



HISTOPATHOLOGY OF GILL, LIVER AND KIDNEY TISSUES OF THE FRESHWATER FISH *Oreochromis niloticus* (LINNAEUS 1758) EXPOSED TO *Tamarindus indica* SEED HUSK POWDER

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ABSTRACT

The study evaluated potential tamarind seed husk powder bioaccumulation and its morphological effects in different organs of the freshwater fish, *Oreochromis niloticus*, after a single acute intra-peritoneal exposure (LC₅₀ of 1.52mg/l) and over progressively longer exposure times (24 h, 48 h, 72 h and 96 h). The tamarind seed husk powder accumulation was differential and time dependent for most target organs (Gill > Liver > Kidney). The liver showed progressive tamarind seed husk powder accumulation, presenting its highest levels only at the 96 h exposure point. Histopathological alterations were observed in kidney, liver and gills from 24 h of tamarind seed husk powder exposure, and the extent of the alterations and their severity increased out to 96 h of exposure. These results showed a correlation between tamarind seed husk powder accumulation and the induced morphological damages in different organs along the time in a tropical fish species *Oreochromis niloticus*, being the histopathology a sensitive technique for the observation of the initial damage from tamarind seed husk powder exposure. The study demonstrated the necessity to regulate the discharge of tamarind seed husk powder in effluents from domestic and industrial sources into aquatic systems.

Keywords: Gill, Histopathology, Liver and kidney, Tamarind seed husk powder, *Oreochromis niloticus*.

INTRODUCTION

Health of aquatic organisms cannot be measured directly. Instead, only indicators of health can be measured and in turn used to assess the health status. Histology and histopathology can be used as biomonitoring tools or indicators of health in toxicity studies as they provide early warning signs of disease (Meyers and Hendricks, 1985). Histopathological alterations are biomarkers of effect of exposure to environmental stressors, revealing prior alterations in physiological and/or biochemical function (Hinton *et al.*, 1992). Fish is a suitable indicator for monitoring environmental pollution because they concentrate pollutants in their tissues directly from water and also through their diet, thus enabling the assessment of transfer of pollutants through the trophic web (Fisk *et al.*, 2001; and Boon *et al.*, 2002). Due to being exposed to pollutants, major structural damages may occur in their target organs, histological structure may change and physiological stress may occur. This stress causes some changes in the metabolic functions. The changes in the functions are initiated with the changes in the tissue and cellular level. Although qualitative data are used in most cases to study the pathologies the



environmental pollutants cause, quantitative data show better reactions of the organisms to pollutants (Jagoe, 1996).

Plants and derivatives of plant play a key role in world health and have long been known to possess biological activity. According to Burns (2000), thirty per cent of all modern drugs are derived from plants. Available evidence suggests that approximately 80% of Africans rely on traditional healthcare practitioners and medicinal plants for their daily healthcare needs (McKay *et al.*, 2007). Natural products have been an overwhelming success in our effort in fighting diseases. They have reduced pain and suffering, and revolutionized the practices of medicine. Natural products are the most important anticancer and anti-infective agents. More than 60% of approved and pre-new drug application (NDA) candidates are either natural products or related to them, not including biological such as vaccines and monoclonal antibodies (Demain, 1999).

Tamarind (*Tamarindus indica*), Family Fabaceae is a tropical leguminous tree. The leaves and husks possess active ingredients such as polyphenols, flavonoids, saponins, tannins and some oils. It is a multipurpose tree of which almost every part finds at least some use (Kumar and Bhattacharya, 2008), either nutritional or medicinal. Virtually every part of the tree (wood, roots, leaves, bark, seed husk and fruits) is of significant value in the subsistence of rural people as well as a number of commercial applications. The unique sweet or sour flavour of the pulp is popular in cooking and flavouring (El-Siddig *et al.*, 2006).

The Nile tilapia *Oreochromis niloticus* is a deep-bodied fish with cycloid scales, silver in colour with olive, grey or black body bars, the Nile tilapia often flushes red during the breeding season (Picker and Griffiths, 2011). It grows to a maximum length of 62 cm, weighing 3.65 kg (at an estimated 9 years of age) (FAO, 2012) and its average size (total length) is 20 cm (Bwanika *et al.*, 2004). *O. niloticus* is surface-feeding omnivore fish belonging to the family Cichlidae. It is the most widely cultured fish in the tropics (Offem *et al.*, 2010) and serves as an important source of high level of animal protein especially in the developing countries where there are high levels of animal protein deficiencies (Fagbenro and Adebayo, 2005; Uchida *et al.*, 2003; and Ogunji *et al.*, 2008).

MATERIALS AND METHODS The Study Area

The experiment was carried out in the Department of veterinary Nursing Department, Ahmadu Bello University, Zaria, Kaduna State. At the end of the experiment, one fish per treatment, that is three fish per concentration were sampled after 96hours exposure for histological analysis, the test organism was killed with a blow on the head, using a mallet and dissected to remove the kidney, gill and liver. The organs were fixed in 10% formalin for three days after which the tissue was dehydrated in periodic acid Schiff's reagent (PAS) following the methods of (Hughes and Perry, 1976) in graded levels of 50, 70, 90 and 100% alcohol for three days, to allow paraffin wax penetrates it. A gill arch of the right side of each fish was collected and fixed in Bouin's fluid for 24 hours, dehydrated in graded ethanol concentrations and embedded in paraffin wax. Sagittal sections (5µm of thickness) were cut and mounted on glass slides. Sections were deparaffinized in xylene, hydratated in ethanol and stained with hematoxylin-eosin (H&E). The liver was quickly dissected, sliced into 3 mm thick slabs, and immersed in Bouin's fixative for 24 hours, dehydrated, and embedded in paraffin; a minimum of 5 pieces resulted. Histological sections (5 µm of thickness) were cut and stained with H&E.



Changes induced by treatment in the gill, liver and kidney tissues were photographed and analyzed by light microscopy (Roberts, 1989).

Experimental Site

The experiment was conducted at the Fish Hatchery Laboratory of the Department of Fisheries and Aquaculture, Bayero University, Kano, Nigeria.

Experimental Fish

Three hundred and sixty (360) *O. niloticus* (mean weight, 19.56 ± 0.7 g and mean length, 12.42 ± 0.3 cm) juveniles were used for the study; fish were purchased from a reputable fish farm from Kano, Kano State.

Source and Processing of Tamarind Seed Husk

The tamarind seed husk was collected within the premises of Bayero University Kano. The plant was authenticated in the Department of Forestry and Wildlife Management, Bayero University Kano where a voucher specimen was maintained for the plant (NO. 1387). The seed husk was removed from the pod and air-dried to a constant weight at (27°C). The dried samples was grounded into fine powdered form and sieved through 0.25mm sieve.

Statistical Analysis

All results were collated and analyzed using computerized, Probit and Logit analysis. The mean lethal concentration LC₅₀ at selected periods of exposure and an associated 95% confidence interval for each replicate toxicity test were subjected to logit and probit analysis (Finney, 1971) using statistical package (SPSS, version 17.0) to determine (LC₅₀).

RESULTS AND DISCUSSION

Behavioral Changes of *Oreochromis niloticus* Juveniles in Tamarind Seed Husk Powder

The results presented in Table 1 summarized the behavioural changes observed in *Oreochromis niloticus* juveniles exposed to different concentrations of tamarind seed husk powder. Abnormal behaviours observed with *O. niloticus* exposed to various concentration of tamarind seed husk powder in this study were excessive gulping for air, erratic swimming behaviour, restlessness, loss of equilibrium, fin and barbell deformation, skin haemorrhage, discolouration and finally death agreed with Omitoyin *et al.* (2006). Ladipo and Doherty (2011) and Okomoda and Ataguba (2011) who worked on Paraquat dichloride glyphasphateisopropylammonium on *Clarias gariepinus*. This also agrees with reports of Bobmanuel *et al.* (2006) who stated that behavioural response of fish to toxicants and different reaction time are due to the effect of chemicals, their concentrations, species, size and specific environmental conditions.

Table 1: Behavioral Changes of *Oreochromis niloticus* Juveniles in Tamarind Seed Husk Powder during 96-hour Exposure at Different Concentrations

Behaviors	Concentration (mg/L)					
	0.00	1.2	1.4	1.6	1.8	2.00
Air gulping	-	-	+	++	+++	+++
Barbel deformation	-	-	-	-	-	-
Discoloration	-	-	+	++	++	++
Erratic swimming	-	-	+	++	+++	+++



Scratching on plastic tank	-	-	-	+++	+++	+++
Jumping	-	-	+	++	++	++
Resting at bottom	-	+	++	++	+++	+++
Hanging vertically in water column	-	+	++	++	++	+++
Fin deformation	-	-	+	++	++	++

Note: + n = slightly present; ++ = moderately present; +++ = highly present; - = absent.

In the study, the fish were observed to be stressed progressively with time before dying eventually. The stressed ailment of respiratory impairment due to the toxic effects of tamarind seed husk powder on gills was similar Omotoyin *et al.* (2006) and Konar (1970) who reported that accumulation of mucous on the gills reduces respiratory activity in fish. The inability of the gills surface to actively carryout gaseous exchange might be responsible for the observed mortalities in this study and was similar to Omitoyin *et al.* (1999), Fafioye (2001) and Omotoyin *et al.* (2002) reports. The behavioural changes observed in this study were similar to those reported for *O. mossambicus*, *T. niloticus* and *O. niloticus* exposed to leaf extracts of *Apodytes dimidiata* and *Thevitia nerifolia* (Sambisivam *et al.*, 2003).

Water Quality Parameters

The water quality parameters recorded (Table 2) were within acceptable ranges for toxicity test (APHA, 1998). They may not have acted synergistically with the toxicant to affect the behaviour as well as mortalities recorded in this study. Similar observations were reported by Onusiriuka (2000) in acute test solutions of Akee apple, *Blighia sapida* and *Kigelia africana* extracts and also reported by Gabriel and Okey (2009) to acute concentrations of *Lepidagathis alopecuroides* to which *C. gariepinus* were exposed.

Table 2: Water Quality Parameters during 96-hr Exposure of *Oreochromis niloticus* Juveniles to Tamarind Seed Husk Powder

Concentrations (mg/L)	Parameters Temp (°C)	Ph	Do (mg/L)	TDS (mg/L)	EC (mg/L)
Control (0)	27.55±0.05 ^a	7.64±0.01 ^a	5.53±0.04 ^a	293.50±2.50 ^a	592.50±5.50 ^c
1.2	27.45±0.05 ^a	7.59±0.03 ^a	5.36±0.01 ^b	301.00±1.00 ^a	608.00±2.00 ^c
1.4	27.55±0.05 ^a	7.56±0.04 ^a	5.32±0.05 ^b	311.50±1.50 ^b	626.00±3.00 ^b
1.6	27.56±0.06 ^a	7.61±0.04 ^a	5.20±0.02 ^c	327.00±2.00 ^c	635.00±1.00 ^b
1.8	27.69±0.01 ^a	7.57±0.05 ^a	4.09±0.02 ^d	346.00±2.00 ^d	683.50±1.50 ^a
2.0	27.48±0.03 ^a	7.51±0.04 ^a	3.58±0.03 ^e	349.50±2.50 ^d	692.00±0.00 ^a



Histopathological changes in the gill, liver, and kidney were observed for all the treatments. Lesions were essentially similar for all treatments and exposure time, although the intensity of cell damage increased with increasing concentration and time of exposure. The normal histology of the gill structure of *Oreochromis niloticus* exposed to 0 mg/l of TSH powder is shown in Figure 1. Following the exposure to Tamarind seed husk powder, changes in histological structure were noted particularly in the filament and lamellae. Figure 2 Shows half of the gill arch have it filament and lamellae degenerated in fish treated with 1.2mg/l. Hypertrophy of the gill arch, degenerated gill filament and lamellae (Figure 3) observed at 1.4 mg/l of Tamarind seed husk powder. Figure 4 shows the central parts of the gill arch have both the lamellae and filament degenerated, while those at both end not affected in fish treated with 1.6mg/l of Tamarind seed husk powder. High degenerated lesion (Figure 5) observed at the gill arch, filaments, and lamellae in concentration of 1.8mg/l. Hypertrophy of gill arch and degenerated gill filament and period complete degenerated filaments and gill arch (Figure 6) in concentration of 2.0 mg/l within 96 hours exposure.

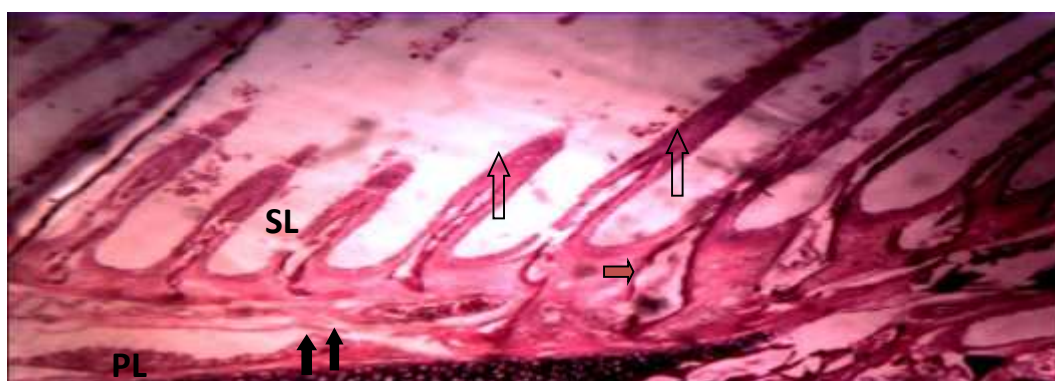


Figure 1: Gills of *Oreochromis niloticus* in the control tank shows normal aspect of the gill filaments (black arrow), the lamellae (L), water channel (*), a pillar cell (white arrow), epithelial lining (arrowhead), primary lamellae (PL) and secondary lamellae (SL).

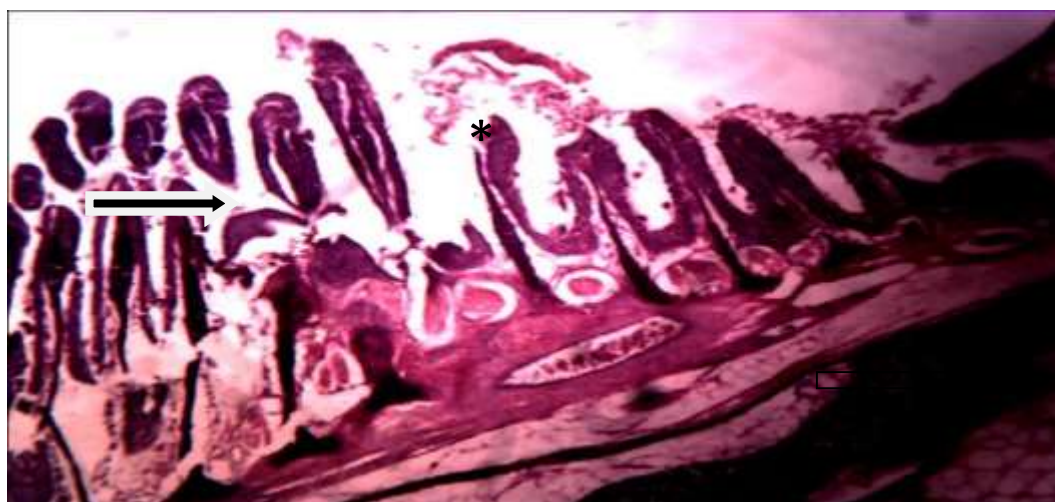




Figure 2: Shows Gills exposed to 1.2mg/l of tamarind seed husk powder alterations seen are lamellar disorganization (black arrow) hyperplasia of lamellar epithelium cell (white arrow), deformed secondary lamellae (D) erosion of epithelial lining of the secondary lamellae (N).

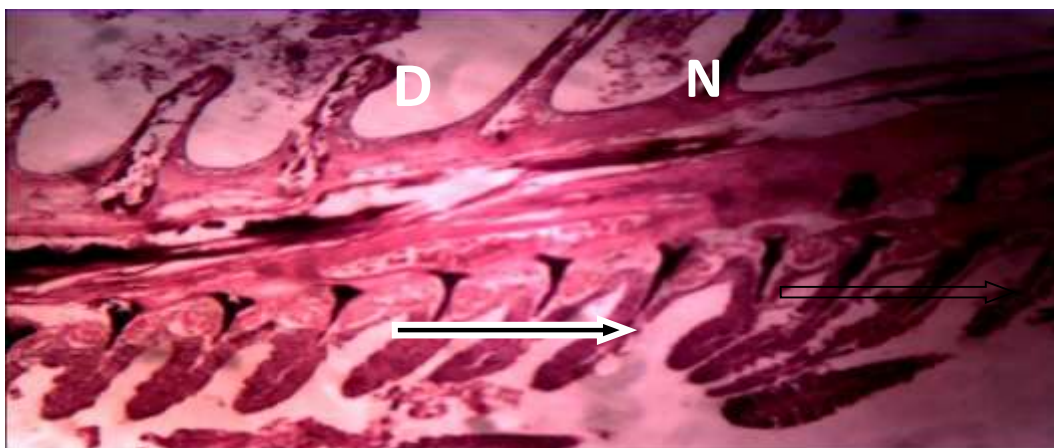


Figure 3: Gills of *Oreochromis niloticus* juveniles exposed to 1.4mg/l of tamarind seed husk powder shows distortion of gill filaments as some have blanketed and eroded (black arrow), epithelium rupture with haemorrhage, blanketing and blood congestion (white arrow).

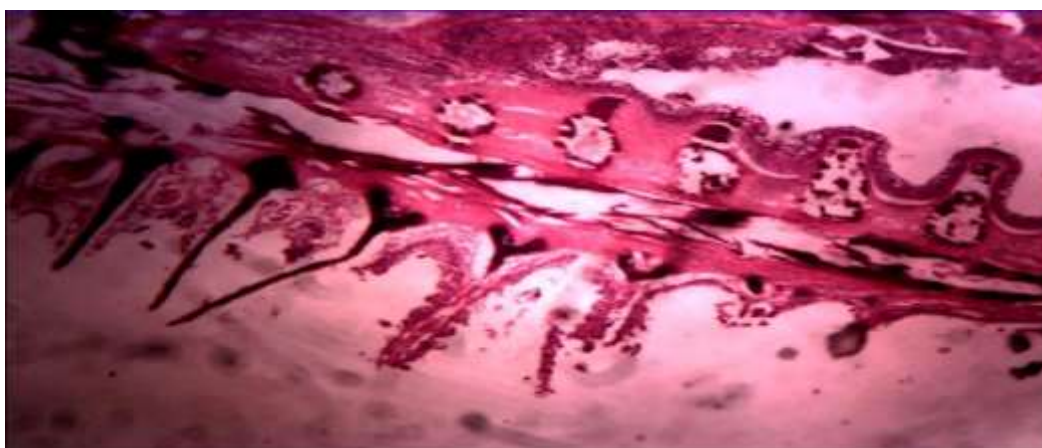


Figure 4: Gills of *Oreochromis niloticus* exposed to 1.6mg/l of tamarind seed husk powder shows distortion of gill filaments as some have blanketed and eroded (black arrow) and epithelium rupture with haemorrhage, intense cellular necrosis and blanketing and blood congestion (white arrow).

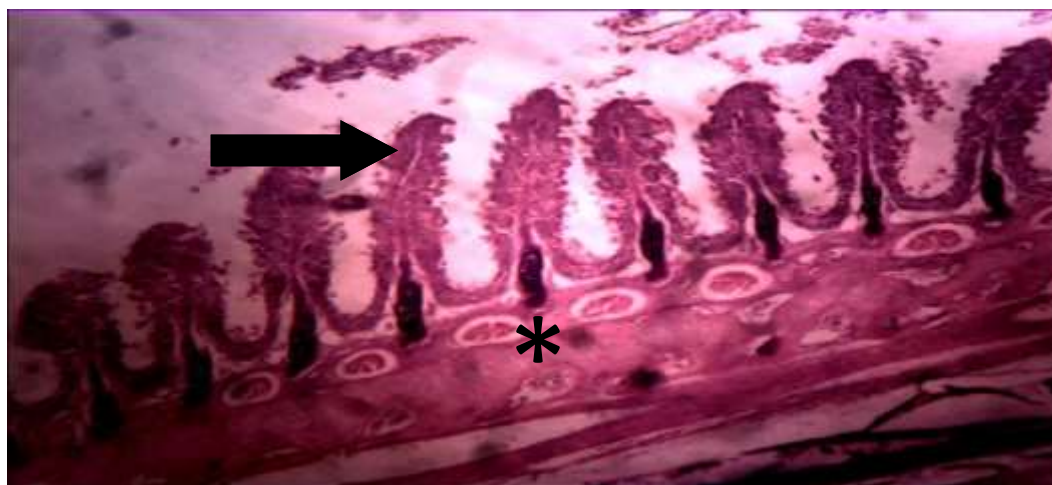


Figure 5: Gills of *Oreochromis niloticus* juvenile exposed to 1.8mg/l of tamarind seed husk powder shows epithelial rupture with haemorrhage (black arrow) hyperplasia of lamellar, blanketing (white arrow) and epithelial and diffusion of secondary lamellae (D).

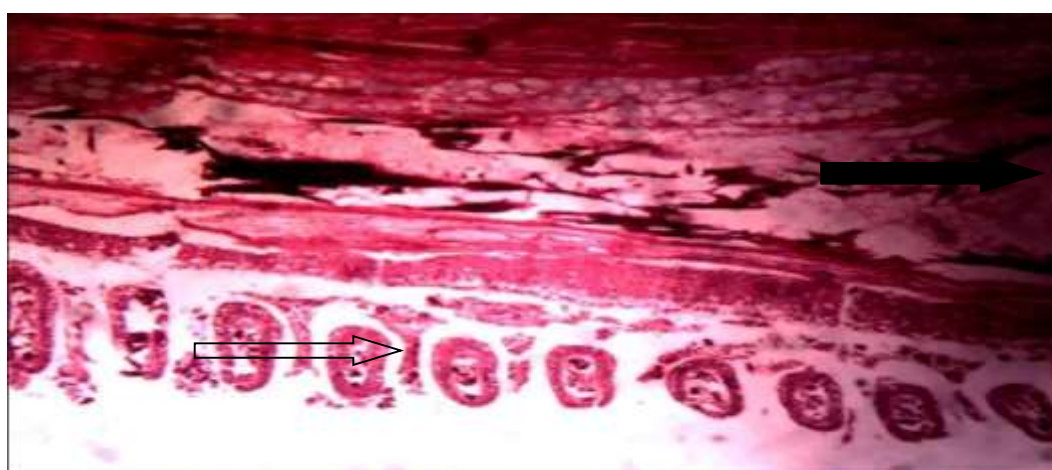


Figure 6: Gills of *Oreochromis niloticus* juveniles exposed to 2.0mg/l of tamarind seed husk powder shows blanketing of the lamellae with distortion of pillar cells (black arrow) and hyperplasia of epithelial cells (white arrow), epithelial lifting and oedema (G)

Figure 7 shows the normal liver cell no pathological lesion observed in the control fish. Disarrangement of hepatic cords, deformed and atrophied hepatocytes with shrunken, displaced nuclei, aggregation of nuclei due to loss of cell boundaries, vacuolation of hepatocytes, and congestion of blood in the blood vessels in Figure 8 exposed to concentration of 1.2ml/g of TSH powder treated fish. Fibrosis, rupture of hepatocytes, haemorrhages and vacuolation of cytoplasm (Figure 9) was recorded in concentration of 1.4 mg/l of tamarind seed husk powder. Cytoplasmic vacuolation, bile pigment disintegration and eosinophilic granules in the cytoplasm (Figure 10) were observed in concentration of 1.6 mg/L of tamarind seed husk powder treated fish. Figure 11 shows liver with hepatic tissue showing focal necrosis, bile stagnation and cytoplasmic degeneration in concentration of 1.8 mg/l of tamarind seed husk powder treated fish. Disarrangement of hepatic cords, shrunken and displaced nuclei and vacuolation of hepatocytes were observed in the highest concentration of 2.0mg/l (Figure 12). This result is similar to the work of Wade *et al.* (2002), Fagbenro and Adebayo, (2005), and

Akinbulumo *et al.* (2005) who worked on the toxicity varying toxicants on *Oreochromis niloticus*.

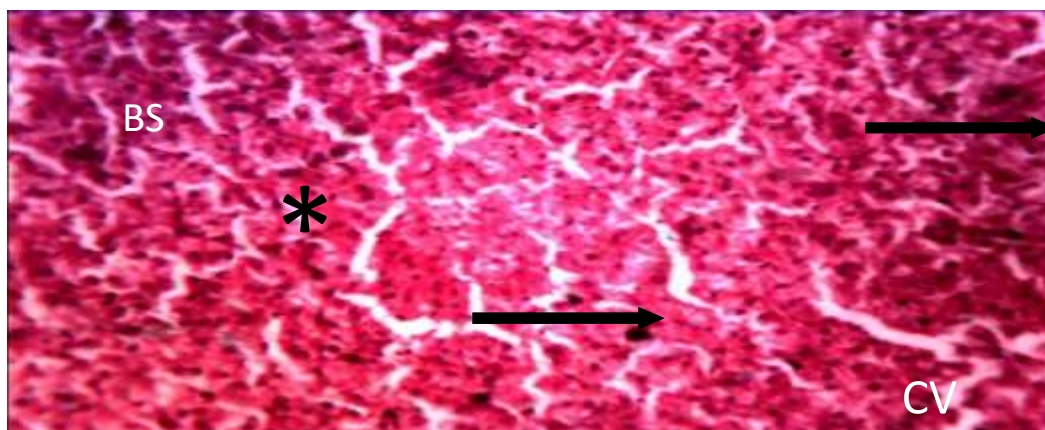


Figure 7: Liver in the control tank shows normal hepatic tissue, hepatocytes with granular cytoplasm, central and round nucleus (black arrow), blood sinusoid (BS) and central veins (CV).

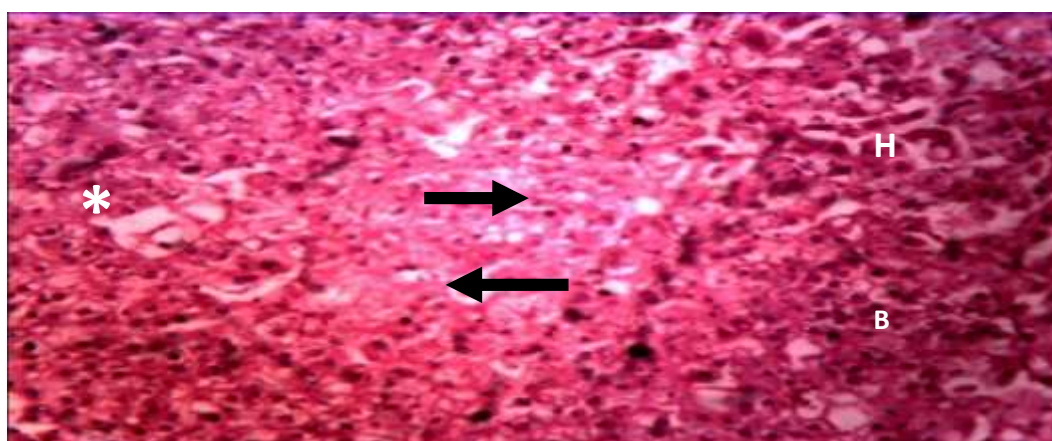


Figure 8: Liver exposed to 1.2mg/l of TSH powder shows disarrangement of hepatic cords, deformed and atrophied hepatocytes (H) with shrunken, displaced nuclei, aggregation of nuclei (black arrows) due to loss of cell boundaries, vacuolation of hepatocytes, congestion of blood in the blood vessels (B).

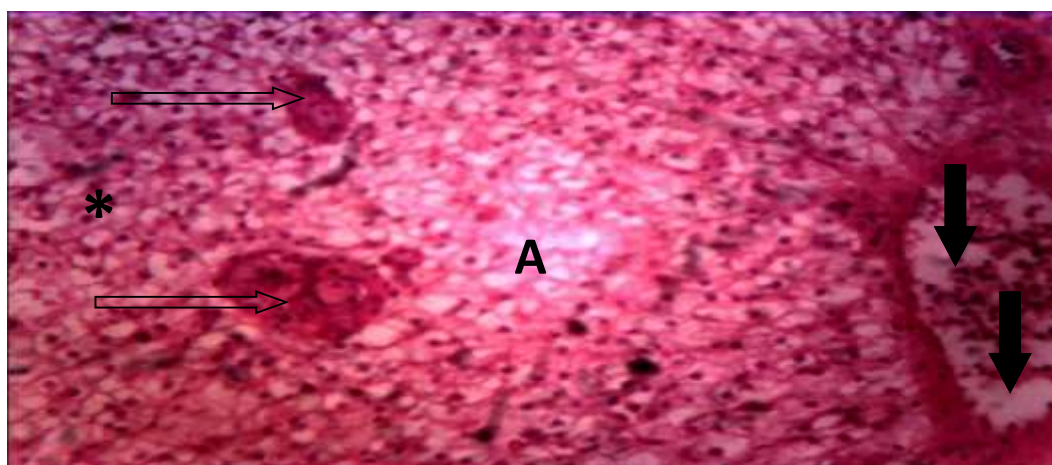




Figure 9: Liver exposed to 1.4 mg/l of TSH powder shows fibrosis (black arrows), rupture of hepatocytes (white arrows), haemorrhages (*) and vacuolation of cytoplasm (A).

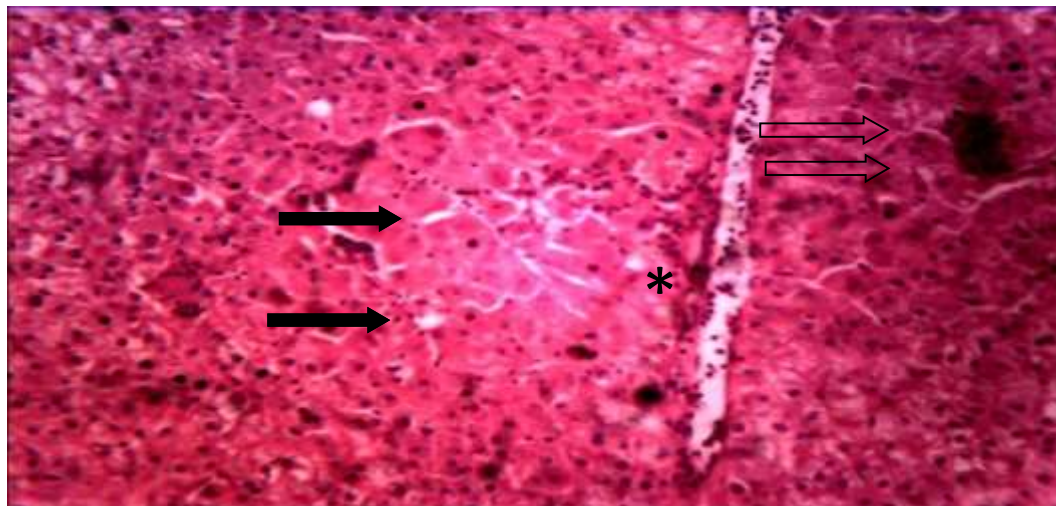


Figure 10: Liver exposed to 1.6mg/l of TSH powder shows cytoplasmic vacuolation (black arrows), bile pigment disintegration (white arrows) and eosinophilic granules in the cytoplasm (*).



Figure 11: Liver expose to (1.8mg/l) TSK alterations observed are liver with hepatic tissue showing focal necrosis (black arrow), bile stagnation (white arrows), and cytoplasmic degeneration (*).

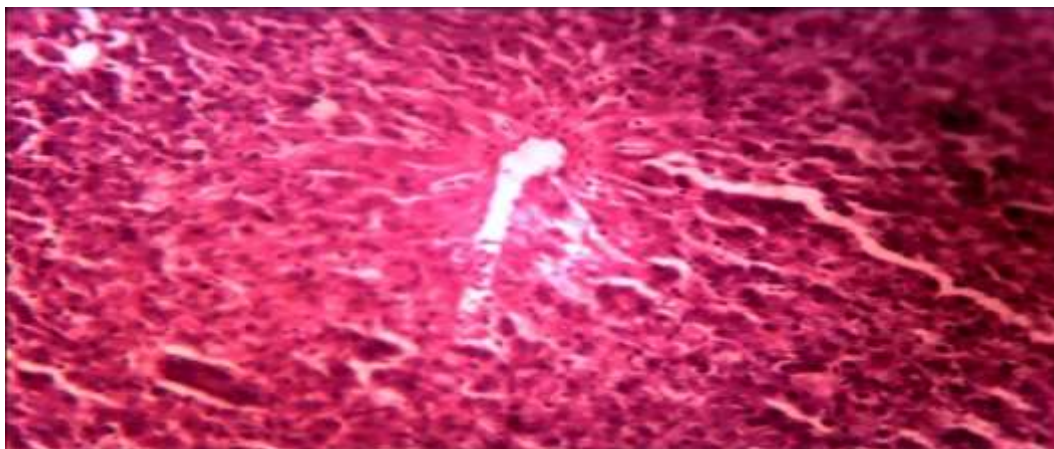


Figure 12: Liver exposed to 2.0mg/l of tamarind seed husk powder show disarrangement of hepatic cords (white arrow) shrunken and displaced nuclei (black arrow) and vacuolation of hepatocytes (L).

Figure 13 shows kidney of *Oreochromis niloticus* in the control tank with normal aspect intestinal cells, Glomerular tissue, collecting tissue, proximal tubule and distal tubule. Figure 14-18 shows kidney exposed to 1.2-2.0mg/l of TSH powder alteration seen are granular degeneration, hyaline droplets, mild damage renal tubules and glomerular oedema, necrosis, cellular hypertrophy, haemorrhages, dilation of Bowman's space and degeneration, degeneration of kidney tubules with bacterial colony fat droplets, necrosis and shrinkage of hepatocytes, cytoplasmic vacuolation, hyaline droplets degeneration and dilation of glomerulus capillaries.

The kidney is a highly dynamic organ in most of the vertebrates. Kidney receives about 20% of the cardiac output. Any chemical substances in the systemic circulation are delivered in relatively high amounts to this organ. Thus a nontoxic concentration of a chemical in plasma could become toxic in the kidney. The kidney of the fish receives largest proportion of postbranchial blood, and therefore renal lesions might be expected to be good indicators of environmental pollution (Ortiz *et al.*, 2003). In the control, kidney of *O. niloticus* revealed normal kidney histology, no noticeable alteration and lesion (figure 13), while other treatments revealed varying levels alteration such as granular degeneration, hyaline droplets, cellular hypertrophy, haemorrhages, dilation of Bowman's space, degeneration mild damage renal tubules and glomerular oedema (Figure 14-18). In a study by Jegede (2007) on toxic effects of sodium chloride on *O. niloticus* fingerlings, it was discovered that at high concentration, the kidney has been distorted.

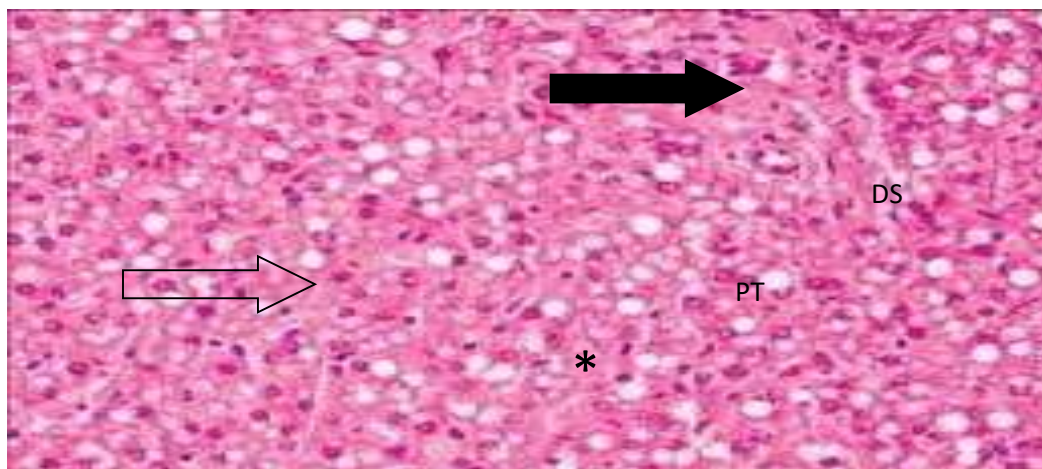


Figure 13: Kidney of *Oreochromis niloticus* in the control tank shows normal aspect intestinal cells (black arrow), Glomerular tissue (white arrow), collecting tissue (*), proximal tubule (T) and distal tubule (DS).

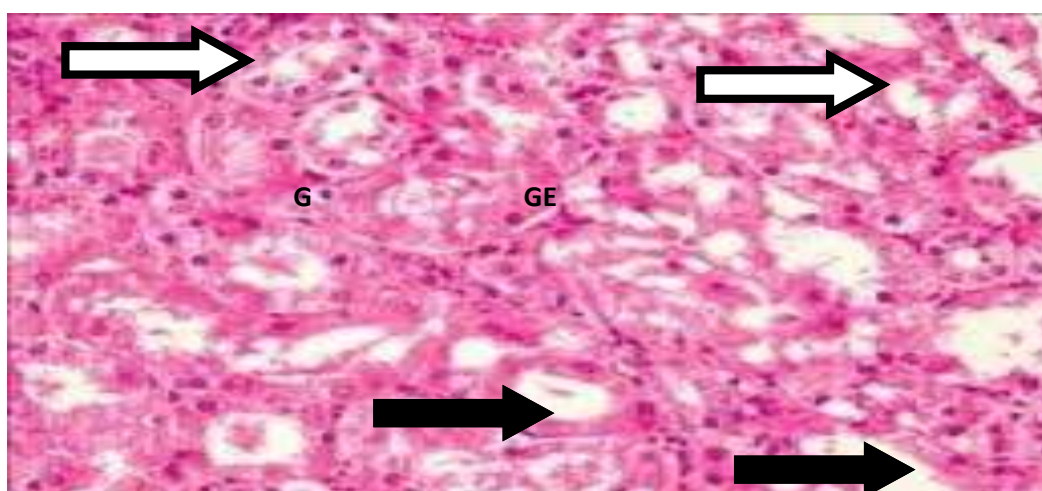


Figure 14: Kidney exposed to 1.2mg/l of tamarind seed husk powder shows granular degeneration (black arrows), hyaline droplets (G), mild damage renal tubules (white arrows) and glomerular oedema (GE).

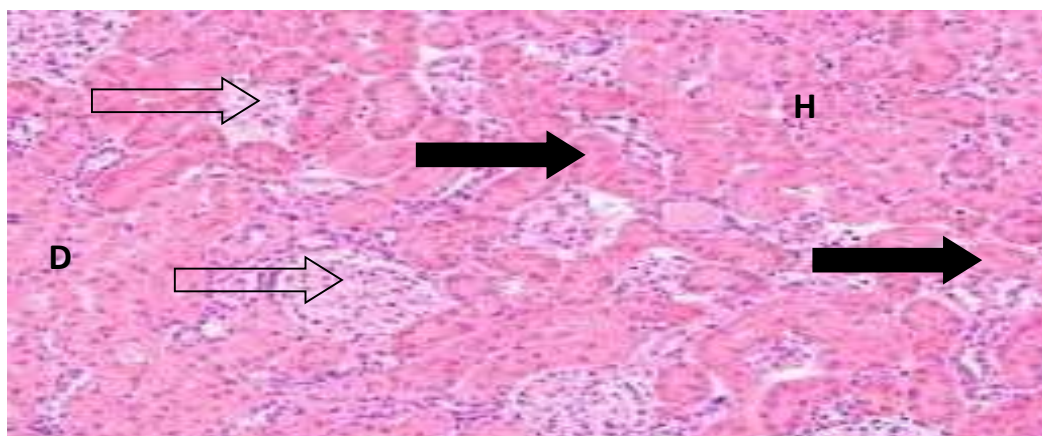




Figure 15: Kidney exposed to 1.4mg/l of tamarind seed husk powder shows tubular necrosis (D), cellular hypertrophy (G), haemorrhages (H), dilation of Bowman's space (black arrows) and degeneration (white arrows).

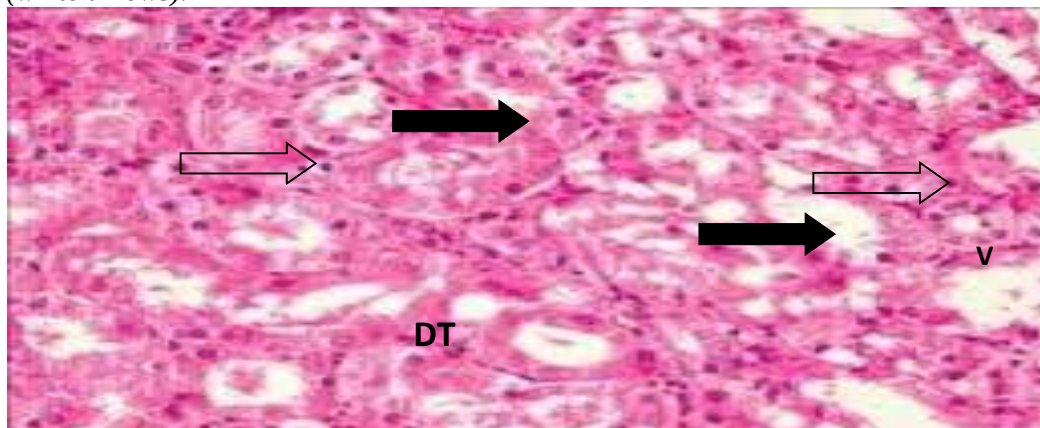


Figure 16: Kidney exposed to 1.6mg/l of tamarind seed husk powder show degeneration of kidney tubules with bacterial colony (DT), vacuum (V), fat droplets (white arrow) and necrosis and shrinkage of hepatocytes (black arrow).

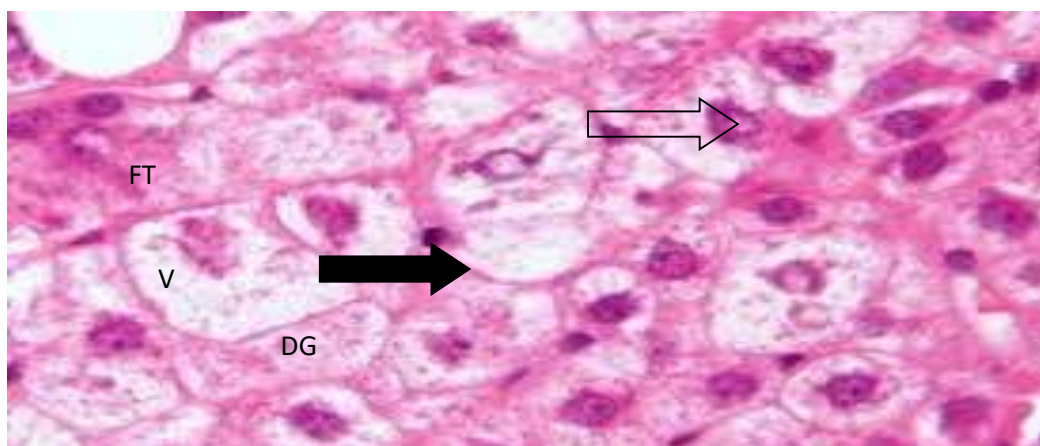


Figure 17: Kidney exposed to 1.8mg/l of tamarind seed husk powder shows dilation of glumerulus (DG), vacuum (V), degeneration of renal tubules (white arrow), necrosis of tubular lumen and fat droplets (FT).

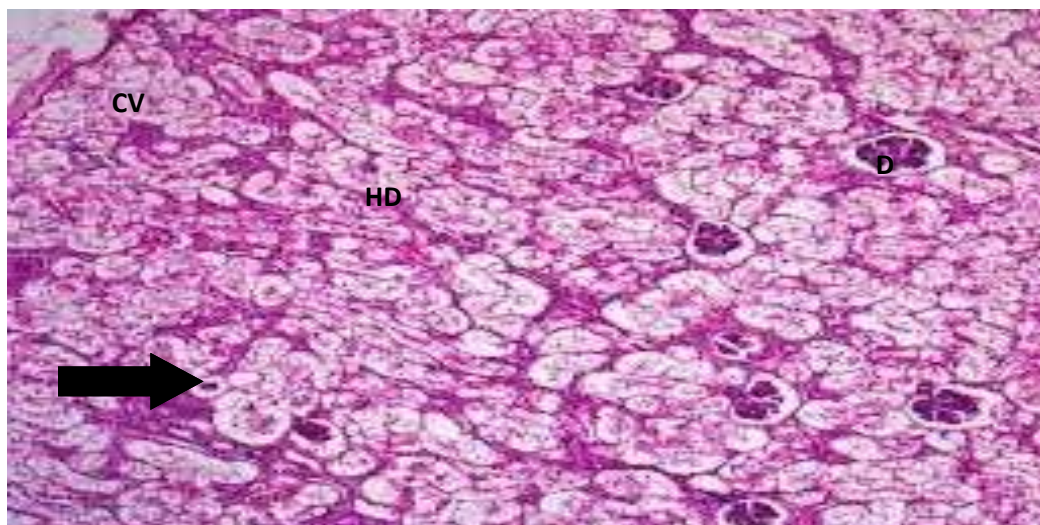


Figure 18: Kidney exposed to 2.0mg/l of tamarind seed husk powder shows Cytoplasmic vacuolation (CV), hyaline droplets degeneration (HD), dilation of glomerulus capillaries (D), cellular hypertrophy (black arrow), haemorrhage in Bowman's space (white arrow).

CONCLUSION AND RECOMMENDATIONS

The study reinforces the application of histopathology as a powerful tool for monitoring anthropogenic contamination within aquatic environments. Histopathological analysis showed morphological alterations in gills, liver and kidney of tamarind seed husk powder-exposed fishes revealing differences in the types and severity of lesions according to increases in exposure time. These results are important in establishing a direct correlation between tamarind seed husk powder accumulation and morphological damage, and therefore help to characterize the mechanism of tamarind seed husk powder-induced pathogenesis. The study demonstrated the necessity to regulate the discharge of tamarind seed husk powder in effluents from domestic and industrial sources into aquatic systems. Whilst links between such pathologies and contaminants are not definitive, such surveillance provides a useful insight into individual, population and overall ecosystem quality. When these pathological endpoints are assessed in conjunction with other parameters such as parasite community structure, sediment and water chemistry, enzyme responses, bile metabolite levels and molecular damage indices, a clearer picture of the complex interactions between anthropogenic and natural environmental modifiers will emerge.

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