Studies on Cassava Mill Effluent and its Toxicological Impact using Histopathological Technique

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ABSTRACT

This study was carried out to determine the pathological effects of cassava mill effluent on Clarias gariepinus. The histological changes in the gills and liver of C. gariepinus exposed to cassava mill effluent at different sub-lethal concentration under static bioassay procedure were experimentally determined. In this study, the most generally encountered type of degenerative changes was congestion, vacuolization of hepatocyte, cellular infiltration and necrosis. The liver of the exposed organisms revealed slight vacuolated cells which is an indication of fatty degeneration of hepatocytes. This study was able to establish that exposure of C. gariepinus to even low concentration of cassava effluent can induce various toxicological effects and histological degradation which depends on the period of exposure and concentration of the pollutant. In the view of the toxicity effect of this effluent, it can be inferred that, indiscriminate discharge of cassava effluent can induce damage to the tissue and organs which might make all the living entities in the polluted environments vulnerable to diseases and eventually resulted to death.

Keywords: Histopathology, sub-lethal, effluent, necrosis

INTRODUCTION

Histopathology is the microscopic study of tissues affected by disease. The procedures adopted for the preparation of material for such studies are known as histological or histopathological techniques. Considerable interest has been shown in recent years in histopathological study while conducting sub-lethal tests in fish (Akinsanya, 2007). Tissue changes in test organisms exposed to a sub-lethal concentration of toxicant are a functional response of organisms which provides information on the nature of the toxicant. Histological changes associated with toxicants in fish have been studied by many authors (Mercy et al., 1996). Histopathological investigations have proved to be a sensitive tool to detect direct effects of chemical compounds within target organs of fish in laboratory experiments (Schwaiger et al., 1996). Fish are widely used to evaluate the health of aquatic ecosystem and physiological changes serves as biomarkers of environmental pollution. Cassava is a major starchy food for more than 300 million people in many tropical countries of the world and many cultivars are toxic. Cassava food products are the most important staples of rural and urban households in southern Nigeria. In Nigeria, traditional foods processed at home or in small-scale cottage operations constitute the principal mode of utilization of cassava (Adeyemo, 2005). Therefore, this study was primarily carried out to determine the histopathological effects of cassava mill effluent to C. gariepinus gills and liver.
METHODOLOGY
To observe the impact of cassava mill effluent on the histopathology of the fish, One hundred (100) apparently healthy adult *Clarias gariepinus* of average body weight of 350g and length ranging from 25.0-27.0cm were purchased from Ministry of Agriculture, Fisheries Division, Ogbomoso. Transportation of the fishes was done in properly aerated container to the Biology laboratory of Ladoke Akintola University of Technology, Ogbomoso, and Oyo State. The fishes were acclimatized for 14 days in the laboratory inside transparent container filled with 50 litres of well dechlorinated water. This experiment was conducted under standard static bioassay procedure (AOAC, 1987). This involves carefully controlled environmental conditions as to define the response of the test organism to the effect of cassava mill effluent. The cassava effluent used for this study was collected from a local ‘garri’ producing industry at Arada Cassava Processing industry in Ogbomoso South Local Government area, Ogbomoso, Nigeria. Collection of the cassava effluent was done every morning to ensure that the cassava effluent collected is fresh and potent i.e active ingredient in the effluent is at its maximum potency.

**Sublethal Test**
All the tested organisms (*C. gariepinus*) into six groups, each group contained ten fishes. The five varying concentrations used ranged from 0.020ml/L, 0.016ml/L, 0.012ml/L, 0.008ml/L, and 0.004ml/L. The volumes of effluent measured into 10Litres of water are 200mls, 160mls, 120mls, 80mls, and 40mls. The experiment was carried out for 14days. The behaviours and morphological features of the fishes were observed every 24hrs. The mortalities were collected and stored in freezer. The sublethal test was done to determine the mean results.

**Histopathology**
Liver, kidney and gill were collected in 10% neutral buffered formalin and processed for paraffin blocks (56-58 °C) and sectioning at 3-5 μm. Stained sections were examined under a Zeiss compound binocular microscope fitted with a photomicrographic attachment.

RESULTS

**Behaviour Responses of the test organisms**
Exposed fish became darker in colour and showed signs of respiratory distress, increased opercular movement were also observed.

**Results of histological examination of selected organs**
The results of histological examination of gill and liver of exposed fish as revealed by Zeiss compound binocular microscope fitted with a photomicrographic attachment are presented in the following plates 1-10. The results obtained showed that the gill and liver sections in the control group shows no lesion in the primary and secondary lamellae. Meanwhile, gill and liver sections of the exposed fish showed significant alterations and abnormalities.
Plate 1: Tissue of gill of *C. gariepinus*(×400) in control.

Plate 2: Gill section of *C. gariepinus* (x400) at 0.004mg/L showing mild cellular infiltration of hepatocytes (CIH).

PLATE 3: Gill structure of *C. gariepinus* (x400) at 0.008mg/L showing more pronounced cellular infiltration of hepatocytes exposed to cassava effluent.
Plate 4: Tissue of gill structure of *C. gariepinus* (x40) at 0.012mg/L showing irregular lamellae epithelium and severe hepatocytes degeneration after being exposed to cassava effluent.

Plate 5: Gill section of *C. gariepinus* (x400) exposed to 0.016mg/L of cassava effluent showing a complete degeneration of hepatocytes (CDH) hence a necrotic condition.
Plate 6: Tissue of liver section of *C. gariepinus* (x400) in the control group showing no significant lesion exposed to cassava effluent.

Plate 7: Liver section of *C. gariepinus* (x400) at 0.004mg/L showing mild glycogen vacuolation and fairly congested central vein after been exposed to cassava effluent.
Plate 8: Liver section of *C. gariepinus* (x400) at 0.004mg/L showing mild glycogen vacuolation (MGV) and fairly congested central vein (FCCV).

Plate 9: Tissue of liver section of *C. gariepinus* (x400) at 0.008mg/L showing diffuse hepatic necrosis (DHN) with severe cellular infiltration (SCI).
Plate 10: Liver section of *C. gariepinus* (x400) at 0.012m/L showing severe infiltration of leukocytes (arrow) and lipid vacuolation (L).

Plate 11: Tissue of liver section of *C. gariepinus* (x400) at 0.016mg/L showing more pronounced hepatic necrosis.

**DISCUSSION**

The exposure of fish to chemical contaminants is likely to induce a number of lesions in different organs (Bucke et al., 1996). Gills (Poleksic et al., 1994), kidney (Bucher and Hofer, 1993), and liver (ICES, 1997) are suitable organs for histological examination in order to determine the effect of pollution. The exposure of aquatic organisms to very low levels or sublethal concentration of pollutants in their environment may result in various biochemical, physiological, and histological alterations in vital tissues.

In this study histological investigation of the liver tissues of *C. gariepinus* not exposed to sublethal concentrations of cassava effluent showed a typical structural organization of the parenchymatous cell appearance of the hepatocytes in the untreated fishes. However, the fishes exposed to the cassava effluent...
showed major histological abnormalities. The abnormalities observed include cellular infiltration, congestion of central vein and cellular necrosis, which showed a progressive architectural distortion at varied concentrations, this observation is in agreement with the submission of Strivastava, (1994). In this study, the most generally encountered type of degenerative changes was congestion, vacuolization of hepatocyte, cellular infiltration and necrosis. The liver of the exposed organisms revealed slight vacuolated cells which is an indication of fatty degeneration of hepatocytes. Cellular necrosis as observed in this study might have resulted from excessive work required by the fish liver to get rid of the toxicants from its body during the process of detoxification. High accumulation of several components of the cassava effluent in the liver is a pointer to the fact that, liver plays a major role in the accumulation and detoxification.

Necrosis became evident as the concentration increases and this could be attributed to the inability of fishes to regenerate new liver cells. It was also observed that the histopathological changes in the liver caused metabolic problems; this lesion is characterized by the remains of the bile in the form of droplets in the cytoplasm of the hepatocyte and this convincingly supported the submission of Pacheco and Santos, (2002) that stated that bile is not being released from the liver which is also an indication of possible damage to the hepatic metabolic functions of the liver. An increased in the degree of damages done to the liver tissue of the fishes (Clarias gariepinus) held in 0.004 mg/L, 0.008 mg/L, 0.012mg/L, 0.016mg/L and 0.02mg/L cassava effluent, is generally related to important hepatic lesions such as degenerative and necrotic processes, this observation was in line with the submission of Chang et al., (1998).

Additionally, the presence of bile stagnation or accumulation and melanomacrophages in great quantity in the liver of exposed C. gariepinus is strong evident in that this organ suffered structural and metabolic damage due to the exposure to the cassava effluent. This is in a way signalling the fact that this environment where the effluent is discharged is grossly polluted and impaired. The histological alterations identified within the hepatocytes in this study may have been the results of various biochemical lesions. Anomalies such as irregular shaped central vein, cellular vacuolation and infiltration may be attributed to the accumulation of lipids and glycogen due to liver dysfunction as a result of exposure to the effects of cassava effluent, this is in conformity with the submission of Fanta et al., (2003).

Pacheco and Santo (2003) also described increased level of vacuolation of the hepatocytes as a signal to the degenerating process that suggest metabolic damage, possibly related to exposure to contaminated water. The liver parenchyma cells of all exposed fish showed signs of degeneration and this has been reported by many authors to be associated with the exposure of fishes to certain toxicants (Chang et al., 1998).

Evidently the damage done to the tissues of the liver and the gills seemed to increase with increase in concentration as shown by the plates 1-11. This result is similar to the work of Wade et al. (2002) who worked on the toxicity of cassava (Manihot esculenta) effluent on the Nile tilapia Oreochromis niloticus, histopathological examination of gill, kidney, and liver indicated damage ranging from oedema and talengiectasis of the gill lamella and gill hyperplasia to vacuolation of the liver cells and necrosis. The Present findings are strongly supported by the works of Cengiz (2006) who observed and reported the histopathological effects of δ-methrin on the gill of the common carp; Cyprinus Carpio (Linn.), the changes at all doses of the test compound were desquamation and necrosis besides aneurism in secondary lamellae, lifting of lamellar epithelium, edema, hyperplasia and fusion of the secondary lamellae were reported.

The present findings are in conformation with the work of Dhanapakiam et al. (1998) who studied histopathological changes in the gills of Channa punctatus (Bloch.) exposed to industrial effluents for 45 days, revealing deformities such as; secondary lamellae of primary filaments showing hyperplasia as compared to the gills of controlled fish indicating that the industrial effluents of river Cauvery induced considerable chemical stress on fish populations. Adhikari (1996) also studied the histopathological changes in the gills of a cat fish; Heteropneustes fossilis on exposure to malathion at different concentrations and different exposure times finding degenerative changes like complete secondary lamellae fusion, edema, hypertrophy and hyperplasia of lamellar cells.
Similarly, the present finding are in agreement with Tilak et al. (2001), Velmurugan et al. (2007) who also observed the same histopathological changes in the gills of various fishes on exposure to different toxins resulting in necrosis, vacuolar degeneration, dystrophy, desquamation, epithelial lifting, edema, shorting of secondary lamellae and lamellar fusion. All these histopathological research studies on the gills of fishes proved to be supporting the results of the present investigation. The observations in the present investigation are also supported by the work of Nagarajan and Kumar (2006) who reported the same histopathological changes in the gills of *Labeo rohita* (Ham.), exposed to sago effluent, which included worn out gill filaments of primary and secondary gill lamellae.

The works of Pandey et al. (1997) provided confirmation to the present investigation who worked on histopathological changes due to the effect of heavy metals on the gills of fishes. Fernandes et al. (2006) observed the histopathological changes in gills epithelium of *Oreochromis niloticus* exposed to copper; they reported edema, lifting of lamellae epithelia, lamellar fusion, thus also providing support for the present investigation.

**CONCLUSION**

This study has been able to establish the fact that; exposure of *C. gariepinus* to even low concentration of cassava effluent can induce various toxicological effects and histological degradation which depends on the period of exposure and concentration of the pollutant. In the view of the toxicity effect of this effluent, it can be inferred that, indiscriminate discharge of cassava effluent can induce damage to the tissue and organs which might make all the living entities in the polluted environments vulnerable to diseases and eventually resulted to death.

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