



Changes in Gonad of Nile Tilapia (*Oreochromis niloticus*) Fed with Crude Extract of Guava (*Psidium guajava* Linnaeus) Leaves

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ABSTRACT: Tilapia is fraught with the problem of prolific breeding which usually leads to stunt growth thus making the fish undesirable for consumers. A56-days study was done to investigate the antifertility potential of *P. guajava* leaves extract on *Oreochromis niloticus* gonad, 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 replicated three times. Fishes were stocked in outdoor concrete tanks supplied with 450 litres of water. Six experimental diets (35% crude protein) containing varying sub-lethal concentrations of *P. guajava* leaf crude extract (0.0, 0.5, 1.0, 2.0, 4.0 and 8.0gkg⁻¹ diets) were formulated (representing O, A, B, C, D and E respectively; O serve as the control). The ovary and testis of fish fed control diet showed normal ovarian tissues and normal distribution of the testicular tissues. Mild atresia and reproduction in the oocytes were observed in the ovaries as the concentration of the crude extract increased from 0.5-8.0gkg⁻¹, while in the testes mild to severe atrophy and cystic seminiferous tubules were observed. This study infers that, for sustainable growth of Tilapia culture, *P. guajava* could be used to solve the problem of prolific breeding, thus energy used in reproduction can be converted to growth.

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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 (Francisa *et al.*, 2005; Dada and Ikuerowo, 2009; Obaroh and Achionye-Nzeh, 2010) and to inhibit reproduction in Tilapia (Francis *et al.*, 2001; Luckstadt *et al.*, 2006; Obaroh and Achionye-Nzeh, 2011). This is because plant materials had been observed to be safe, cheap and environmentally friendly when compared with synthetic substances. *Psidium guajava* leaf is a tree that grows very fast it had been reported to reach a height of 15-20m, it is an evergreen tree with fairly dense crown. The photochemical 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 (Biu *et al.*, 2009). Biological and Pharmacological activities attributed to different parts and extracts of these plant include anti-plasmodial, anti-trypanosoma, antioxidant, anticancer, antibacterial, antiviral, larvicidal, and fungicidal activities. Other include antiulcer, spermicidal, anthelmintic, anti-diabetes, anti-implantation,

immnomodulative, molluscicidal, nematocidal, immunocontraceptive, insecticidal, antifeedant and insect repellent effects (Ganguli, 2002; Meymand *et al.*, 2004; Atawodi and Atawodi, 2009). The antifertility effects of *Psidium guajava* leaf on white mice (Sri Retno *et al.*, 2008) have been reported. Ekanem and Okoronkwo, (2003) observed disintegrated cells in the high dose treatment when some group of *Oreochromis niloticus* were fed with feed containing pawpaw seeds. Abdelhamid *et al.*, (2010) reported coagulation necrosis in yolk granules and atresia of ripe oocytes when 2.0g therigon/kg diets were fed to *Oreochromis niloticus*. Titiana *et al.*, (2009) also reported that *Oreochromis niloticus* liver treated with 280mg/kg of *Hyptidendron canum* crude ethanol extract showed slight capillary dilation.

There is little or no detailed information on the effects of sub-lethal concentrations of *P. guajava* crude extract on gonads of *O. niloticus*. Thus, the aim of this study was to study the structural changes in gonads of *O. niloticus* due to different sub-lethal concentrations of *Psidium guajava* leaf extract.

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MATERIALS AND METHODS

Identification and Preparation of Plant Materials: *Psidium guajava* fresh leaf were collected within New-Bussa metropolis, they were authenticated at the herbarium section of Department of Biological Science Kebbi State University of Science and Technology Aliero, 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 100g of dried powdered sample in 250ml of ethanol for 24 hours the filtrate was concentrated into jelly-like semi solid substance and stored in the refrigerator.

Preparation of Experimental Diets: The feedstuffs were obtained locally from the market. Basal feed was formulated to provide 35% crude protein (Table 1). *Psidium guajava* leaf extract was added to the basal diet at sub-lethal concentration of 0.5, 1.0, 2.0, 4.0 and 8.0gkg⁻¹. The feedstuff were thoroughly mixed in a pelleting/mixing machine, hot water was added at intervals to gelatinize the starch, feeds were pelletized using 2mm diameter die, air dried and each packed in a polythene bag labeled and stored in the refrigerator till when needed. The proximate compositions of the experimental diets were analyzed using (AOAC, 1990) method of analysis.

Experimental Design: One hundred and eighty *Oreochromis niloticus* of average size 29.30±2.02 – 31.79±3.11g were obtained from National Institute for Freshwater Fisheries Research. New Bussa, Nigeria. Fishes were acclimatized for one week, after acclimatization they were divided into 6 groups and each group was replicated three times, each replicate consist of 10 fishes, these were stocked in outdoor concrete tank (2x2x1.25m) supplied with 450 litres of water. The water quality parameters were within the acceptable range for tilapia culture (Dissolved oxygen 5.00±0.06 – 5.65±0.45mg/L, pH 7.51±0.05 – 7.70±0.05 and Temperature 27.00±1.00 – 28.50±0.50°C). Fish were fed 3% body weight/day with the 6 experimental diets (represented as O, A, B, C, and E respectively) at two installments 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.

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 and testes. 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 6µ thickness, stained with Hematoxylin-Eosin (Morrison, 2007) and examined using the light microscope and the photomicrographs were taken by the use of mounted digital camera (Sony DSC-S3000 model).

RESULTS AND DISCUSSION

Ingredients and Proximate Composition of the Experimental Diet: Table 1 shows the ingredients used in formulating the basal diet and the sub-lethal concentrations of *P. guajava*, ingredients were obtained from the local market. Table 2 shows the proximate composition of the six experimental diets. Addition of the crude extract of *P. guajava* leaf at different sub-lethal concentration was observed not to have significant effect on the proximate composition of the experimental diets. Similar observation was reported by Dada and Ikuero, (2009) when *Garcinia kola* extract was used in formulating feed.

Table 1: Ingredients composition of experimental diets

Ingredients	Treatments					
	O	A	B	C	D	E
Fishmeal	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
Groundnut cake	08	08	08	08	08	08
Vitamin premix	03	03	03	03	03	03
Cassava starch	02	02	02	02	02	02
Crude Extract	0.0	0.5	1.0	2.0	4.0	8.0

Vitamin/mineral premix: Vitamin A, 1.U.; Vitamin D, 11252U; Vitamin E, 71 U, Vitamin K3, 2mg; Vitamin B12, 0.015mg; Panthothenic 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

Parameters	Treatments					
	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

Effects of Crude Extract of *P. guajava* 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 *P. guajava*

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 0.5gkg^{-1} diet (A) showed mild atrophy of the testicular tissues, testis of fish fed 1.0gkg^{-1} diet (B) showed mild atrophy of the testicular tissues and cystic seminiferous tubules. Testis of fish fed 2.0gkg^{-1} diet (C) showed mild atrophy of the testicular tissues. Testis of fish fed 4.0gkg^{-1} diet (D) showed severe atrophy of the testicular tissues, while testis of fish fed with 8.0gkg^{-1} diet (E) showed atrophy of the testicular tissues and mild cystic seminiferous tubules. Mild to severe 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 high doses have been reported in rabbits and rats (Kassem *et al.*, 2006; Udoh and Ekpenyong, 2001). Meymand *et al.*, (2004) also observed abnormality in the spermatogenesis and sperms production in some seminiferous tubules of rats that were administered varying concentration of *P. guajava*.

Effects of Crude Extract of *P. guajava* 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 *P. guajava* 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 0.5gkg^{-1} diet (A) the ovary showed mild atrophy of the oocytes (AT). Ovary of fish fed 1.0gkg^{-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 2.0gkg^{-1} diet (C) showed severe atrophy of the oocytes. Slight distortion of the secondary oocyte (OS) and wall of the oocytes and clumping of the yolk granules, ovary of fish fed 4.0gkg^{-1} diet (D) showed severe atrophy of the ripe oocytes and shrinking of the wall of the oocytes while ovary of fish fed with 8.0gkg^{-1} diet showed necrosis and hyperplasia of the oocytes.

The histopathological study of *O. niloticus* gonads in this work showed increase in the alteration of these organs as the crude extract inclusion level increases. Mild to severe necrosis and atrophy were observed in the ovaries of the fish that were treated with the crude extract of *P. guajava* leaves. Jegede and Fagbenro, (2008) reported similar observation in ovaries of *Tilapia zillii* that were fed with different inclusion of *Azadirachta indica* meal. Ekanem and Okoronkwo (2003), reported swollen nuclei in the low dose treatment and disintegrated cells in the high dose treatment when some group of male *Oreochromis*

niloticus were fed with feed containing low and high dose of pawpaw seed. But in contrast, Abdelhamid *et al.*, (2010) observed coagulation necrosis in yolk granules at low concentration when a commercial feed additive was fed to *O. niloticus*.

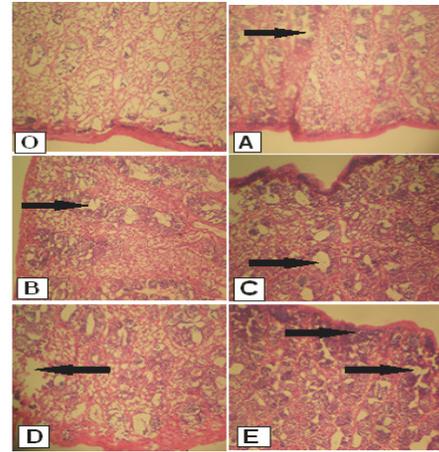


Plate 1: Sections of testes of *O. niloticus* fed with different inclusion levels of *P. guajava* leaf, Haematoxylin and Eosin stained.

O: Fish fed with the control diet (0.0 g/kg) showing the normal testicular tissues with the spermatozoa well distributed, (x150). **A:** Fish fed with 0.5 g/kg diet, showing mild atrophy of the testicular tubules, (x150). **B:** Fish fed with 1.0g/kg diet, showing mild atrophy of the testicular tubules, (x150). **C:** Fish fed with 2.0g/kg diet, showing mild atrophy of the testicular tubules, (x150). **D:** Fish fed with 4.0g/kg diet, showing slight degeneration of the testicular tubules, (x150). **E:** Fish fed with 8.0g/kg diet, showing mild atrophy of the testicular tubules and cystic seminiferous tubules, (x150).

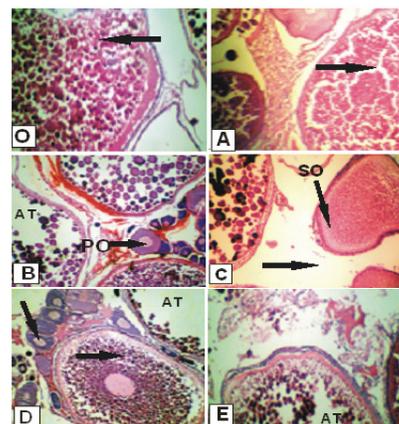


Plate 2: Sections of ovaries of *O. niloticus* fed with different inclusion level of crude extract of *P. guajava* leaf, (Haematoxylin and Eosin stained).

O: Fish fed with the control diet (0.0g/kg), showing normal ovarian tissues at the ripe stage of the oocytes, (x300). **A:** Fish fed with 0.5g/kg diet, showing mild atresia of the oocytes, (x300). **B:** Fish fed with 1.0g/kg diet, showing slight reduction in the number of oocytes, (x300). **C:** Fish fed with 2.0g/kg diet, showing large space between the ripe oocyte and the secondary oocyte with slight distortion in the shape of the secondary oocyte, (x300). **D:** Fish fed with 4.0g/kg diet, showing several primary oocytes around a matured secondary oocyte, mild atresia and atrophy of the oocyte, (x300). **E:** Fish fed with 8.0g/kg diet, showing slight reduction in the number of oocytes (AT) and mild shrinking of the wall of the oocyte, (x300).

Conclusion: This study infers that, the potential use of *P. guajava* as phytoadditive in aquaculture could be exploited especially at low concentration. Furthermore, a baseline information has been established as to the effects of crude extract of *P. guajava* leaf on gonads of *Oreochromis niloticus*.

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