance with ethical standards

Acknowledgments

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Disclosure of conflict of interest

The authors declare that they have no conflict of interest.

Statement of ethical approval

Experimental rats were cared for according to standard international [16] as approved by the Department of Veterinary Physiology and Biochemistry animal ethical committee of the College of Veterinary Medicine, University of Agriculture, Makurdi, Benue State, Nigeria.

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