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Histological Changes in Gonads and Liver of *Oreochromis niloticus* (L.) Fed Crude Extract of *Azadirachta indica* Leaf

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Authors' contributions

This work was jointly carried out in collaboration between the two authors. Author IOO managed the analyses of the study, managed the literature searches and performed the statistical analysis while author GCN designed the study, wrote the protocol, wrote the first draft of the manuscript. Both authors read and approved the final manuscript.

Original Research Article

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ABSTRACT

Structural changes in the gonads and liver of *Oreochromis niloticus* fed sub-lethal concentration of crude extract of *Azadirachta indica* leaf was investigated. The sub-lethal concentrations were earlier reported to inhibit reproduction in the fish species. *Oreochromis niloticus* of mean weight 29.30±2.02-31.79±3.11 were divided into 6 groups. Each group was replicated three times. Fishes were stocked in out door concrete tanks (2x2x1.25 m) supplied with 450 litres of water. Six experimental diets (35% crude protein) containing varying sub-lethal concentrations of *A. indica* leaf crude extract (0.0, 0.5, 1.0, 2.0, 4.0 and 8.0 gkg^{-1} diets) were formulated (representing O, A, B, C, D and E respectively; O serves as the control). Fish were fed 3% body weight/day at two feeding instalments between 0900-0930 and 1700-1730 for 56 days. Ovary, testis and liver of fish fed control diet showed normal ovarian tissues, normal distribution of the testicular tissues and normal structure of the vein. Mild to severe necrosis and granulation of the interstitial tissue were observed in the ovaries as the concentration of the crude extract increased from 0.5 – 8.0 gkg^{-1}. The testes showed mild to severe atrophy and cystic seminiferous tubules, while in the fish liver; increase in the number of rodlet cells and distortion of the

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vein walls were observed as the sub-lethal concentrations of the crude extract in the diets increased. Changes in the histology of the ovaries and testes showed the antiifertility potency of *A. indica* leaf, while changes in the liver histology revealed the sub-lethal toxicity effect of *A. indica* leaf all at moderate level of inclusions.

Keywords: Crude extract; necrosis; atrophy; diets; effects.

1. INTRODUCTION

In recent years there has been interest in the use of plant extracts especially at sub-lethal concentrations as phyto-additive in fish feed to promote growth [1,2,3], and to inhibit reproduction in Tilapia [4,5,6,7]. This is because plant materials had been observed to be safe, cheap and environmentally friendly when compared with synthetic substances. *Azadirachta indica* is a tree that grows vey fast it has been reported to reach a height of 15 – 20 m; it is an evergreen tree, with fairly dense crown. The phytochemical screening of the leaf showed a high concentration of saponins, moderate concentration of tannin and glycosides while alkaloids, terpenes, flavonoids reducing sugar, and pentoses showed low concentration [8].

Biological and Pharmacological activities attributed to different parts and extracts of these plant include antiplasmodial, antitrypanosoma, antioxidant, anticancer, antibacterial, antiviral, larvicidal, and fungicidal activities. Others include antiulcer, spermicidal, anthelminthic, antidiabetes, antiimplantation, immnomodulative, molluscicidal, nematicidal, immnocontraceptive, insecticidal, antifeedant and insect repellent effects [9,10,11]. The antiifertility effects of *Azadirachta indica* leaf on Tilapia [12,6] and rats [13,14] have been reported. [15] observed visible lesion within the seminiferous tubules when male rats were administered with 400 mg/kg *Morinda lucida* extract, while [16] observed disintegrated cells in the high dose treatment when some group of *Oreochromis niloticus* were fed with feed containing paw paw seeds. [17] reported coagulation necrosis in yolk granules and atresia of ripe oocytes when 2.0 g therigon/kg diets were fed to *Oreochromis niloticus*. [18] also reported that *Oreochromis niloticus* liver treated with 280 mg/Kg of *Hyptidendron canum* crude ethanol extract showed slight capillary dilation.

There is little or no detail information on the effects of sub-lethal concentrations of *A. indica* crude extract on gonads and liver of *O. niloticus* structural. Thus the aim of this study was to study the structural changes in gonads and liver of *O. niloticus* due to different sub-lethal concentrations of *Azadirachta indica* leaf extract.

2. MATERIALS AND METHODS

2.1 Identification and Preparation of Plant Materials

Azadirachta indica fresh leaf were collected within Ilorin metropolis, they were authenticated at the herbarium section of Department of Plant Biology University of Ilorin, Nigeria and then shade-dried for 2 weeks. The dried leaves were ground into fine powder using an electrical blender and sieved. The crude ethanol extract was prepared by soaking 100 g of dried powdered sample in 250 ml of ethanol for 24 hours the filtrate was concentrated into jelly-like semi solid substance and stored in the refrigerator.

2.2 Preparation of Experimental Diets

The feedstuffs were obtained locally from the market. Basal feed was formulated to provide 35% crude protein (Table 1). *Azadirachta indica* leaf extract was added to the basal diet at sub-lethal concentrations of 0.5, 1.0, 2.0, 4.0 and 8.0 gkg^{-1}. The feedstuff were thoroughly mixed in a pelleting/mixing machine, hot water was added at intervals to gelatinized the starch, feeds were pelletized using 2 mm diameter die, air dried and each packed in a polythene bag labelled and stored in the refrigerator till when needed. The proximate compositions of the experimental diets were analyzed using [19] method of analysis.

2.3 Experimental Design

One hundred and eighty *Oreochromis niloticus* of average size 29.30±2.02 - 31.79±3.11 g were obtained from Ministry of Agriculture and Natural Resources Hatchery Farm Ilorin, Nigeria. Fishes were acclimatized for one week, after acclimatization they were divided into 6 groups each group was replicated three times, each replicate consist of 10 fishes, these were stocked in out door concrete tanks (2x2x1.25 m) supplied with 450 litres of water. The water parameters were within the acceptable range for tilapia culture (Dissolved oxygen 5.00±0.06 - 5.65±0.45 mg/L, pH 7.51±0.05 - 7.70±0.05 and Temperature 27.00±1.00 - 28.50±0.50ºC). Fish were fed 3% body weight/day with the 6 experimental diets (representing O, A, B, C, D and E respectively) at two instalments between 0900-0930 and 1700-1730 for 56 days. Tanks were drained and washed twice a week and replenished with fresh water. The experiment conforms to the standard guidelines on animal use.

2.4 Tissues Preparation

Fish samples were randomly selected according to their sexes from each of the experimental group they were rapidly decapitated and dissected according to the standard procedures to remove the ovaries, testes and livers, these organs were immediately fixed in 10% formaldehyde solution before they were taken to the laboratory for sectioning. The organs were subjected to normal histological routines, sectioned into 6 µ thickness, stained with Hematoxylin-Eosin [20] and examined using the light microscope and the photomicrographs were taken by the use of mounted digital camera (Sony DSC-S3000 model).

3. RESULTS

3.1 Ingredients and Proximate Composition of the Experimental Diet

Table 1 shows the ingredients (in grammes) used in formulating the basal diet and the sub-lethal concentrations of *A. indica*, ingredients were obtained from the local market. Table 2 shows the proximate composition of the six experimental diets. Addition of the crude extract of *A. indica* leaf at different sub-lethal concentration was observed not to have significant effect on the proximate composition of the experimental diets.

Table 1. Ingredients composition of experimental diets

	Treatments					
Ingredients	**O**	**A**	**B**	**C**	**D**	**E**
Fish meal	30	30	30	30	30	30
Yellow maize	25	25	25	25	25	25
Soybean meal	20	20	20	20	20	20
Blood meal	12	12	12	12	12	12
Ground nut cake	08	08	08	08	08	08
Vit/min premix	03	03	03	03	03	03
Cassava starch	02	02	02	02	02	02
Cassava starch	0.0	0.5	1.0	2.0	4.0	8.0

Vitamin/mineral premix: Vitamin A, I.U.; Vitamin D, 11252U; Vitamin E, 71 U; Vitamin K3, 2mg; Vitamin B12, 0.015mg; Pantothenic acid 5mg; Nicotinic acid 14mg; Folic acid, 0.4mg; Biotin, 0.04mg; Choline, 150mg; Cobalt 0.2mg; Copper, 4.5mg; Iron, 21mg; Manganese, 20mg; Iodine, 0.6mg; Selenium, 2.2mg; Zinc, 20mg; Antioxidant, 2mg.

Table 2. Proximate composition of experimental diets

	Treatments					
Parameters	**O**	**A**	**B**	**C**	**D**	**E**
% crude protein	35.23±0.44	35.14±0.39	34.78±1.01	35.01±0.63	35.17±0.33	35.33±0.71
% crude fat	12.01±0.68	11.95±0.85	11.98±1.03	12.05±0.73	12.11±0.49	11.88±0.63
% ash content	15.07±0.92	15.01±1.00	15.23±0.61	15.31±0.58	15.09±0.49	14.99±0.77
% moisture	10.13±1.05	10.19±1.10	09.86±1.56	10.33±0.88	09.99±1.12	10.21±0.88

n=3.

3.2 Effects of Crude Extract of *A. indica* Leaf on Testes of *O. niloticus*

Plate 1 shows, the histology of the testes of *O. niloticus* (Haematoxylin and Eosin stained) fed with varying concentrations of *A. indica* leaf extract; it was observed that testis of fish fed with the control diet (O) showed normal testicular tissues and spermatozoa, testis of fish fed 0.5 gkg^{-1} diet (A) showed mild atrophy of the testicular tissues, testis of fish fed 1.0 gkg^{-1} diet (B) showed mild atrophy of the testicular tissues and cystic seminiferous tubules. Testis of fish fed 2.0 g/kg diet (C) showed mild atrophy of the testicular tissues. Testis of fish fed 4.0 gkg^{-1} diet (D) showed severe atrophy of the testicular tissues, while testis of fish fed with 8.0 g/kg diet (E) showed severe atrophy of the testicular tissues and mild cystic seminiferous tubules.

3.3 Effects of Crude Extract of *A. indica* Leaf on Ovaries of *O. niloticus*

Plate 2 shows, the histology of ovary of *O. niloticus* fed with varying concentrations of the crude extract of *A. indica* leaf, it was observed that the ovary of fish fed with the control diet (O) showed normal ovarian tissues with primary oocytes (PO) and ripe oocytes stage, in fish fed with 0.5 gkg^{-1} diet (A) the ovary showed mild atrophy of the oocytes (AT), ovary of fish fed 1.0 gkg^{-1} diet (B) showed, atrophy of the ripe oocytes, slight distortion of the secondary oocytes and shrinking of the wall of the oocytes. Ovary of fish fed 2.0 gkg^{-1} diet (C) showed severe atrophy of the oocytes, slight distortion of the secondary oocytes (OS) and wall of the oocytes and clumping of the yolk granules, ovary of fish fed 4.0 gkg^{-1} diet (D) showed severe atrophy of the ripe oocytes and shrinking of the wall of the oocytes, while ovary of fish fed with 8.0 gkg^{-1} diet (E) showed necrosis and hyperplasia of the oocytes.

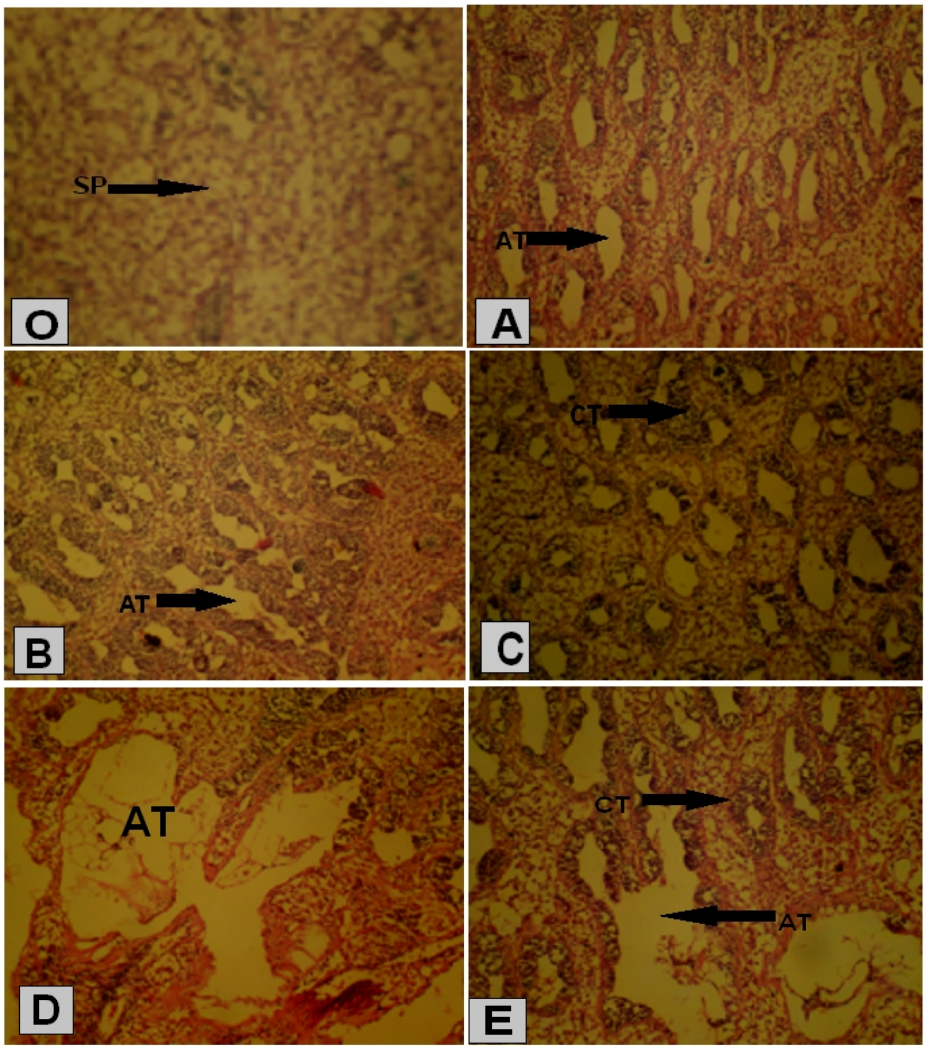

Plate 1: Sections of testes of *O. niloticus* fed with varying inclusion level of crude extract of *A. indica* leaf, (Haematoxylin and Eosin stained).

O: Fish fed with the control diet (0.0 gkg^{-1}) showing normal testicular tissues and spermatozoa (SP), (150x). A: Fish fed 0.5 gkg^{-1} diet, showing mild atrophy (AT) of the testicular tissues, (x150). B: Fish fed 1.0 gkg^{-1} diet, showing mild atrophy (AT) of the testicular tissues, (150 x). C: Fish fed 2.0 gkg^{-1} diet, showing severe atrophy (AT) of the testicular tissues and cystic seminiferous tubules (CT), (150 x). D: Fish fed with 4.0 gkg^{-1} diet showing severe atrophy (AT) of the testicular tissues, (200 x). E: Fish fed 8.0 gkg^{-1} diet showing severe atrophy of the testicular tissues (AT) and cystic seminiferous tubules (CT), (150 x). (Length of arrow bar 15 mm)

3.4 Effects of Crude Extract of *A. indica* Leaf on Liver of *O. niloticus*

Plate 3 shows the histology of the liver of *O. niloticus* (Haematoxylin and Eosin stained) fed with varying concentrations of crude extract of *A. indica* leaf, it was observed that liver of fish fed with the control diet (O) showed normal structure of the vein with blood spot and normal polyhedral hepatocytes, liver of fish fed 0.5 gkg^{-1} diet (A) showed normal

polyhedral hepatocytes and vein with little growth of rodlet cells around the vein, liver of fish fed 1.0 gkg^{-1} diet (B) showed normal polyhedral hepatocytes and distortion, liver of fish fed 2.0 gkg^{-1} diet (C) showed distortion of the vein wall and more growth of rodlet cells around the wall of the vein, liver of fish fed 4.0 gkg^{-1} diet (D) showed distortion of the vein wall and growth of rodlet cells around the wall of the vein, while liver of fish fed 8.0 gkg^{-1} diet (E) showed severe distortion of the vein wall and high growth of rodlet cells around the wall of the vein.

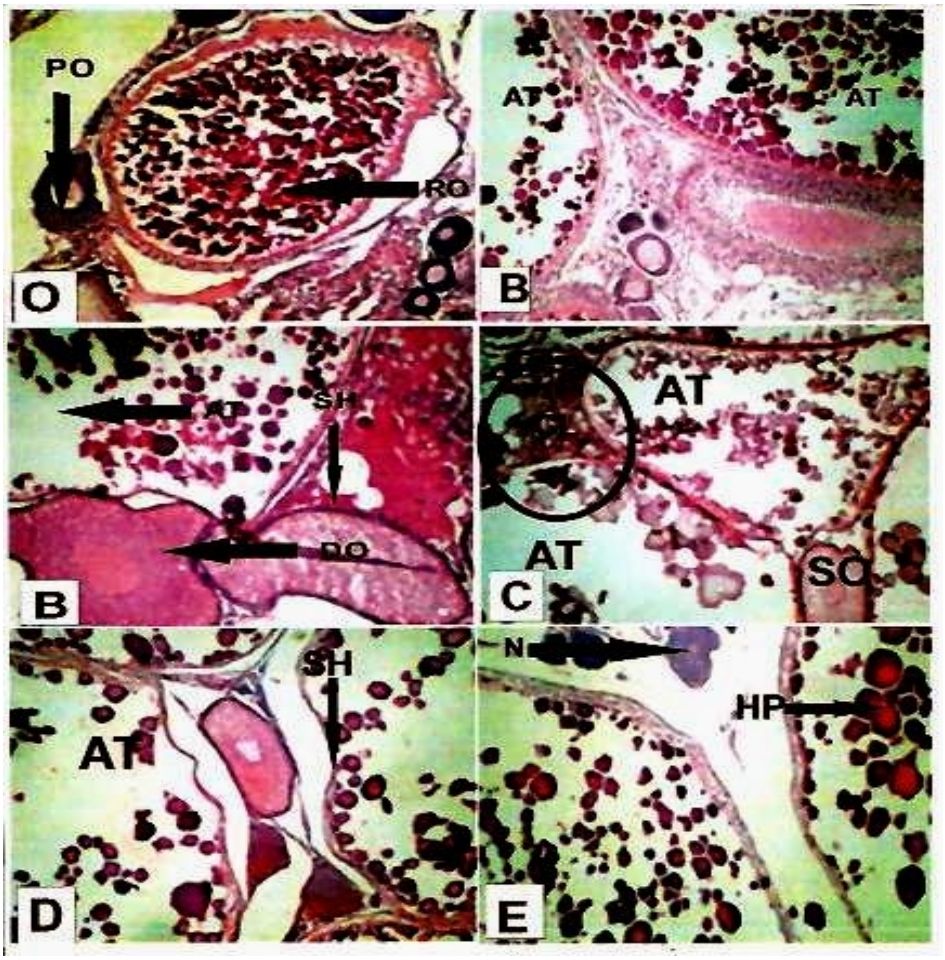

Plate 2: Sections of ovaries of *O. niloticus* fed with varying inclusion level of crude extract of *A. indica* leaf, (Haematoxylin and Eosin stained).

O: Fish fed with the control diet (0.0 gkg^{-1}), showing normal ovarian tissues with primary oocytes (PO) and a ripe oocyte (RO). (150x). A: Fish fed 0.5 gkg^{-1} diet, showing mild atrophy of the oocytes (AT), (150x). B: Fish fed 1.0 gkg^{-1} diet showing atrophy (AT) of the ripe oocyte, slight distortion (DO) of the secondary oocyte and shrinking (SH) of the wall of the oocytes, (200x). C: Fish fed 2.0 gkg^{-1} diet, showing severe atrophy of the oocyte, slight distortion of the secondary oocyte (SO) and wall of the oocyte and clumping (CL) of the yolk granules, (150x). D: Fish fed 4.0 gkg^{-1} diet, showing severe atrophy of the ripe oocyte and shrinking (SH) of the wall of the oocytes, (150x). E: Fish fed 8.0 gkg^{-1}, diet showing mild necrosis (N) and hyperplasia (HP) of the oocytes, (150 x).
(Length of arrow bar 15 mm)

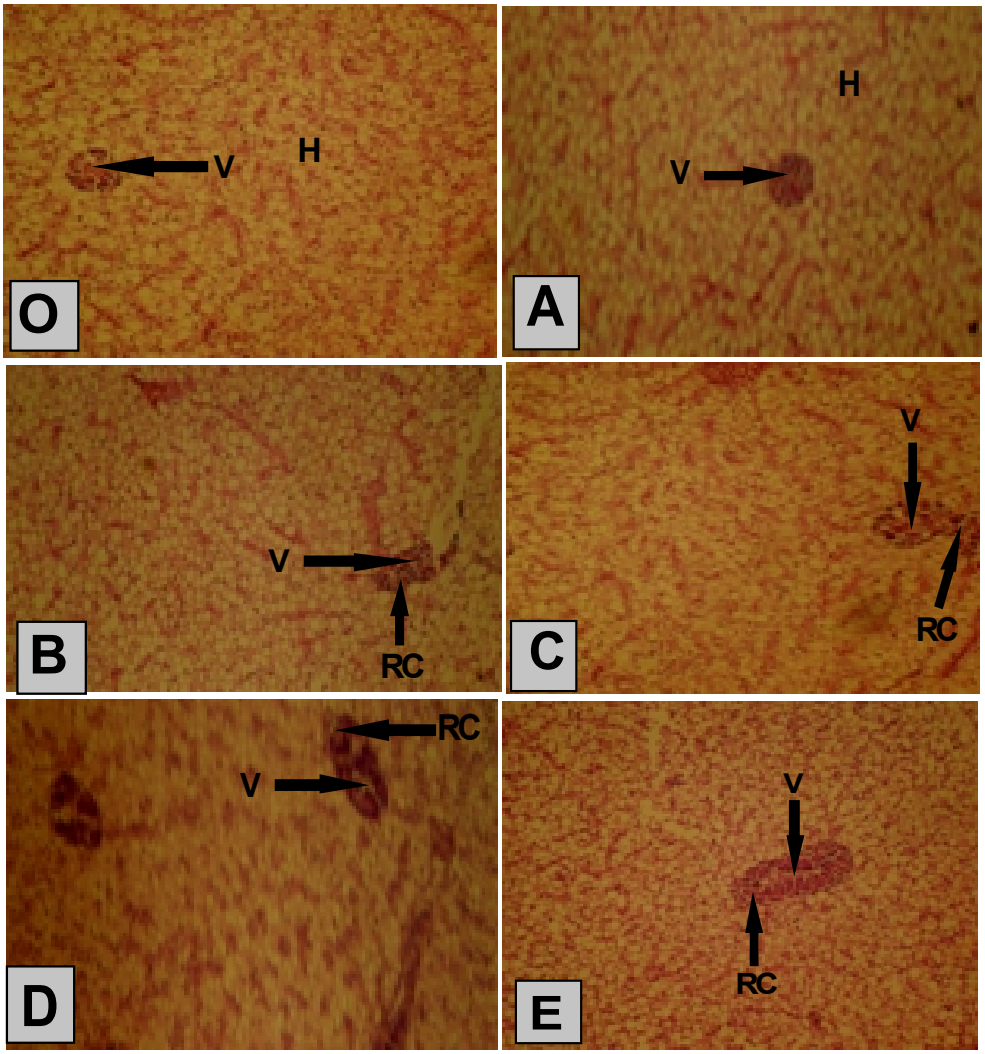

Plate 3: Sections of livers of *O. niloticus* fed with varying inclusion level of crude extract of *A. indica* leaf, (Haematoxylin and Eosin stained).

O: Fish fed with the control diet (0.0 gkg^{-1}), showing normal structure of the vein (V) with spot of blood and normal polyhedral hepatocytes (H). (150x). A: Fish fed 0.5 gkg^{-1} diet, showing normal polyhedral hepatocytes and vein with slight growth of rodlet cells around the vein, (150x). B: Fish fed 1.0 gkg^{-1} diet, showing normal polyhedral hepatocytes and distortion in the structure of the vein with mild growth of rodlet cells (RC) around the vein, (150x). C: Fish fed 2.0 gkg^{-1} diet, showing distortion of the vein wall and more growth of rodlet cells around the wall of the vein, (150x). D: Fish fed 4.0 gkg^{-1} diet, showing distortion of the vein wall and growth of rodlet cells around the wall of the vein, (150x). E: Fish fed 8.0 gkg^{-1} diet, showing severe distortion of the vein wall and high growth of rodlet cells (RC) around the wall of the vein, (150x). (Length of arrow bar 15 mm).

4. DISCUSSION AND CONCLUSION

The inclusion of the crude extract of *Azadirachta indica* leaf had no effect on the proximate composition of the experimental diets as shown in Table 1, similar observation was reported

by [2]. The histopathological study of *Oreochromis niloticus* gonads and liver in this work showed increase in the alteration of these organs as the crude extract inclusion level increases. Mild to severed necrosis and atrophy were observed in the ovaries of the fish that were treated with the crude extract of *Azadirachta indica* leaves, [12] reported similar observation in ovaries of *Tilapia zillii* that were fed with different inclusion of *Azadirachta indica* meal.

Mild to severed atrophy and cystic seminiferous tubule were observed in the testes of the fish that were fed with different inclusion levels of the crude extract, similar damage to the seminiferous tubules, interstitial tissues and complete degeneration of sperm in the testicular tubules at a very high doses have been reported in rabbits and rats [21,22]. [10] also observed abnormality in the spermatogenesis and sperms production in some seminiferous tubules of rats that were administered varying concentration of *Azadirachta indica*.

Gradual distortion in the structure of the veins and increase in the number of rodlet cells were observed in the livers of the fish that received different inclusion level of the crude extracts, [23] suggested that the rodlet cells (RCs) may be stimulated by certain substances produced as a result of tissue injury or related factors, and is reminiscent of leukocyte responses and various chemothatic stimuli. Increase in the rodlet cells observed in this study suggests possible toxic effect of the plant extract at a higher level of inclusion on. Bioassay-guided studies and phytochemical analyses utilizing modern state-of-the-art techniques such as HPLC-MS, GC-MS, NMR and Infra Red spectroscopy have revealed phytocompounds like azadirachtins, nimocinol, isomeldenin, azadirachtol, 2,3-dehydrosalanol gedunin, nimbin, nimolicinol, odoratone, azadironolide, isoazadironolide, naheedin and mahmoodin present in *Azadirachta indica* are responsible for the varied biological, pharmacological and toxicological properties usually observed [11]. This study has further established a base line as to the effects of crude extract of *Azadirachta indica* leaf on gonads and livers of *Oreochromis niloticus*

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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