Original Research Article

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 aquatic environment and to aquatic inhibitors such as fish. The aim of this study was 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 a period of 2 months. The organ (testis) changes in antioxidant biomarkers' histology and histomorphometry, and somatic indices compared with the control. There was reduction in SOD, MDA and GSH activity as the concentration of the toxicant increases 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 increase in testis somatic indices as the concentration of the plant extract increases when compared with the control. Bitter leaf extract was shown to have toxic effect on *Clarias gariepinus* juveniles. As such, the pesticidal use of the plants near aquatic environment should be discouraged.

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

1. INTRODUCTION

With the world's population expected to reach 8.2 billion people by 2030, and with 842 million people estimated as having been undernourished in the period 2011–13, food supply will present a growing challenge in the next two decades (Lem, 2014). With increases in income along with demographic changes related to family size, population aging and urbanization, and consumer trends such as concerns for healthy eating and sustainable production, there will be great shifts in demand and major changes in the composition of demand (Lem, 2014). This scenario will in turn have an impact on food supply, which will need to increase and become more efficient if it is to grow within the constraints presented by the availability of natural resources and existing technology (FAO, 2014).

Currently, fish consumption by population has been increasing worldwide, mainly due to the availability, access and price in relation to meat consumption, such as beef, pork, and poultry

(Lem, 2014). Consequently, some concerns begin to emerge, primarily regarding the method of harvesting fishes from natural water bodies and fish treatment by using piscicides by aquaculturist which may have effect on aquatic environment and quality of fish available in the market. Bioactive ingredient could be present in any product of animal origin causing economic losses and putting into a risk human and animal health. According to Bostock *et al*. (2010), the 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 species of catfish of the family Clariidae and comprises the most cultivated fishes in Nigeria. It is the most and highly demanded freshwater fish in the world due to its resistance to stress, ability to tolerate a wide range of environmental conditions and high stocking densities under culture conditions, and relatively fast growth (APHA, 1988). They are found throughout Africa and the Middle East, and live in freshwater lakes, rivers, and swamps, as well as human-made habitats, such as oxidation ponds or even urban sewage systems. The African catfish can never be failed to mention when talking about traditional fish capture. In the present system of aquaculture practices, they 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 also a good sentinel for 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.

Over the last several decades, botanical extracts have been shown much interest in aquaculture to control fish parasites, fish fry predators and unwanted fishes from aquaculture ponds as attempts to replace chemical pesticides and piscicides, since extensive and indiscriminate use of these non-biodegradable synthetic chemicals results in harmful impact on aquatic environment and presents high risk to the non-targeted organisms (Kumar *et al*., 2010; Das, 2013).

Various plants are reputed for their medicinal and antimicrobial values (Nascimento *et al*., 2000; Adamu *et al*., 2005), including their pesticidal, acaricidal and trypanocidal properties (Jhingran, 1975, Mgbojikwe and Okoye, 1998; Atawodi, 2005). Yet, some are known as potent arrow and fish poisons (Geidam *et al*., 2007; Kamalkishor and Kulkarni, 2009) depending upon the type and the concentrations of their bio-active constituents. This is because, plants contain structurally diverse biological substances with varying properties (Istvan, 2000). Plant extracts are considered promising agents because of their eco-friendliness, ease of availability, high efficiency, rapid biodegradability and reduced toxicity to non-targeted animals (Yunis Aguinaga *et al*., 2014). To date, a good number of plants have been investigated in different countries to evaluate their pesticidal (Rahuman *et al*., 2008; Dubey *et al*., 2010; Miresmailli and Isman, 2014) and piscicidal activities (Kulakkattolickal, 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 available plant products are still limited, and hence, efforts should be made to find out new sources of botanical pesticides and piscicides for rapidly growing pisciculture (Ramanujam and Ratha, 2008). Plant extracts are referred to piscicides if they exert toxicological effects on fishes and cause death to these aquatic animals (Burkill, 1995). Plant piscicides are obtained from a variety of plants belonging to different families and species that may vary considerably not only for their taxonomic variations but also for the plant parts used (leaves, barks, fruits and seeds), mode of use, mode of extraction and species of target fishes (Van Andel, 2000; Neuwinger, 2004). Botanical materials contain a number of bioactive compounds that work either individually or synergistically as piscicides (Obomanu *et al*., 2007; Ramanujam and Ratha, 2008). The degree of toxicity as well as piscicidal activity of any plant extract can be assessed by exposing fishes to it and subsequent estimation of the median lethal concentration (LC50) (Ramanujam and Dominic, 2012). One of the common toxicological effects of plant extract is to bring about changes in haematological, biochemical parameters and antioxidants of fishes (Winkaler *et al*., 2007; Kavitha *et al*., 2012), which affect biological and physiological activities of fish. Adeyemo (2005) observed the haematological and histopathological effects of *Cassava mill* effluent in *Clarias gariepinus*.

The activities of man have been responsible for introducing contaminants into the environment in the last few decades (Awoyinka *et al.*, 2019). Environmental factors such as pH, turbidity, alkalinity, dissolved oxygen, temperature and conductivity influence the rate of reaction of the pollutants entering the water or the lethal effects on the aquatic organisms (Fagbenro, 2002). Nowadays, pollution of natural ecosystems is increasing so that with increasing human activities is becoming a fundamental problem. Determination of the toxic compounds in aquatic environments and their effects on aquatic organisms are a fundamental issue in ecotoxicology science. Toxic substances present in the environment can be determined by chemical analysis, but their effects on aquatic organisms in aquatic ecosystems cannot be determined by chemical analysis. Hence, to assess the environmental impact of toxic compounds, the use of bioassay experiments is necessary (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 definitely 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 ± 2°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 size 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 Zoology Department, Nasarawa State University, Keffi. The fish was transferred into aquaria containing dechlorinated municipal tap water and allowed to acclimatize to the laboratory conditions for a period of 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 Finney calculator. *V. amygdalina* crude leaves extract was 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 chocked 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 8 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±2.43g~~ and mean total length ~~12.501±0.39cm~~ 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, weighted 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 respective tissues with chilled phosphate buffer (0.1 M, pH 7.4), and 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 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 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 × 105 M - 1 cm -1. Results were expressed as μ mol -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 each 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 for the examination of the gills, kidney and liver of *O. niloticus* fingerlings exposed to aqueous crude leaves extract of *B. aegyptiaca*. The harvested gills, kidney and liver of *C. gariepinus* were fixed in 10% formalin for 3 days, cut into thin slices of 5 x 2 x 1 mm thick and then processed with the SPIN Tissue Processor (STP) 120 (Thermoscientific 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 embedding 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 ThermoScientific)) set at 4 micromes, 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 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^oC 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 histomorphometrical 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 ± standard errors of mean.

3. RESULTS

There is reduction in SOD activity as the concentration of the toxicant increases, the highest value 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 reduction in GSH activity as the concentration of the toxicant increases, the highest and lowest value 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 value 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 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 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.

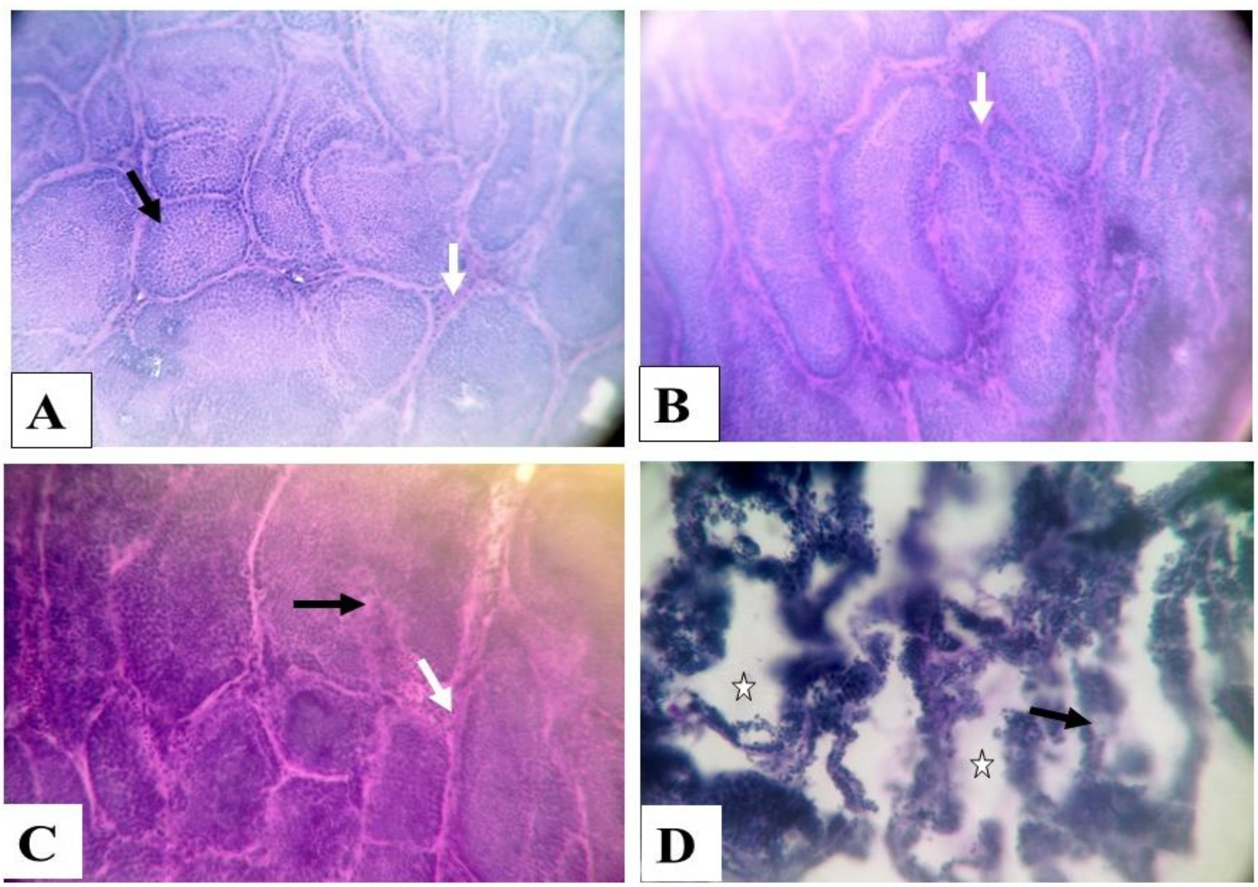

Plate A-D: Photomicrographs of the Testes of *Clarias gariepinus* Exposed to Graded Concentrations of *Vernonia amygdalina*.

Key: A. Control (0.00 g/L): The testes present normal numerous tubular structure 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 depletions (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.

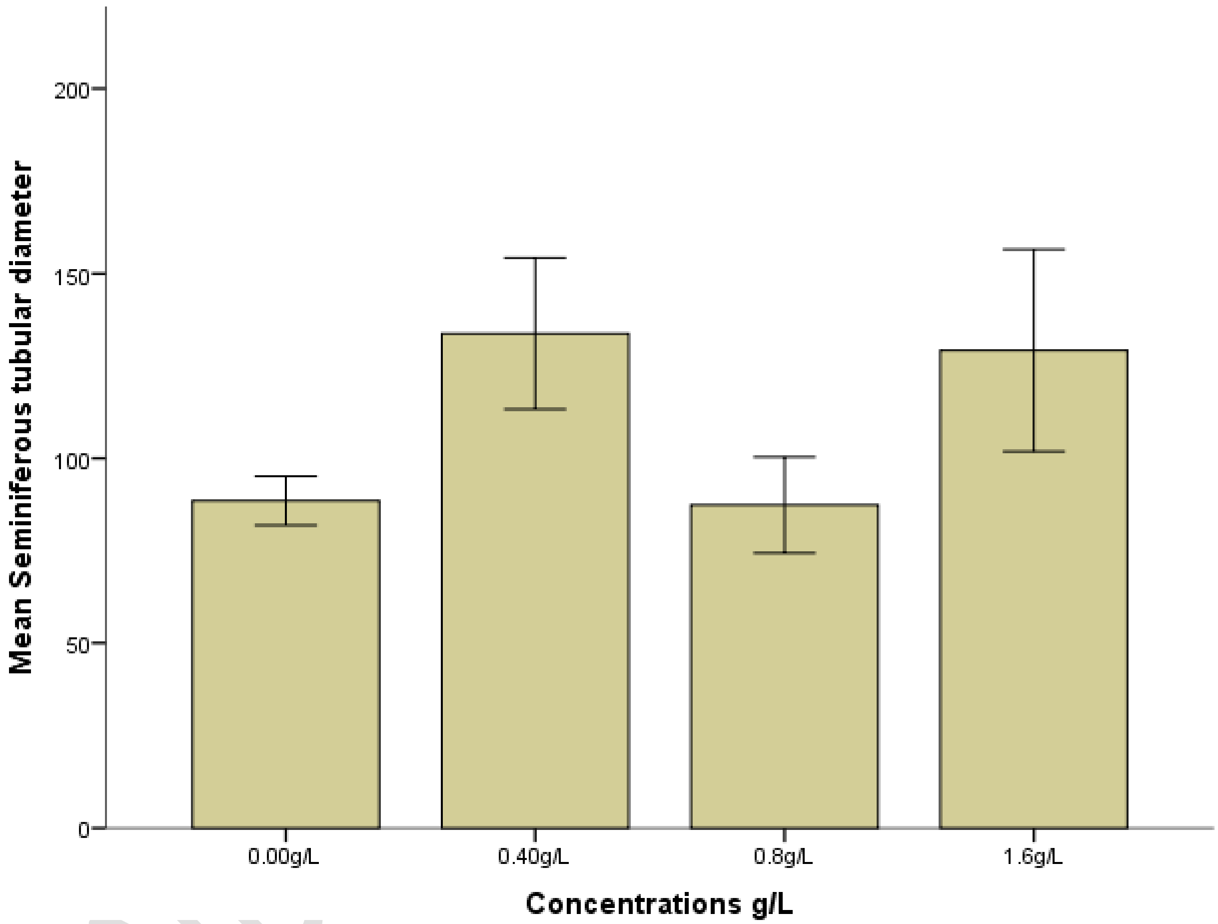

Figure 1: Mean Testes Somatic Index of *C. gariepinus* Juveniles Exposed to Concentrations of Aqueous Crude Leaves Extract of *V. amygdalina.*

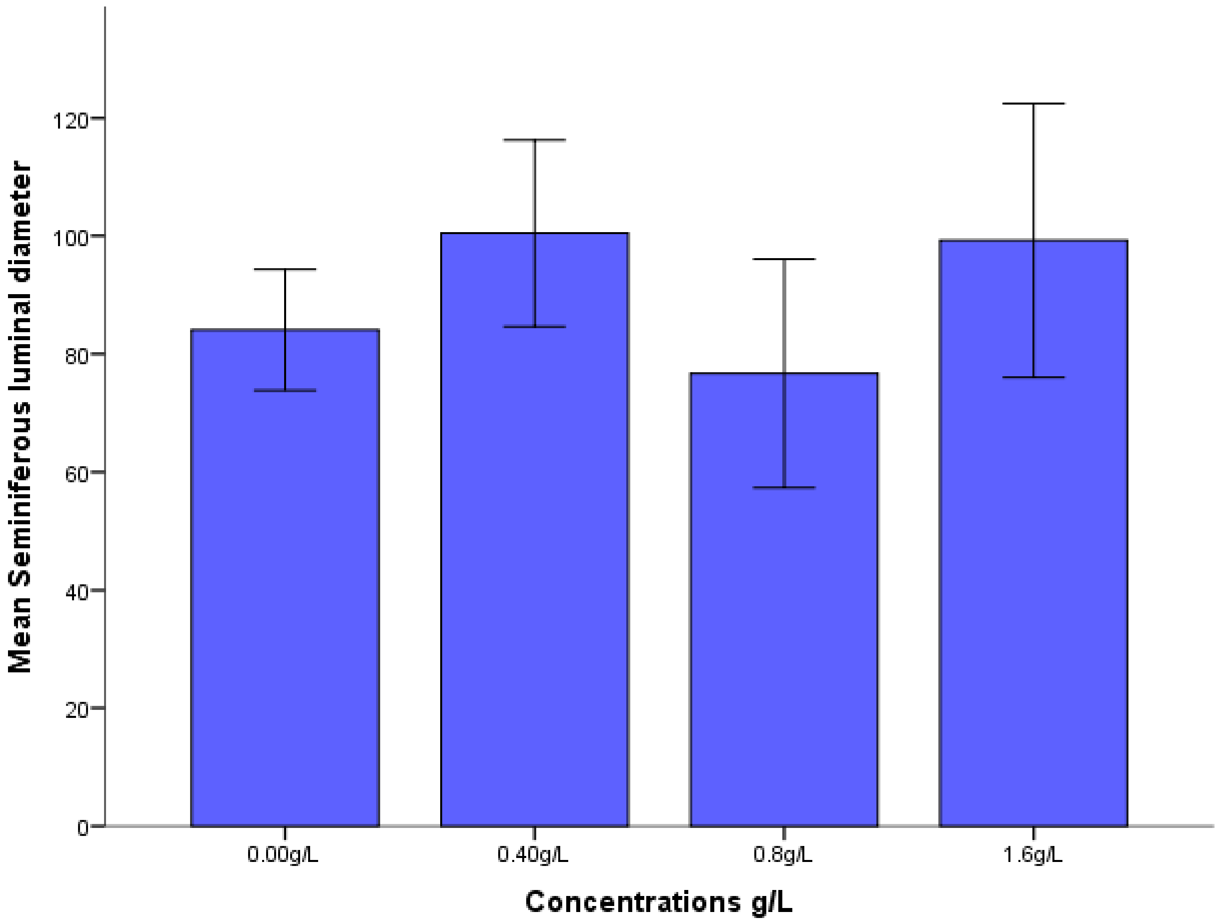

Figure 2: Mean Testes Somatic Index of *C. gariepinus* Juveniles Exposed to Concentrations of Aqueous Crude Leaves Extract of *V. amygdalina.*

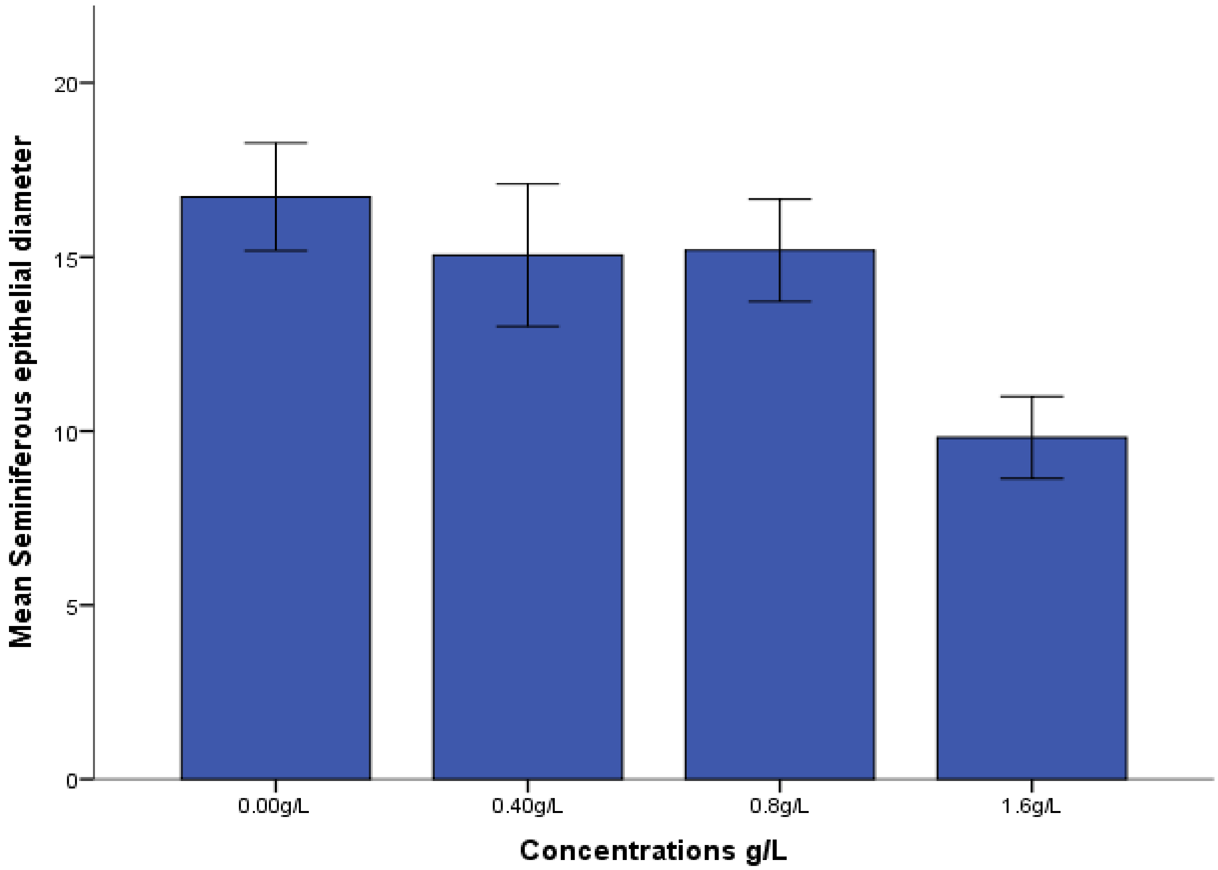

Figure 3: Mean Testes Somatic Index of *C. gariepinus* Juveniles Exposed to Concentrations of Aqueous Crude Leaves Extract of *V. amygdalina.*

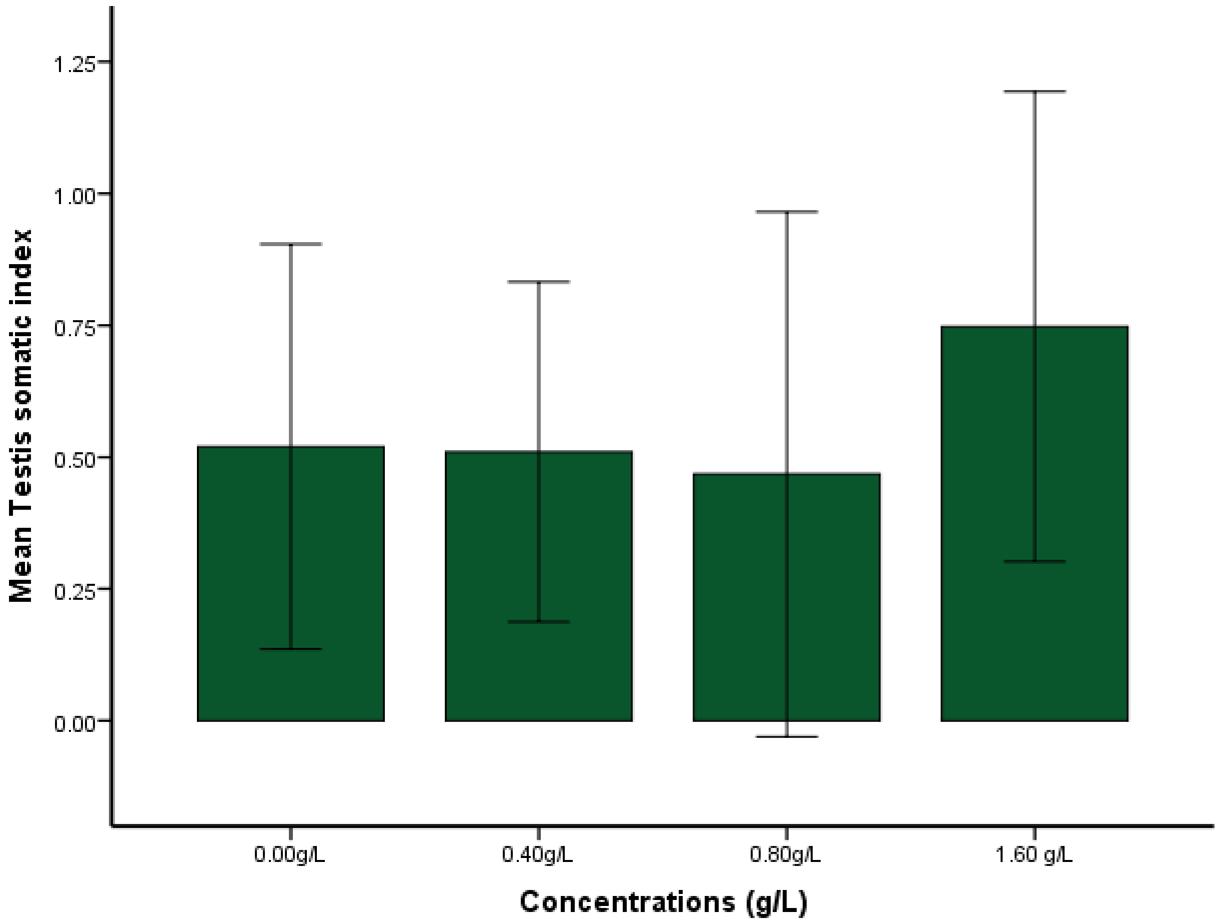

Figure 4: Mean Testes Somatic Index of *C. gariepinus* Juveniles Exposed to Concentrations of Aqueous Crude Leaves Extract of *V. amygdalina.*

4. DISCUSSION

Variation in testes antioxidant biomarkers (MDA, GSH, SOD) indicated oxidative stress in testes which 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 report from Alaa and Rania (2016) who opined that there was 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 μg/L 4-nonylphenol and quince (*Cydonia oblonga*) to fishes exposed for a period of 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 a 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 histology of the testis by 30th day of exposure, considerable reduction in spermatozoa in the testis when freshwater fish (*Rasbosa dandia)* was exposed to mercury. But they reported that there was 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 significant difference in the testis somatic indices of *C. gariepinus* with respect to 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 was 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 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 a period of 45 days. Gupta (2002) also reported that there was distortion of the seminiferous tubules in the testis of freshwater fish *Oreochromis mossambicus* in response to plant nutrient which is in line with the findings of this research work. But, he also recorded that there was 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 LC_{50} of phenol, whereas significant reduction was observed in the case of male fish subjected to 40% and 80% 96-h LC_{50} 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 booster of catfish should be discouraged.

6. RECOMMENDATION

Further toxicity research works of the effect of the plants on 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 use products in our area of research and country. There is absolutely 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 any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

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