

Effect of Sub-Lethal Concentrations of *Vernonia amygdalina* (Bitter Leaf) on Testes of *Clarias gariepinus* (African Catfish) Juveniles

ABSTRACT

Vernonia amygdalina has recently been linked to insecticidal and pesticidal properties that could replace the harmful agrochemical pesticide usage around the aquatic environment and to aquatic inhibitors such as fish. This study aimed to determine the effect of sub-lethal concentrations of *Vernonia amygdalina* (bitter leaf) on testes of *Clarias gariepinus* (African catfish) juveniles. The fishes were exposed to 0.00, 0.40, 0.80 and 1.60 g/L graded concentrations of aqueous crude leaves extract of *Vernonia amygdalina* for two months. The organ (testis) changes in antioxidant biomarkers' histology, histomorphometry, and somatic indices compared with the control. There was the reduction in SOD, MDA and GSH activity as the concentration of the toxicant increased compared with the control. Mild interstitial oedema, mild tubular germ cell, interstitial cell depletions, severe depletion of seminiferous luminal content and sloughing off of the seminiferous tubular boundary tissue were observed in various concentrations of *Vernonia amygdalina* compared with the control. There was an increase in testis somatic indices as the plant extract concentration increased compared with the control. Bitter leaf extract was shown to have a toxic effect on *Clarias gariepinus* juveniles. As such, the pesticide use of the plants near the aquatic environment should be discouraged.

Keywords: Sub-Lethal Concentrations; *Vernonia amygdalina*; *Clarias gariepinus*; Testes; Juveniles.

1. INTRODUCTION

With the world's population estimated to cross 8.2 billion by 2030 and 842 million people estimated to be undernourished in 2011–13, food supply will be an increasing challenge over the next twenty years (Lem, 2014). With rising income and demographic changes such as family size, population ageing, and urbanisation, as well as consumer trends such as concerns about healthy eating and sustainable production, there will be major changes in demand and significant changes in the composition of demand (Lem, 2014). This scenario will have an effect on food supply, which will need to enhance and become more effective if it is to grow within the constraints imposed by natural resource availability and existing technology (FAO, 2014). Fish consumption by people is on the rise around the world, owing to the availability, accessibility, and cost of meats like beef, pork, and poultry (Lem, 2014). Consequently, some concerns begin to emerge, primarily regarding harvesting fishes from natural water bodies and fish treatment by using piscicides by aquaculturists which may affect the aquatic environment and quality of fish available in the market. The bioactive ingredient could be present in any product of animal origin, causing

economic losses and putting human and animal health at risk. According to Bostock *et al.* (2010), aquaculture contributes nearly half of all food of aquatic origin intended for human consumption as a vital part of the global food industry.

The African catfish (*Clarias gariepinus*) is a Clariidae catfish species that is the most widely grown fish in Nigeria. Due to its stress resilience, capacity to survive a wide range of environmental conditions, high stocking density under culture conditions, and relatively fast development, it is the most widely requested freshwater fish on the planet (APHA, 1988). They live in freshwater lakes, rivers, swamps, and man-made environments such as oxidation ponds and even urban sewage systems throughout Africa and the Middle East. The African catfish can never be mentioned when talking about traditional fish capture. The current aquaculture practices, are similar to tilapia in terms of culture (Ayotunde *et al.*, 2011). The *C. gariepinus* is a native of Algeria, Angola, Benin, Botswana, Burkina Faso and an excellent sentinel to study of plant crude extracts. It feeds on detritus. It inhabits the streams, rivers, lakes, estuaries and brackish lagoons, found all year round, and its feeding habits reflect the local contamination simply because it is a bottom dweller.

Botanical extracts have piqued interest in aquaculture for controlling fish parasites, fish fry predators, and unwanted fishes from aquaculture ponds to substitute chemical pesticides and insecticides during the previous several decades. Because the widespread and indiscriminate use of these non-biodegradable synthetic compounds has a negative influence on the aquatic environment and puts non-targeted creatures at danger (Kumar *et al.*, 2010; Das, 2013).

Various plants are known for their therapeutic and antibacterial capabilities, including pesticidal, acaricidal, and trypanocidal properties (Nascimento *et al.*, 2000; Adamu *et al.*, 2005). (Jhingran, 1975, Mgbojikwe and Okoye, 1998; Atawodi, 2005). Depending on the nature and quantities of their bioactive ingredients, some are known as strong arrow and fish poisons (Geidam *et al.*, 2007; Kamalkishor and Kulkarni, 2009). This is due to the fact that plants contain a wide range of biological compounds with different characteristics (Istvan, 2000). Because of their eco-friendliness, ease of availability, high efficiency, rapid biodegradability, and low toxicity to non-targeted animals, plant extracts are regarded potential agents (Yunis, *et al.*, 2014). To far, a large number of plants have been studied in many countries to determine their pesticidal (Rahuman *et al.*, 2008; Dubey *et al.*, 2010; Miresmailli and Isman, 2014) and piscicidal activity (Rahuman *et al.*, 2008; Dubey *et al.*, 2010; Miresmailli and Isman, 2014). (Kulakkattoilickal, 1989; Chiayvareesajja *et al.*, 1997; Neuwinger, 2004; Tiwari and Singh, 2004; Obomanu *et al.*, 2007; Murthy *et al.*, 2010; Ramanujam and Dominic, 2012). However, commercially accessible plant products are still limited, thus new sources of botanical pesticides and piscicides for the constantly expanding pisciculture industry should be sought (Ramanujam and Ratha, 2008). Piscicides are plant extracts that have toxicological effects on fish and induce mortality in these aquatic creatures (Burkill, 1995). Plant piscicides are made from a variety of plants from various families and species, which might differ significantly not only in terms of taxonomic differences, but also in terms of plant components used (leaves, barks, fruits, and seeds), method of application, mode of extraction, and target fish species (Van Andel, 2000; Neuwinger, 2004). Botanical materials contain a number of bioactive chemicals that act as piscicides separately or in combination (Obomanu *et al.*, 2007; Ramanujam and Ratha, 2008). Any plant extract's toxicity and piscicidal activity can be determined by exposing fish to it and then calculating the median lethal concentration (LC50) (Ramanujam and Dominic, 2012). Alteration in haematological, biochemical, and antioxidant parameters of fishes (Winkaler *et al.*, 2007;

Kavitha et al., 2012), as well as biological and physiological activities of fish, are some of the common toxicological effects of plant extract (Winkaler et al., 2007; Kavitha et al., 2012). In *Clarias gariepinus*, Adeyemo (2005) found the haematological and histological impacts of Cassava mill effluent.

Man's activities have been responsible for introducing contaminants into the environment in the last few decades (Awoyinka et al., 2019). pH, turbidity, alkalinity, dissolved oxygen, temperature, and conductivity are all environmental parameters that determine the pace at which pollutants enter the water and their fatal effects on aquatic life (Fagbenro, 2002). Nowadays, contamination of natural ecosystems is on the rise, and it is quickly becoming a major issue as human activities increase. A basic topic in ecotoxicology study is determining toxic substances in aquatic environments and their consequences on aquatic animals. Chemical analysis can reveal the presence of toxic compounds in the environment, but it cannot determine their effects on aquatic animals in aquatic ecosystems. As a result, bioassay experiments are required to assess the environmental impact of harmful substances (Bagheri, 2007).

Information regarding the adverse effect of sub-lethal concentrations on the reproductive system of *C. gariepinus* is scanty. Healthy testes of fish are important determinants of its breeding potential, and thus any toxicological factor adversely affecting the histopathology of testes will reduce the gross production of fish.

Vernonia amygdalina has been reported to be used as growth enhancer, phyto-additive, antibacterial, milt booster and insecticidal by *C. gariepinus* farmers (Kayode, 2021). However recent studies showed that *V. amygdalina* has negative effect on glucose level of *C. gariepinus* (Alagoa and Osakwe, 2021). Acute toxicity studies showed that the plant is toxic to catfish (Audu et al., 2017). There is scarcity of information on the testis somatic indices, antioxidant biomarkers and histology and histomorphometry of *C. gariepinus* fish exposed to sub-lethal concentrations of *V. amygdalina*. Hence, this study examined the effect of sub-lethal concentrations of *Vernonia amygdalina* (bitter leaf) on testes of *Clarias gariepinus* (African catfish) juveniles.

2. MATERIALS AND METHODS

2.1 Description of Study Area

Vernonia amygdalina was collected from Keffi Local Government Area of Nasarawa State, Nigeria. This study was carried out at the Department of Zoology Laboratory, Faculty of Natural and Applied Sciences, Nasarawa State University, Keffi.

2.2 Procurement and Processing of Experimental Plant (*Vernonia amygdalina*)

The leaves were air-dried at room temperature ($27 \pm 2^\circ\text{C}$) in the Laboratory of Zoology Department, Faculty of Natural and Applied Sciences, Nasarawa State University, Keffi. The dried leaves were pounded with laboratory mortar and pestle into powder, sieved with a 30 μm mesh sieve.

2.3 Collection, Transportation and Acclimatization of Experimental Animal

C. gariepinus was purchased from Rayuwa Fish Farm, Karu Local Government Area, Nasarawa State, Nigeria. Experimental animals were transported in tanks containing water from the pond to the Laboratory Unit of the Zoology Department, Nasarawa State University, Keffi. The fish was transferred into aquaria containing dechlorinated municipal tap water and acclimatized to the laboratory conditions for two weeks. During this period, the fish was fed to satiation at 8:00 a.m. and 6:00 p.m. with commercial fish feed (Vital feed®).

2.4 Sub-Lethal Concentrations of Aqueous Crude Leaf Extract of *V. amygdalina* and Test Procedure

Mortality (0, 1, 2, 5, 7 and 10) of *C. gariepinus* juveniles exposed to acute concentrations of 0.0, 0.19, 0.38, 0.75, 1.50 and 3.00 g/L of crude leaf extract of *A. amygdalina* was reported by Audu *et al.* (2018). The LC_{50} was calculated as 0.82 g/L by using the Finney calculator. *V. amygdalina* crude leaves extract administered at sub-lethal concentrations 1/5, 1/10, 1/20 of the LC_{50} (Abubakar *et al.*, 2018) in a static renewable bioassay system.

The weighed samples were macerated in 1 L of distilled water for 24 hours at room temperature (27.0°C) to obtain the stock solution. The obtained stock solution was filtered through a funnel choked with non-absorbent cotton wool, and each filtrate (1.6, 0.8 and 0.4 g/L) was transferred into tanks A1, B1, and C1, respectively, with D1 as the control. Each of the filtrates was diluted by adding 9 L of dechlorinated municipal tap water. The same procedure was repeated in replicate tanks A2, B2 and C2, while tanks D1 and D2 which were not inoculated with the test materials, served as the controls. The sub-lethal toxicity test lasted for two months (56 days or eight weeks). The test fish was fed 3% body weight at 0800 and 1800hrs with photoperiod being natural.

2.5 Experimental Design

The experiments consist of four circular rubber tanks and one hundred and twenty *C. gariepinus* juveniles, mean weight 23.13 ± 2.43 g and mean total length 12.501 ± 0.39 cm in a non-randomized block design. Each of the eight tanks was filled with 9 L of dechlorinated municipal tap water. Six of the filled tanks were inoculated with various concentrations (powder earlier macerated in 1 L of water) of aqueous crude leaves extract of *V. amygdalina* and *C. gariepinus* fingerlings were introduced into each.

2.6 Assay for Biomarkers of Oxidative Stress

2.6.1 Preparation of the Tissue Samples for the Study

At the end of the experiment, fishes were carefully netted to minimize stress, weighed and sacrificed. Tissues such as liver, testes and gills were carefully removed and weighed. The tissues were washed in chilled phosphate buffer saline. Sample homogenates were made of individual tissues with chilled phosphate buffer (0.1 M, pH 7.4), centrifuged in a centrifuge at 9,000 rpm for 20 min at 4°C (Charity *et al.*, 2018). The supernatant obtained was used for further analysis. Samples were transported to Biochemistry Units of National Veterinary Research Institute (NVRI), Vom, Plateau State, Nigeria.

2.6.2 Determination of Glutathione Peroxidase

Glutathione peroxidase (GPx) activity was assayed as described by Paglia and Valentine (1967) with modifications according to Lawrence and Burke (1978). The reaction mixture contained 50 mM potassium phosphate buffer (pH 8.3), 1 mM EDTA, 1 mM sodium azide, 0.2 mM nicotinamide adenine dinucleotide phosphate (NADPH), and 1 U/mL glutathione reductase. The reaction was initiated with the addition of 1.5 mM cumene hydroperoxide. The enzyme activity was estimated from the rate of oxidation of NADPH. The reagents were mixed, and the absorbance was measured at 340 nm. Enzyme activity was expressed in mmol/minute/milligram protein.

2.6.3 Determination of Superoxide Dismutase Activity

Superoxide dismutase (SOD) activity was assayed as described by Misra and Fridovich (1972). The assay was based on the ability of SOD to inhibit the autoxidation of epinephrine at an alkaline pH. 0.5 ml of tissue homogenate was diluted with 0.5 ml of distilled water, to which 0.25 ml of ice-cold ethanol and 0.15 ml of ice-cold chloroform was added. The mixture was properly mixed using a cyclo mixer for 5 minutes and centrifuged at 2500 rpm. To 0.5 ml of supernatant, 1.5 ml of carbonate buffer (0.05 M, pH 10.2) and 0.5 ml of EDTA solution (0.49 M) were added. The reaction was initiated by the addition of 0.4 ml of epinephrine (3 mM), and the change in optical density/minute was measured at 480 nm against the reagent blank. SOD activity was expressed as units/mg protein. Change in optical density per minute at 50% inhibition of epinephrine to adrenochrome transition by the enzyme was taken as the enzyme unit.

2.6.4 Determination of Lipid Peroxidation

The malondialdehyde (MDA) content, a measure of lipid peroxidation, was assayed in the form of thiobarbituric acid reactive substances (TBARS) using the method of Buege and Aust (1978). 1.0 mL of the sample was added to 2 mL of (15% (w/v) trichloroacetic acid, 0.375% (w/v) thiobarbituric acid (TBA), 0.25 M HCl). The solution containing plasma and reagent was heated in a boiling water bath for 15 min and then cooled. Precipitates were removed by centrifuging at 1000 rpm for 10 minutes. The supernatant was removed, and the absorbance read at 535 nm against a blank. MDA was calculated using the molar extinction coefficient ϵ for MDA $1.56 \times 10^5 \text{ M}^{-1} \text{ cm}^{-1}$. Results were expressed as μmol^{-1} .

2.7 Extraction of Organs of *C. gariepinus* Juveniles Exposed to Sub-Lethal Concentrations of Aqueous Crude Leaves Extract of *Vernonia amygdalina*

Following the exposure of *C. gariepinus* juveniles to sub-lethal concentrations of crude leaf extract of *V. amygdalina*, four fishes from treatment and control tanks were removed, sacrificed and dissected to obtain the testis, gill, liver and kidney. Each removed organ was rinsed with distilled water to wash off traces of blood. Each organ was preserved in 10 ml specimen bottle containing 5 ml of formal saline before transportation to NVRI, Vom, Plateau State, Nigeria, for histopathological analyses of the tissues.

2.7.1 Determination of Organ Somatic Indices of *C. gariepinus* Juveniles Exposed to Sub-Lethal Concentrations of Aqueous Crude Leaves Extract of *V. amygdalina*

The calculation of the testes somatic index (TIS) was done by random selection of four individual fishes from each treatment group and total weight of the individuals were noted (Shalaka and Pragna, 2013). The organs of the fishes like testes were removed carefully and weighed in an electronic weight machine, after removing moisture by blotting paper (Pamela *et al.*, 2018).

Organs Somatic Index was calculated using:

$$\text{OSI} = \frac{\text{Weight of organ}}{\text{Weight of fish}} \times 100$$

2.8 Procedure for Histological Examination of Organs of *C. gariepinus* Juveniles Exposed to Sub-Lethal Concentrations of Aqueous Crude Leaves Extract of *V. amygdalina*

The routine Paraffin wax method and haematoxylin-eosin staining techniques of tissue processing described by Drury and Wallington (1967) and Avwioro (2011) were adopted to examine the gills, kidneys and liver of *O. niloticus* fingerlings exposed to aqueous crude leaves extract of *B. aegyptiaca*. The harvested gills, kidneys and liver of *C. gariepinus* were fixed in 10% formalin for three days, cut into thin slices of 5 x 2 x 1 mm thick and then processed with the SPIN Tissue Processor (STP) 120 (Thermo Scientific Model). The tissues were buffered in 10% formalin before passing through the following levels of hydrocarbons for two hours each: 70% Alcohol, 80% Alcohol, 90% Alcohol, 95% Alcohol, Absolute Alcohol I, Absolute Alcohol II, Absolute Alcohol III, Xylene I, Xylene II, Paraffin Wax Oven I, Paraffin Wax Oven II.

Tissues were embedded in molten paraffin wax using embedding moulds. The tissues were embedded using cassettes on a tissue Tek Embedding Centre (SLEE MPS/P2) and cooled rapidly on the cooling component. Tissues were sectioned using a rotary microtome (MICROM HM340E Thermo Scientific) set at four microns, picked on slides and ready for staining.

Haematoxylin and eosin staining technique was used for the staining of the tissues. Tissue sections were dewaxed and hydrated by passing through two changes of xylene and through descending levels of alcohol (100%, 80%, 70%) for three min each and then into the water before staining in Harris' haematoxylin solution for 5 min and washed in running water. They were differentiated in 1% Acid alcohol and then washed thoroughly in water, blued in Scott's tap water substitute for 5 min and rinsed briefly in distilled water. Each tissue was then counterstained in 1% aqueous eosin for 2 min and then washed in water, dehydrated in descending grades of alcohol before clearing in xylene and mounted in Distrene, Plasticizer and Xylene (DPX). Sections were then placed in slide carriers and placed in a 40°C oven to dry overnight. Each tissue was read microscopically. Photographs of the prepared sections were taken using a mounted photo-microscopic camera.

2.9 Histomorphometrics of Organs of *Clarias gariepinus* Juveniles Exposed to Sub-Lethal Concentrations of Aqueous Crude Leaves Extract of *Vernonia amygdalina*

Ten fishes from each experimental group were randomly selected. In addition, hepatocyte histomorphometric measurements such as hepatocyte nuclear diameter and hepatocytes surface area were determined by using Motic image plus 2.0 (Motic Asia, Hong Kong) software (Audu *et al.*, 2017).

2.10 Statistical Analysis Method

Data collected were subjected to analysis of variance (ANOVA) using SPSS version 16. Significant means were set at $p < 0.05$ and separated using Duncan new multiple range test. Results were presented as means $\pm$ standard errors of the mean.

3. RESULTS

There is a reduction in SOD activity as the concentration of the toxicant increases. The highest and lowest value of 19.4462 ± 0.98461 and 18.1615 ± 1.10000 were recorded in control (0.00 g/L) and highest concentration (1.60 g/L). There is also a reduction in GSH activity as the concentration of the toxicant increases, the highest and lowest values of 10.2500 ± 9.1250 were recorded in 0.80 g/L and the highest value recorded in 0.40g/L. There is also reduction in GPx activity as the concentration of the toxicant increases, the highest and lowest values of 2.9746 ± 1.9046 were recorded in 0.40g/L and the highest value recorded in 0.80g/L (Table 1).

There is mild interstitial oedema, mild tubular germ cell, interstitial cell depletions, severe depletion of seminiferous luminal content and sloughing off of the seminiferous tubular boundary tissue observed in testes of the exposed fishes as seen in Plate A-D.

Morphometric parameters such as STD with the highest and lowest value of 133.79 ± 9.799 and 87.35 ± 6.211 shows a significant difference compared to the control recorded in 0.40 g/L and 0.80 g/L. SLD with the highest and lowest value of 100.45 ± 7.561 and 76.70 ± 9.241 shows the significant difference when compared to the control recorded in 0.40 g/L and 0.80 g/L, while SED with the highest and lowest value of 16.72 ± 0.740 and 9.82 ± 0.563 shows significant difference when compared to the control recorded in 0.00 g/L and 1.60 g/L (Table 2).

TSI shows no significant difference compared with the control with the highest and lowest value of 0.7475 ± 0.14014 and 0.4675 ± 0.15638 recorded in the highest concentration (1.60g/L) and (0.80 g/L) (Table 3).

Table 1: Mean Testes Superoxidase Dismutase, Lipid Peroxidation and Glutathione Peroxidase of *Clarias gariepinus* Juveniles Exposed to Concentrations of Aqueous Crude Leaves Extract of *V. amygdalina*

Concentrations (g/L)	SOD	GSH	GPx
0.00	19.4462 ± 0.98461	9.2500 ± 0.50000	2.153 ± 0.34223
0.40	18.2000 ± 0.41538	10.2500 ± 0.50000	1.9046 ± 0.21081

0.80	18.4615 ± 0.75385	9.1250 ± 0.37500	2.9746 ± 0.64136
1.60	18.1615 ± 1.10000	9.5000 ± 0.75000	2.3272 ± 0.54893
P-value (0.05)	0.7050	0.5370	0.4940

Key: SOD = Superoxidase Dismutase; GSH = Glutathione Peroxidase; GPx = Lipid Peroxidation.

Table 2: Sub-Lethal Effects of Bitter Leaves Crude Extract on Testes Morphometry Parameters

Concentrations (g/L)	STD	SLD	SED
0.00	88.49±3.174 ^A	84.07±4.902	16.72±0.740 ^B
0.40	133.79±9.799 ^B	100.45±7.561	15.05±0.978 ^B
0.08	87.35±6.211 ^A	76.70±9.241	15.19±0.701 ^B
1.60	129.24±13.068 ^B	99.23±11.098	9.82±0.563 ^A

Key: Values with different letters in the same row are significantly different (P<0.05) compared with the control.
 STD = Seminiferous Tubular Diameter; SLD = Seminiferous Luminal Diameter;
 SED = Seminiferous Epithelial Diameter.

Table 3: Mean Testes Somatic Index of *Clarias gariepinus* Juveniles Exposed to Concentrations of Aqueous Crude Leaves Extract of *V. amygdalina*

Concentrations	TSI
1.60	0.7475 ± 0.14014

0.80	0.4675 ± 0.15638
0.40	0.5100 ± 0.10141
0.00	0.5200 ± 0.12076
Total	0.5613 ± 0.06515

Key: TSI = Testes Somatic Index.

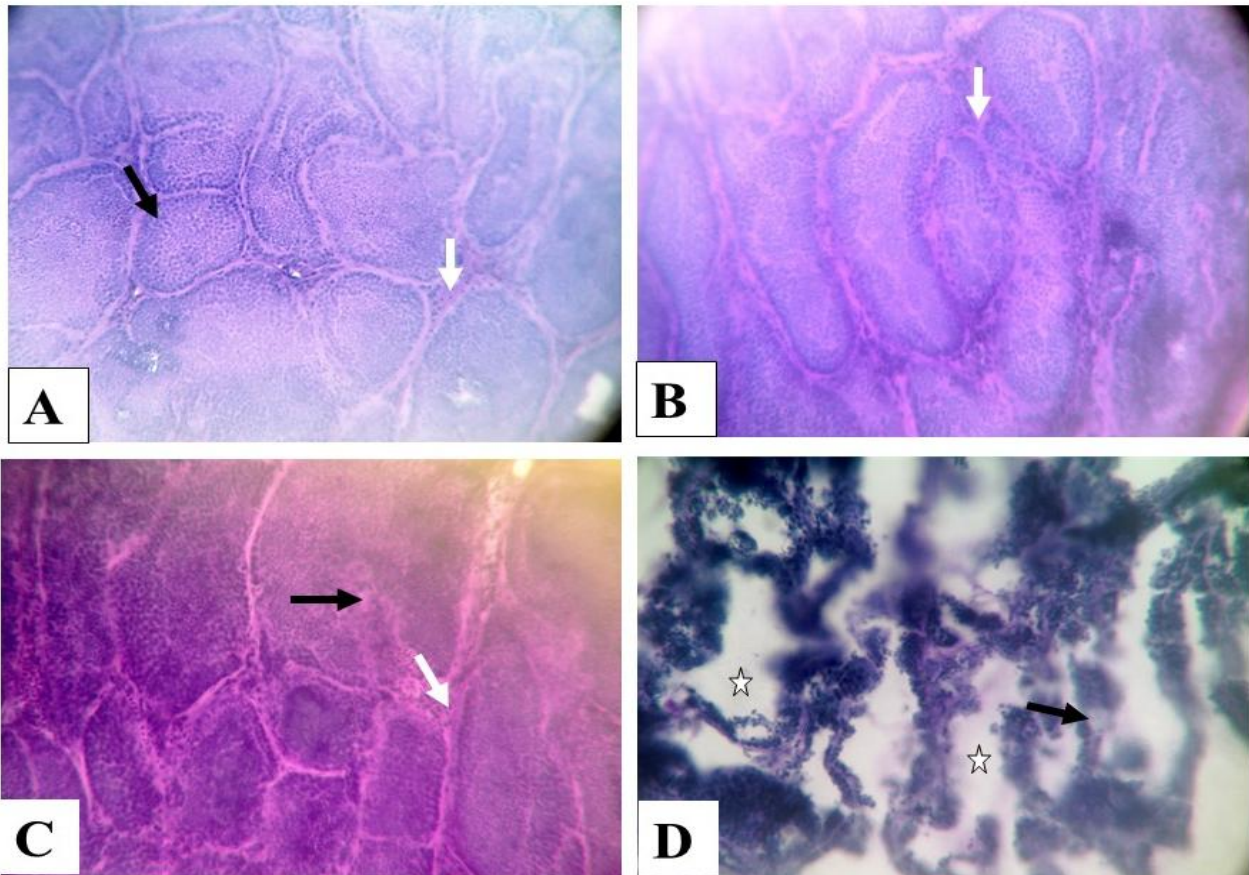


Plate A-D: Photomicrographs of the Testes of *Clarias gariepinus* Exposed to Graded Concentrations of *Vernonia amygdalina*.

Key: **A. Control (0.00 g/L):** The testes present normal numerous tubular structures housing different spermatogenic cell series (black arrow) and distinct interstitium between the tubules (white arrow). **B. 0.4 g/L:** There was no visible lesion except for mild interstitial oedema (white arrow). **C. 0.8 g/L:** Presence of mild tubular germ cell (black arrow) and interstitial cell depletion (white arrow). **D. 1.6 g/L:** Severe depletion of seminiferous luminal content (star) and sloughing off of the seminiferous tubular boundary tissue (black arrow). Magnification: x400; Stain: Haematoxylin-eosin.

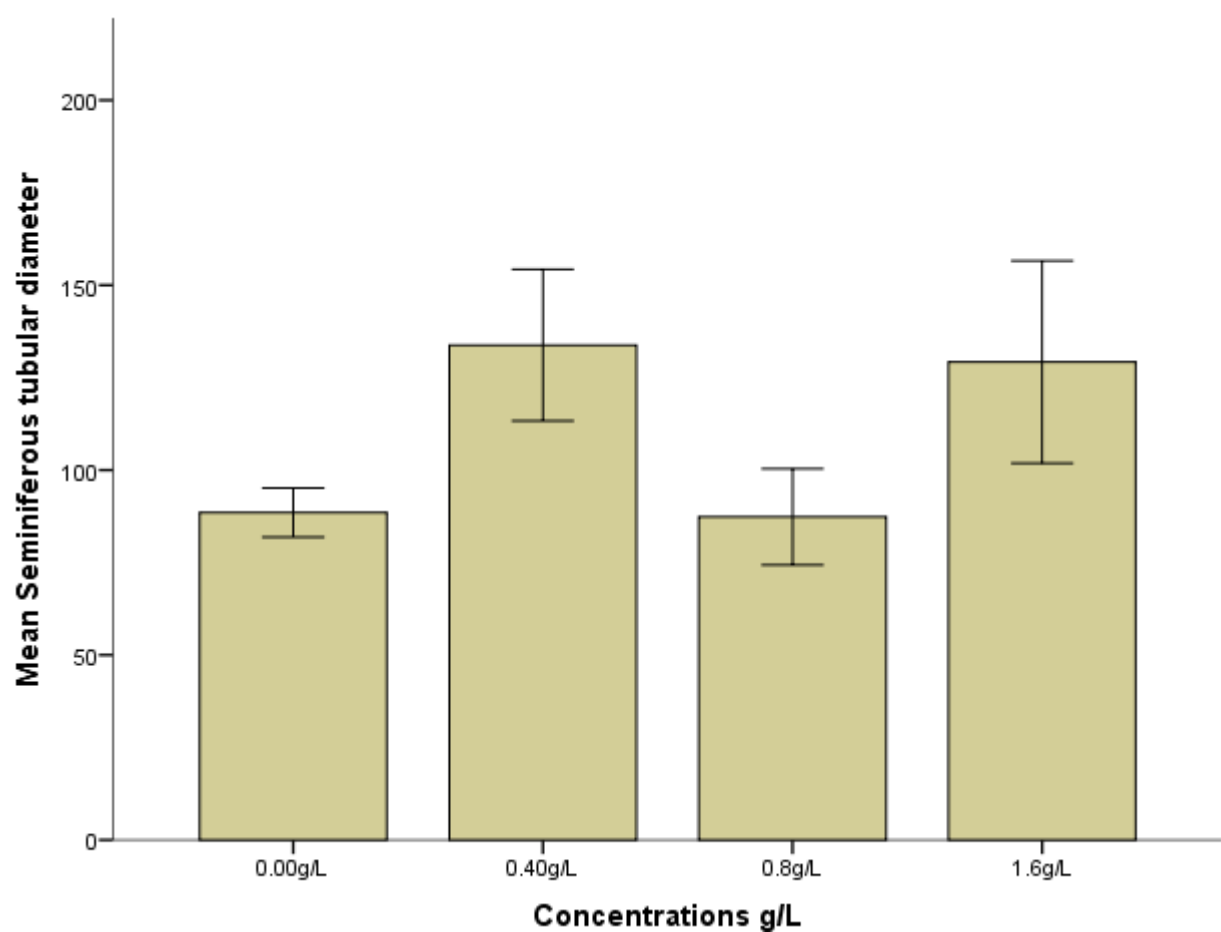


Figure 1: Mean Testes Somatic Index of *C. gariepinus* Juveniles Exposed to Concentrations of Aqueous Crude Leaves Extract of *V. amygdalina*.

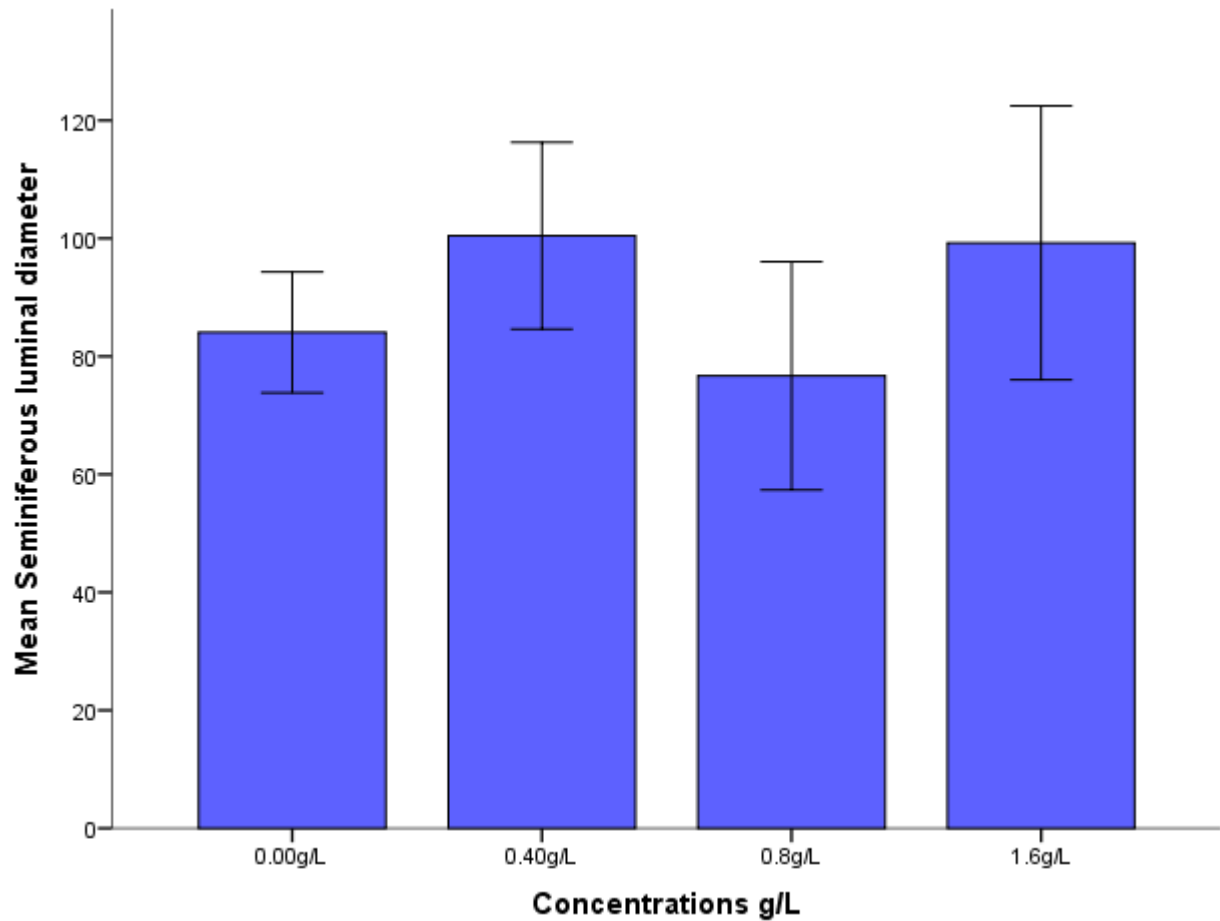


Figure 2: Mean Testes Somatic Index of *C. gariepinus* Juveniles Exposed to Concentrations of Aqueous Crude Leaves Extract of *V. amygdalina*.

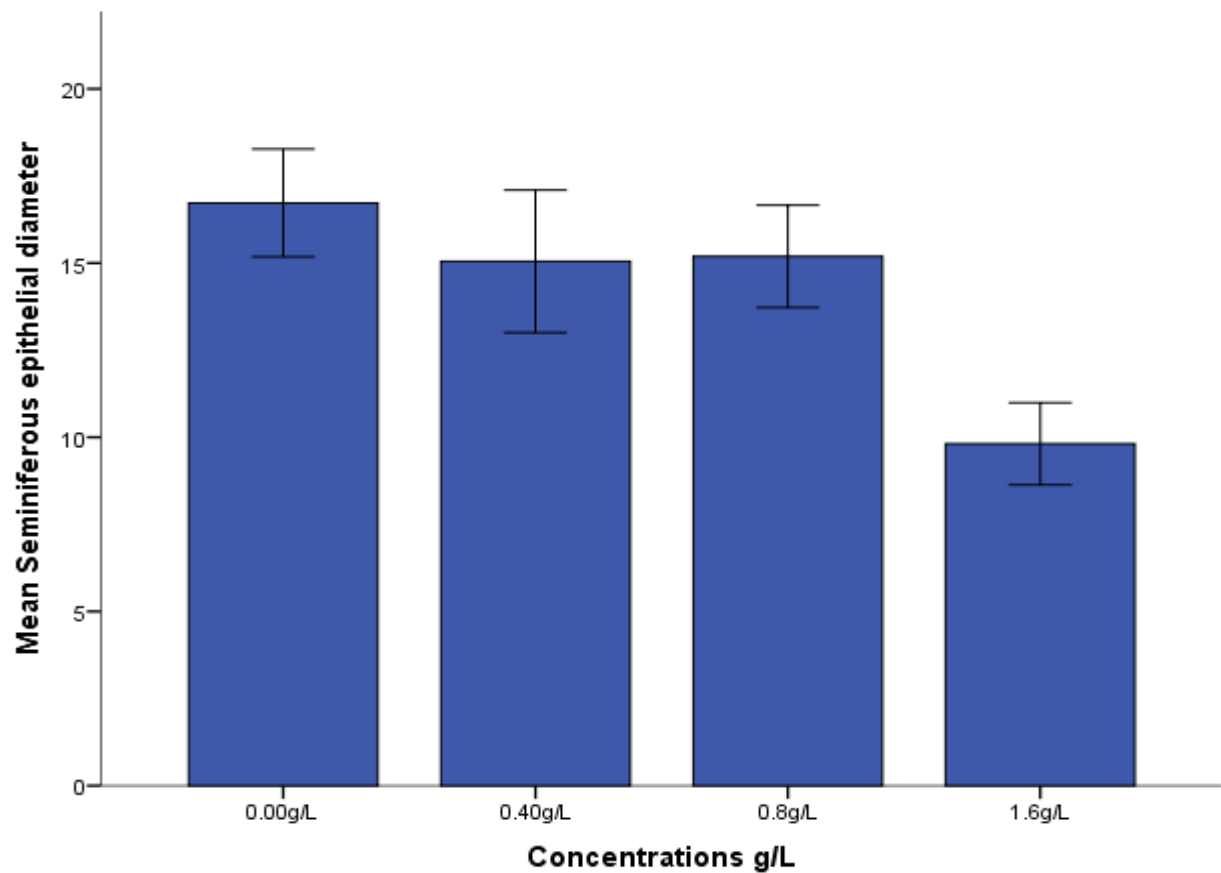


Figure 3: Mean Testes Somatic Index of *C. gariepinus* Juveniles Exposed to Concentrations of Aqueous Crude Leaves Extract of *V. amygdalina*.

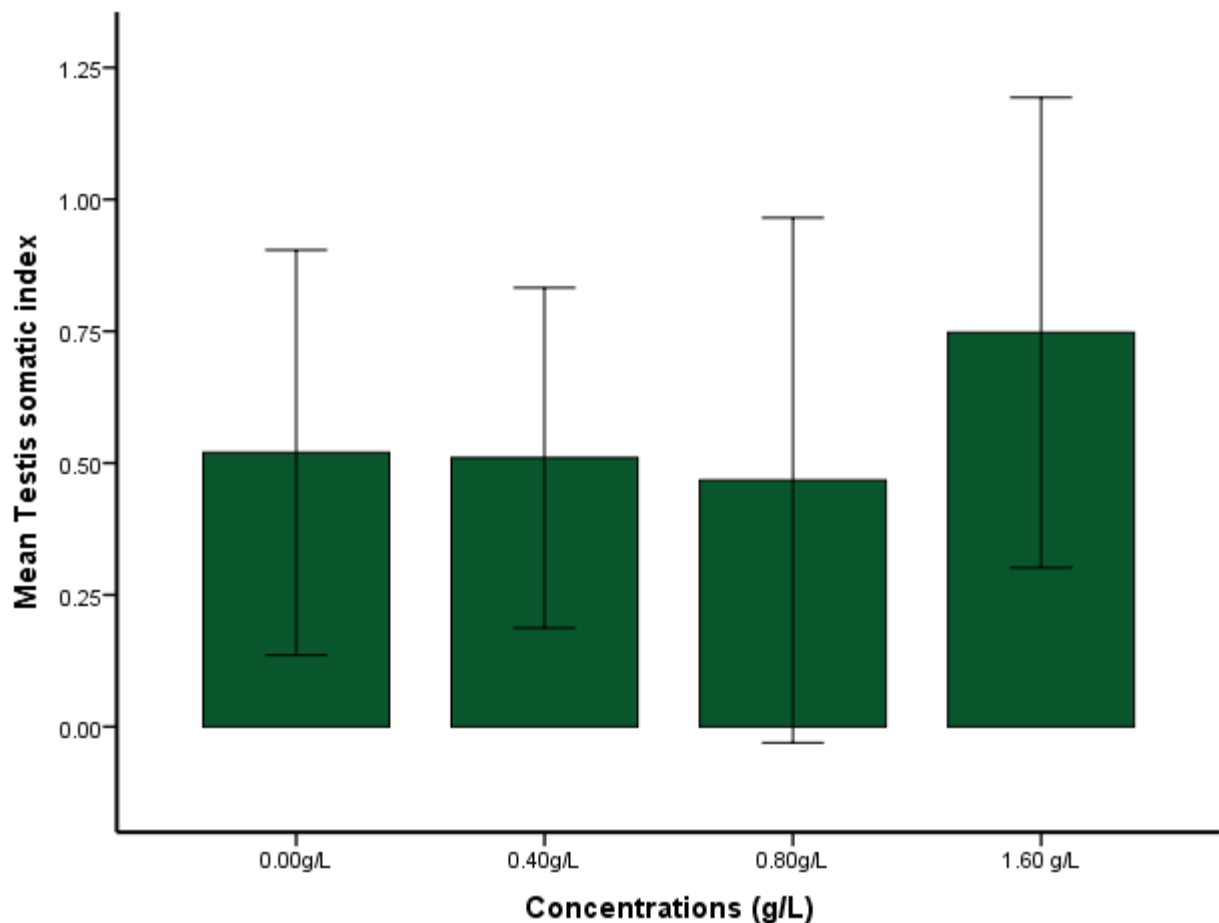


Figure 4: Mean Testes Somatic Index of *C. gariepinus* Juveniles Exposed to Concentrations of Aqueous Crude Leaves Extract of *V. amygdalina*.

4. DISCUSSION

Variation in testes antioxidant biomarkers (MDA, GSH, SOD) indicated oxidative stress in testes which is commensurate with the finding of Burak *et al.* (2016) in the testes of fish from the Lake Van and Karasu River of Van fish *Alburnus tarichi*, who reported that there was an unchanged MDA, GSH and SOD level. This research finding is also in line with a report from Alaa and Rania (2016), who opined that there was a significant increase ($p < 0.05$) in activities of serum SOD, glutathione s-transferase (GSH), and malondialdehyde (MDA) when adult *C. gariepinus* were exposed to pure 100 $\mu\text{g/L}$ 4-nonylphenol and quince (*Cydonia oblonga*) to fishes exposed for 15 days when compared to the control, but in this research, CAT was not recorded.

The severe sloughing off of the seminiferous tubular boundary tissue and severe depletion of seminiferous luminal content reported in this research work is in line with findings from Priya and Balu (2013) who reported in their work that there was severe deterioration of the testis histology by the 30th day of exposure, considerable reduction in spermatozoa in the testis when freshwater fish (*Rasbosa dandia*) was exposed to mercury. However, they reported a congestion of blood vessels and proliferation of interstitial tissues and vacant spaces enlarged which was not seen in this research work. The result shows that there is a significant difference in the testis

somatic indices of *C. gariepinus* concerning the graded concentrations of *V. amygdalina* when compared with the control, and this research work is not in line with the finding of Ada *et al.* (2015), who reported that there were no changes in gonad somatic indices in *C. gariepinus* exposed to Atrazine and coconut water after the fishes were exposed for a period of 14 days. This research work is in line with the findings of Claramma and Radhakrishnan (2016), which reported that there were histopathological changes observed as a distortion of seminiferous tubules, disorganization of spermatogonia, spermatocytes and spermatids with cytoplasmic vacuolization and nuclear pycnosis after exposing *Clarias batrachus* to sub-lethal concentrations of chromium for 45 days. Gupta (2002) also reported distortion of the seminiferous tubules in the testis of freshwater fish *Oreochromis mossambicus* in response to plant nutrients which is in line with the findings of this research work. However, he also recorded an increase in vacuolization, condensation of spermatocytes besides inflammation and inter-tubular vacuolation, which was very prominent.

There is an increment in the value of testis somatic index as the concentration of the plant increases which is not in line with the findings of Malik and Naeem (2020) that reported decline in the value of TSI of the fish with increase in the concentrations. Nassr-Allah (2007) also reported that there was a serious reduction in the GSI of blue tilapia juveniles as concentration levels of phenol increases with no significant increase reported for male fish subjected to 20% 96-h LC₅₀ of phenol, whereas significant reduction was observed in the case of male fish subjected to 40% and 80% 96-h LC₅₀ of phenol. Findings from this research work are in line with findings from the work of Shalaka and Pragna (2013), who reported that there was an increase in the GSI value when freshwater fish *Oreochromis mossambicus* were increasingly fed with plant nutrient.

5. CONCLUSION

Variation in testis antioxidant biomarkers (MDA, GSH, and SOD) indicated oxidative stress in testes. Alteration in testes histological and histomorphometric parameters also indicated that the bitter leaf is toxic to catfish and that the constant and indiscriminate use of bitter leaves as sperm boosters of catfish should be discouraged.

6. RECOMMENDATION

Further toxicity research works on the effect of the plants on the reproductive organs of other commercial fish species should be conducted.

COMPETING INTERESTS DISCLAIMER:

Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly used in our research area and country. There is no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for litigation but the advancement of knowledge. Also, the research was not funded by the producing company rather, it was funded by the personal efforts of the authors.

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