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Original Research Article

Effect of Ethanol Extract of *Ficus thonningii* on Sperm Parameters and Testicular Morphology in Amlodipine-Induced Toxicity in Wistar Rats

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Abstract

The histology of the testis and sperm parameters of Wistar rats was investigated with a view to determining the potential of *Ficus thonningii* ethanol leaf extract in correcting the testicular damage caused by high dosage of Amlodipine. Five groups of Wistar rats were used for the study. They included, the control that received sterile water (vehicle), 0.2 ml/ 100 g rat; a drug-only group which received a dosage of 0.07 mg/kg, p.o.; a drug and low dose extract group that received 0.07 mg/kg, p.o. and extract of 300 mg/kg, p.o.; a drug and high dose extract group that received 0.07 mg/kg, p.o. and extract of 400 mg/kg, p.o.; and an extract-only group which received 300 mg/kg, p.o. of extract, all for a duration of 64 days (8 weeks). The values obtained from the control and treatment groups were compared statistically using Analysis of Variance (ANOVA) of the Statistical Package for Social Sciences (SPSS). Results showed a statistically significant (p<0.05) difference relative to the control group for sperm parameters. In conclusion, ethanol leaf extract of *Ficus thonningii* had ameliorative and prophylactic abilities to counter damage induced on the testis by Amlodipine when the extract was administered at both low and high doses in test animal subjects.

Keywords: Ficus thonningii, Ethanol extract, Amlodipine, Sperm parameters, Testicular damage.

INTRODUCTION

Ficus thonningii, commonly known as Blume Moraceae, known by the TIV people of Benue State Nigeria as "AKINDE" a fig of great nutritional value, is very rich in proteins and minerals (Dangarembizi *et al*., 2013). It has been used locally for the treatment of infertility by the TIVs (Shomkegh *et al*., 2016). It has also been extensively used in African ethnomedicine for the treatment of a number of disease conditions, which include diarrhea, urinary tract infections, diabetes mellitus, gonorrhea, respiratory infections and mental illnesses (Dangarembizi *et al.*, 2013).

The fertility enhancing capacity of the plant extract has been reported in numerous studies. In recent times, paramount attention is being shifted from synthetic drugs to natural products. Some wild herbs and spices have been shown to be most effective, relatively non-toxic, and have substantial scientific documentation attest to their efficacy in infertility management (Saalu *et al.,* 2013). The specific objectives of the research were: to determine the effect of ethanol leaf extract of *Ficus thonningii* on sperm parameters in rats; to evaluate the effect of Amlodipine on sperm parameters in rats; and to examine the effect of the extract on testosterone concentration and testicular histo-architecture of rats.

MATERIALS AND METHODS

Plants Collection and Extraction

The plant material was obtained at a village near Judges' Quarters in Makurdi Local Government Area of Benue State, North Central Nigeria, in the Month of April, 2020, and was taken to the herbarium of the Department of Biological Sciences, Benue State University, for authentication. The extraction was carried out using 300 g of pulverised leaves of *Ficus thonningii* in 800 ml of absolute ethanol for about 72 h.

The filtrate was concentrated to dry residue *in vacuo* in a rotary evaporator under reduced pressure at 40 °C. The extract generated was stored in the refrigerator at 4 °C until required for investigation.

Animals

Twenty (24) adult male Wistar rats with average weight of 200 g were used for the experiment. Acclimatisation was done prior to the experiment on the animals. They were divided into five groups (n=5), except the fifth group which had four rats in total. They were obtained and maintained in the animal facility of the Department of Anatomy, College of Health Sciences, Benue State University, Makurdi. The animals were fed with finisher's mash procured from Vital Feeds Limited, Makurdi, Benue State, Nigeria. They were allowed access to drinkable water placed in troughs within the cages.

Drug and Extract Administration

The drug used for the research was procured at HESERA Pharmacy, located at Terwase Agbadu junction, along George Akume Way, Makurdi, Benue State, North Central Nigeria. It was manufactured by Pfizer Pharmaceuticals Inc., New York, USA. The drug constitutes 5 mg/kg of Amlodipine according to the pharmaceutical specifications. The drug was reconstituted to 0.07 mg/kg by diluting with appropriate ratio of sterile water.

The rats in the treated groups were administered drug and extract thus: Group 2 received Amlodipine 5 mg/70 kg (0.07 mg/200 g) given orally, once per day for 64 days (8 weeks).

Group 3 received *Ficus thonningii* extract of 300 mg/kg (60 mg/200 g) for 64 days by oral gavage.

Group 4 received both Amlodipine and *Ficus thonningii* extract at a dose of 5 mg/70 kg (0.07 mg/200 g) of Amlodipine given orally once a day, and 300 mg/kg (60 mg/200 g) extract of *Ficus thonningii* given *via* oral gavage once per day for 64 days.

Group five received Amlodipine and *Ficus thonningii* extract at a dose of 5 mg/70 kg (0.07 mg/200 g) of Amlodipine and 400 mg/kg (80 mg/200 g) extract all through the oral route.

Animal Sacrifice and Sample Collection

Each rat at the time of sacrifice was weighed then anaesthetised by placing them in a closed container, containing cotton wool sucked in chloroform anaesthesia. The abdominal cavity was then opened up through a midline abdominal incision to expose the reproductive organs. Then the testes were excised and trimmed of all fat. The testes and epididymis from the rats were carefully dissected out, and the testes were carefully removed, trimming them of epididymides and adjoining tissues.

The samples were then used for experimental analysis. The values obtained from the control and treatment groups were recorded and compared statistically using Analysis of Variance (ANOVA) Test of the Statistical Package for Social Sciences (SPSS).

RESULTS

The findings from the research revealed the following:

Acute Toxicity and LD_{50}

The oral median lethal dose (LD_{50}: >3000 mg/kg, p.o.) of *Ficus thonningii* leaf extract was found to be relatively non-toxic and safe in animal subjects (Aniagu *et al.,* 2008).

Sperm Count

The sperm count of the drug-only group (2) was the lowest, (17.33±11.75) showing damage, and the other experimental groups have shown improvement when treated with extract of *Ficus thonningii*. The positive control group (2) has a statistical mean value of (28.80±3.22) which shows a statistical significant improvement relative to the drug only group (2) $P<0.05$. The low dose extracts group (4) has statistical mean value of (23.20±5.67) which also shows improvement over group (2). The high dose extracts group with statistical mean value of (34.80±4.91) massively improved relative to the drug only group $P<0.05$. And although the statistical mean values did not improve past the negative control group (1), they did improve significantly from the damage caused by Amlodipine when they were counter treated with extracts of *Ficus thonningii* $P<0.05$.

Testosterone Concentration

The testosterone concentration of the damage group was lowest, statistically (1.92 ± 0.12) across all groups. That of the positive control group (3) was highest, (2.41 ± 0.09*) and significantly improved relative to group (1), the control group with statistical mean value of (2.08 ± 0.06). The low dose group improved over both the drug only group and the control group, with the mean value of (2.24 ± 0.05) although the improvement was not significant relative to group 1, it was statistically significant at $P<0.05$ relative to the drug-only group. The high dose extracts group significantly improved relative to the control group and the drug only group with statistical mean value of (2.27 ±0.02*) at $P<0.05$.

Sperm Morphology

From table 1 above, the mean values of the normal sperm, that is sperm with normal morphology, improved for positive control group, that was treated with only *Ficus thonningii* extract (300 mg/kg, p.o.) which is group (3), with the value (87.96±8.11) over the control group, with mean value (85.63±6.15) and was

also a massive improvement over group (2), administered with only the damage inducing drug, Amlodipine (0.07 mg/kg, p.o.) with mean value (77.23±17.40). Both groups (4) and (5) that received 0.07 mg/kg, p.o. of drug and a low and high dose of *Ficus thonningii* of 300 mg/kg, p.o. and 400 mg/kg, p.o. respectively, also improved over the control group, and the group induced with damage, with mean values of (86.26±1.85) and (98.23±1.91). The result shows that the damage had occurred, since the damaged group has the lowest mean normal value (77.23±17.40).

Sperm Progressivity

The progressivity did not quite improve for the experimental groups except the fifth, which was the high dose extract group with the mean value of (3.66±0.58), the highest mean progressive value across all groups. Evidence of damage occurred in progressivity also, as the group (2) was lower (2.66±0.57) when compared with control group (3.33±0.57).

Sperm Motility

The sperm motility of the group (2) drug-only-group was lowest statistically, (68.47±14.00) across all groups. The positive control group that received only the *Ficus thonningii* extract with the mean value of (70.97±12.65) appeared to be an improvement over the damaged group (2). The high dose extract group improved both on the drug-only group (2) and the control group (1) with statistical mean value of (94.00±4.16). This was statistically significant at $P<0.05$.

Table 1: Sperm Parameters and Testicular Morphology of Experimental Wistar Rats

Treatment Group	% Normal	Progressivity	Motility	Count x10^6	Testicular Weight (mg)	Testosterone Level
1	85.63±6.15	3.33±0.57	82.53±8.16	36.20±8.30	1.67±0.15	2.08 ± 0.06
2	77.23±17.40	2.66±0.57	68.47±14.00	17.33±11.75*	1.43±0.15	1.92 ± 0.12
3	87.96±8.11	2.66±0.57	70.97±12.65	28.80±3.22#	1.77±0.25	2.41 ± 0.09*#
4	86.26±1.85	2.33±0.57	66.60±5.45	23.20±5.67	1.67±0.06	2.24 ± 0.05#
5	98.23±1.91#	3.66±0.58#	94.00±4.16#	34.80±4.91#	1.67±0.15	2.27 ±0.02*#

Mean and Standard Deviation of various sperm parameters in Wistar rats. Asterisk was used to indicate significant difference between control and experimental groups taken at ($P<0.05$), while # represented statistically significant difference relative to group two (2) connective tissues containing Leydig cells and Sertoli cells.

Sections obtained from rats in group two showed a marked de-population of spermatogenic cells within the seminiferous tubules and a moderate tissue distortion.

Sections obtained from rats in group three also showed normal histological architecture of the testis. The sections showed numerous spermatogenic cells and numerous matured sperm cells.

Sections obtained from rats in group four showed moderate de-population of spermatogenic cells; a visible distortion of the cells.

Sections obtained from rats in group five showed improved population of mature sperm cells, normal arrangement of the seminiferous tubules and spermatocytes. There is also a normal appearance of the interstitial connective tissues carrying the Leydig cells.

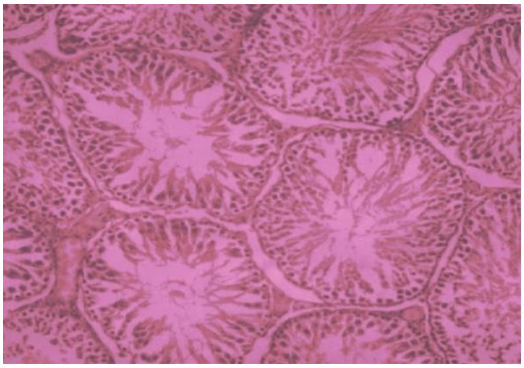

Plate 1: Testicular section of the control group showing the Lumen L; Normal seminiferous tubules ST; Spermatogenic Cells S; Interstitial Connective Tissue carrying Leydig Cells X. Magnification x100. Stain H&E.

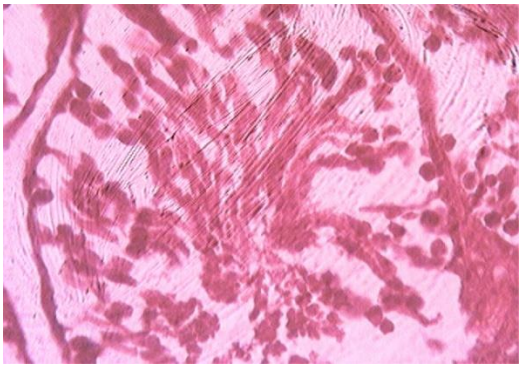

Plate 2: Testicular section of the control group showing Normal seminiferous tubules ST; Spermatogenic Cells S; Interstitial Connective Tissue carrying Leydig Cells X. Magnification x400. Stain H&E.

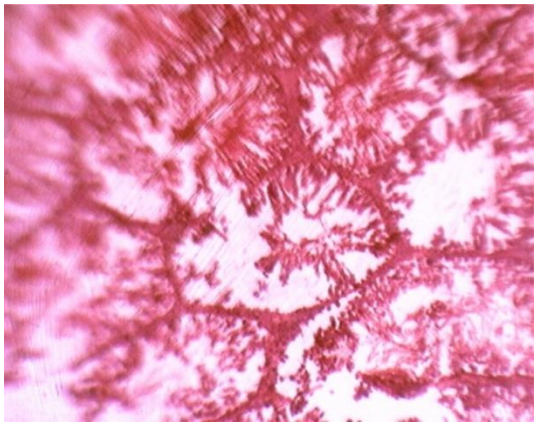

Plate 3: Testicular sections from group two (2) showing marked depopulation of the spermatocytes S within the seminiferous tubules ST and a visible tissue distortion markedly across the interstitial connective tissue X. Magnification x100. Stain H&E.

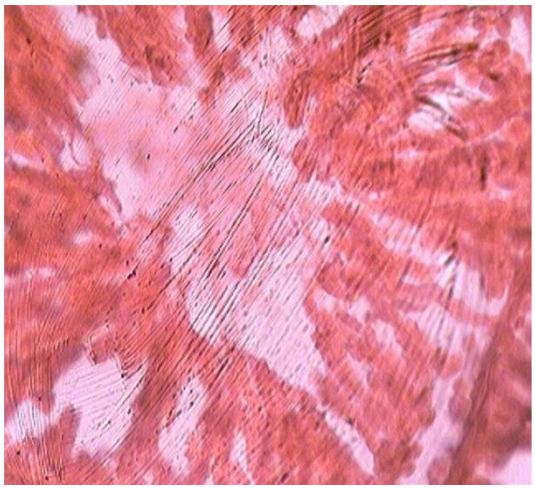

Plate 4: Testicular sections from group two (2) showing marked depopulation of the spermatocytes S within the seminiferous tubules ST and a visible tissue distortion markedly along the interstitial connective tissue X. Magnification x400. Stain H&E.

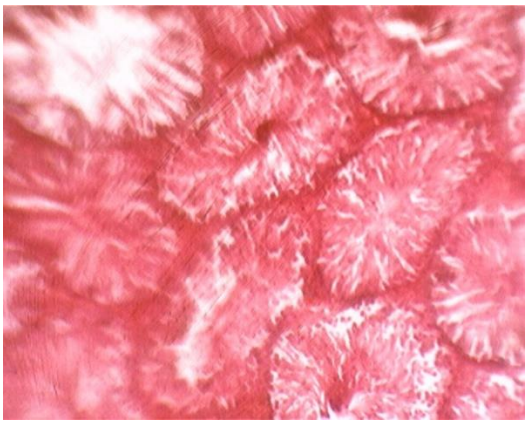

Plate 5: Testicular section of group three (3) showing normal appearance of testicular histology with normal seminiferous tubules ST, a very populated spermatogenic cells S within the seminiferous tubules and normal interstitial connective tissue carrying Leyding cells X. Magnification x100. Stain H&E.

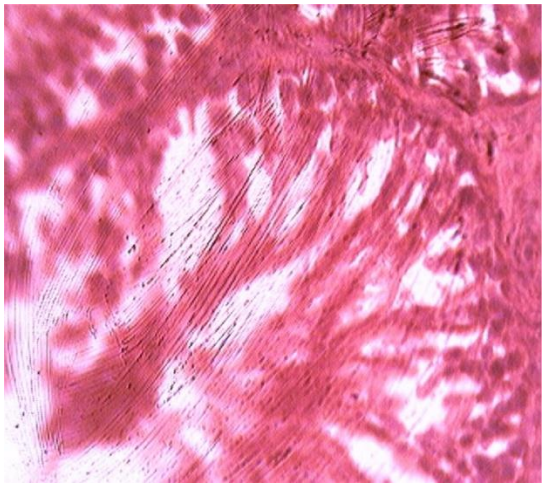

Plate 6: Testicular section of group three (3) showing normal appearance of testicular histology with normal seminiferous tubules ST, a very populated spermatogenic cells S within the seminiferous tubules and normal interstitial connective tissue carrying Leyding cells X. Magnification x400. Stain H&E.

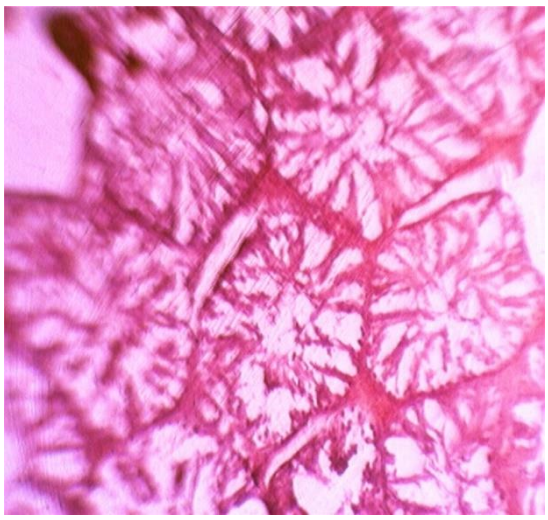

Plate 7: Testicular sections of group four (4) showing moderate depopulation of the spermatogenic cells S within the seminiferous tubules ST and a visible cellular distortion. Magnification x 100. Stain H&E.

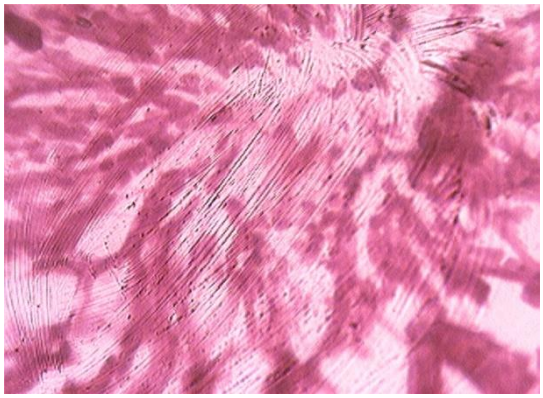

Plate 8: Testicular sections of group four (4) showing moderate depopulation of the spermatogenic cells S within the seminiferous tubules ST and a visible cellular distortion. Magnification x400. Stain H&E.

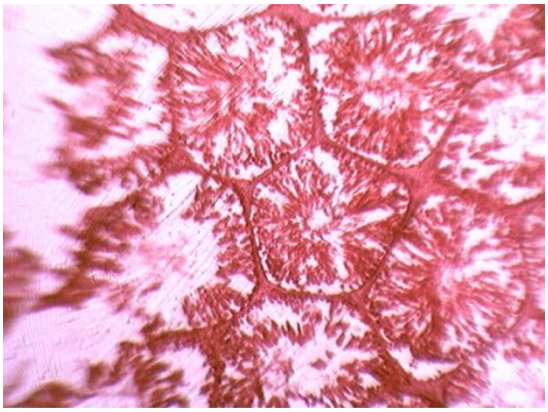

Plate 9: Testicular sections of group five (5) showing a significant improvement in the population of spermatogenic cells S, improved population of mature sperm cells in the lumen L a normal appearance of the interstitial connective tissue carrying Leydig cells X . Magnification x 100. Stain H&E.

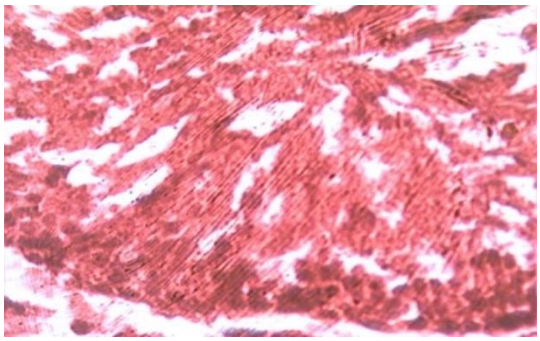

Plate 10: Testicular sections of group five (5) showing a significant improvement in the population of spermatogenic cells S, improved population of mature sperm cells in the lumen L a normal appearance of the interstitial connective tissue carrying Leydig cells X . Magnification x400. Stain H&E.

DISCUSSIONS

The study examined the effects of ethanol leaf extract of *Ficus thonningii* on sperm parameters and testicular morphology in amlodipine-induced toxicity in Wistar rats, with a view to determining the potential of *Ficus thonningii* ethanol leaf extract in correcting the testicular damage caused by high dosage of Amlodipine, a calcium channel receptor antagonist.

The results obtained from the research as displayed in the table showed that treatment of Wistar rats with Amlodipine caused testicular damage, and co-administration with ethanol extract of *Ficus thonningii* leaves caused changes in the sperm parameters, histological profile, and hormonal profile relative to control animals. Observations made are hereby discussed below.

From the results that were obtained from semen analysis, the drug used to induce testicular damage was effective for the purpose, when treated groups were compared with the control. These changes were observed from the mean values for each sperm parameter: sperm count of rats treated with Amlodipine alone was 17.33±11.75 as against 36.20±8.30 recorded in the control group. Sperm motility recorded for drug-treated group was 68.47±14.00 as against 82.53±8.16 recorded for the control animals. Sperm progressivity was 2.66±0.57 as against 3.33±0.57 recorded in the control. Sperm morphology was 77.23±17.40 as against 85.63±6.15 recorded in the control. The marked reduction in the mean values of the various sperm parameters recorded from the rats treated with only-the-drug is in agreement with works of other researchers such as (Adesanya *et al*., 2012) and (Dena and Andrienne, 2019) which suggests that calcium channel blockers of which Amlodipine is one of them, contribute to low sperm count, in that calcium influx is an absolute requirement for physiological acrosome reaction in sperm in both invertebrates and mammals. Calcium channel blockers act by changing the cholesterol content of the sperm cell membrane, and this makes sperm cells non-functional. Calcium channel antagonists may double male contraceptive, in that long-term treatment with them might be associated with significant testicular regression and reduction in serum testosterone. Calcium channel blockers have also been shown to suppress spermatogenesis. These actions of calcium channels antagonists do not favour the testis and the process of spermatogenesis, hence the negative results produced from the group that was treated with only-the-drug.

The results obtained from the research as well as that found from other researchers agree that, treatment with high dose of Amlodipine causes toxicity to the testis and its attendant negative impact on the quality of sperm. However, co-administration with ethanol leaf extract of *Ficus thonningii* markedly improved the quality of the sperm at both low and high doses in the animals treated with the extract. This demonstrates the capacity of the extract to correct the damage caused by the drug. According to Grassie *et al*., 2010, orientin, vitexin and isovitexin which are flavonoids isolated from *Ficus thonningii* are good antioxidants which scavenge and reduce free radical formation, especially orientin. The antioxidant properties of these *glucoysl flavonoids* may have been responsible for the ameliorative potential shown by the ethanol leaf extract of the plant. According to Olas *et al.*, 2008, apart from the flavonoids antioxidant activity, the stibens present in *Ficus thonningii* also exhibit antioxidant activity, and also, resveratrol and its methylated derivative, trans-3.3', 5.5' tetrahydroxystibene, possess antioxidant effects against oxidative stress induced by reactive nitrogen species and reactive oxygen species. According to Ahur *et al.*, 2010, due to the activity of antioxidants isolated from ethanol leaf extract of *Ficus thonningii*, which antagonise the depletion of glutathione, prevention of free radical species generation as a result of oxidative

stress, is achieved. This further explains the ameliorative potentials shown by the extract obtained from the ethanol leaf extract of *Ficus thonningii.* The result corroborates the findings of other researchers such as Saalu *et al.,* 2013, Victoria *et al.,* 2018 and Naghdi *et al.,* 2016, who have also investigated other phytochemical constituents of other plants as remedy against free radical species (e.g., hydrogen peroxide, superoxide anion and hydroxyl radical).

In the results obtained from the profile of serum testosterone of the Wistar rats, there was a marked negative impact of Amlodipine on serum testosterone level relative to the control animals. That is 1.92±0.12 of the drugs-only- group as against 2.08±0.06 of the control group. The result is in concordance with the findings of other researchers, such as Dena & Adrienne, 2019 and Adesanya *et al.,* 2012, which suggest that calcium channel antagonists, such as Amlodipine may double as male contraceptive, in that long-term treatment with them might be associated with significant testicular regression and reduction in serum testosterone. The high dosage of Amlodipine used in the experimental animals corroborates these findings.

However, the animals treated with both the low and high doses of the ethanol leaf extract of *Ficus thonningii* have shown a marked increase in the serum testosterone levels relative to Amlodipine- treated group. That is, 2.08±0.06 and 2.27±0.02* of low and high doses respectively, as against 1.92±0.12 of Amlodipine treated group, the high dose group significantly ($P \leq 0.05$) increased over the Amlodipine-treated group.

The rats in group 3 which were treated with only the ethanol leaf extract of *Ficus thonningii* also significantly ($P < 0.05$) increased in serum testosterone levels when compared with both the control and the Amlodipine-treated group. That is, 2.41±0.09* recorded in group 3 as against 2.08±0.06 and 1.92±0.12 recorded in the control and Amlodipine- treated groups respectively.

Sections obtained from the histological profile across all groups were observed to have the following changes from different treated groups. Although, the control group plate showed normal histological arrangements of the testis with the seminiferous tubules, spermatocytes, interstitial connective tissue carrying Leyding cells appearing normal, the plate from Amlodipine-treated group demonstrated significant de-population of the spermatocytes within the seminiferous tubules relative to control. The low dose extract group showed only a moderate de-population of the spermatocytes showing the ameliorative potentials of the extract. The high dose extract group improved markedly on the histological arrangements within the seminiferous tubules, appearing almost the same as the control, although, there are visible cellular distortions which may have occurred from the damage by Amlodipine, but were corrected by the extract of *Ficus thonningii*. The rats treated with only the ethanol leaf extract of *Ficus thonningii* showed sections with normal arrangement of testicular histology. It showed numerous spermatocytes and mature sperm cells within the seminiferous tubules. Also, there is normal interstitial connective tissue arrangement. The results from this also, can be attributed to the antioxidant activity of the flavonoids and other phytochemical constituents isolated from *Ficus thonningii*, which antagonise the action of free radicals (reactive oxygen species) caused by oxidative stress, which may have been induced by the high dosage of Amlodipine administered to the animals, according to Ahur *et al.,* 2010.

CONCLUSION

The study concluded that the ethanol leaf extract of *Ficus thonningii* has ameliorative, mitigating and prophylactic potentials to counter the damaging effect induced on the testis by Amlodipine when the extract was administered at doses of 300 mg/kg and 400 mg/kg orally. It also improved the testicular histo-architecture and sperm parameters when only 300 mg/kg, p.o. of the extract was administered. This was probably due to the bioactive constituents of *Ficus thonningii*, which scavenge and antagonise the action of reactive oxygen species.

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COMPETING INTERESTS

Authors have no conflicts of interest to disclose.

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