GLOBAL JOURNAL OF PURE AND APPLIED SCIENCES VOL. 8, NO. 2, MARCH 2002:193 - 196
COPYRIGHT(C) BACHUDO SCIENCE CO. LTD. PRINTED IN NIGERIA. ISSN 1118-0579

THE EFFECT OF ETHANOLIC EXTRACT OF *PIPER GUINEENSE* ON THE HISTOLOGY OF SOME ORGANS OF *OREOCHROMIS NILOTICUS* (LIMN) PISCES: CICHLIDAE

A. OKOKON OKON

(Received 21 July 2000; Revision accepted 29 November 2001)

ABSTRACT

The effect of the sub-lethal dose 3.0mg/l (of dechlorinated water) of *Piper guineense* on the histology of Liver and Kidney of *Oreochromis niloticus* exposed for 21 days in a static bioassay revealed some pathological changes in liver and kidney of the fish. These include vacuolation, liver cord disarray/necrosis and the distortion of the organized cellular pattern. These findings revealed the destructive property of *P. guineense* as an ichthyotoxic plant. The effects of ichthyotoxic plants vis-à-vis other fish poisons are discussed in the wild and natural environment.

Keywords Ethanolic extract, *Piper guineense*, static bioassay, ichthyotoxic plants, Liver, Kidney.

INTRODUCTION

Obnoxious fishing practice involves the introduction of poisonous substances such as chemicals, ichthyotoxic plants and explosives into the aquatic ecosystem to narcotize and/or kill both find and shell fish thus rendering them liable to capture. Such substances usually in liquid forms include Gamalin 20, Adrex 40, and Didimac 25. The ichthyotoxic plants include *Tephrosia vogelli, Accacia pennata, Boehvia coccinea, Mundulea sericea, Tetrapleura tetraptera, Baillonela toxisperma*. The explosive devices which are used include dynamites, hand grenades and bombs (Tobor, 1992).

The practice is obnoxious and highly destructive because the poisonous substances are non-selective in their effects, and cause mass mortality of aquatic organisms, thus leading to unsustainable fishing. Some ichthyotoxic plants and chemicals have non-biodegradable components which make them environmentally unfriendly and can be biomagnified along the food chain which brings untold ecological damage to the milieu (BrockNeely, Branson and Bau, 1994; Crossby, 1975; Hamelik, Waybrant and Ball, 1977).

P. guineense (the West African black pepper) is an ichthyotoxic plant which belongs to the family piperaceae. It is distributed throughout the tropical and subtropical regions of the world. It is sold locally as spice and medicine for curing stomach disorder (Dalzie, 1948).

The reported toxicity of Piper guineense to the nymph and adult of grasshopper. *Zonocerus variegatus* (L) was attributed to piperine, the active ingredient acting with guineensine (Ivbijaro and Agbaje, 1986). Little literature exist on its effects on fish (Okorie, Ugwumba and Okon, 1992).

However, sublethal effects of other toxicants on fish tissues have been documented. Mattiessen and Brafield, 1973 revealed that liver of mercury exposed fish was congested and also caused serious histological effect on stickle back, *Gasterosteus aculeatus* (L) such as detachment and sloughing of epithelial cells, coalescing of adjacent secondary lamella epithelia. The cytoplasmic abnormalities included extensive vacuolation, followed by swelling of nuclei and mitochondria leading to cellular disintegration.

A wide spread cyanide-induced degenerative necrosis of Hepatocytes was

A. OKOKON OKON, Department of Fisheries & Aquaculture, Faculty of Agriculture, University of Uyo, Uyo, Nigeria

observed in rainbow trout exposed for 18 days (Dixon and Leduc, 1981). Sastry and Malik, (1979) have reported the sublethal effect of Dimecron on digestive system of fresh water fish. *Channa punctatus* exposed for 20 days. The most conspicuous pathological changes in the liver were vacuolation of the cytoplasm of Hepathocytes, enlargement of the nuclei, rupture of the cell membrane, liver cord disarray and damage of connective tissue.

The present investigation is aimed at revealing the effect of sublethal concentration of ethanolic extract of *P. guineense* on the histology of the liver and kidney of the fish, *Oreochromis niloticus*.

MATERIALS AND METHODS

Collection of test organisms

The juveniles of Oreochromis niloticus (weight range 6.2 – 20.7g, total length 7.2 – 10.0 cm) were collected from Oyo State Fisheries Department at Agodi, Ibadan. They were transported to the laboratory in a ploythene bag containing aerated water. In the laboratory the fish were held in batches in aerated glass aquaria, containing dechlorinated tap water to avoid stressful condition. The fish were fed once a day with formulated feed The faecal pellet and left over feed were siphoned out each day. The water in each tank was replaced twice a week to avoid contamination. The fish were acclimatized for a minimum of seven days. They were accepted as being fully adapted where no death was observed for four consecutive days (FAO, 1986). Feeding was stopped 24 hrs prior to each set of experiments.

Preparation of Ethanolic Extract of *Piper guineense* (EEPG)

Fruit of *P. guineense* were dried at 60°C for 72hrs, then ground using an electric blender. The powder was stored in an air-tight bottle until used. The homogenized sample of 347g was later extracted with ethanol. The extract was evaporated to dryness in a Roto-evaporator at about 60°C to obtain a crude, brownish, semi-solid substance of weight 100.8g. The substance as wrapped with a black material to avoid light penetration and stored in a refrigerator until needed.

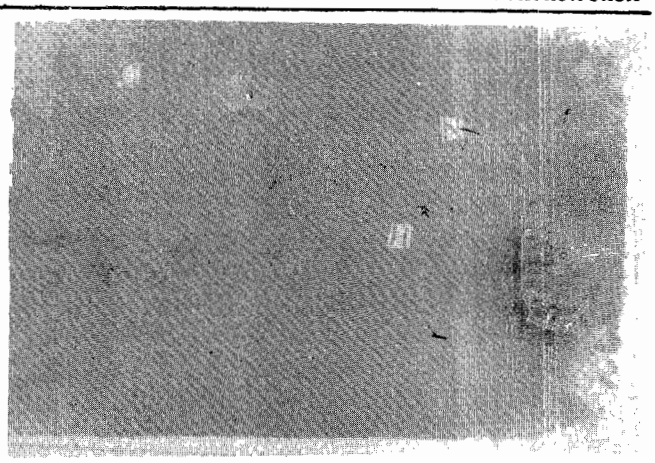

Fig. 1a: Control liver tissue, with hepatocytes (H) arranged in definite chord-like pattern and with the nucleus (N) of each hepatocytes being spherical or slightly ovoid in shape and containing scattered chromatin granules (C).

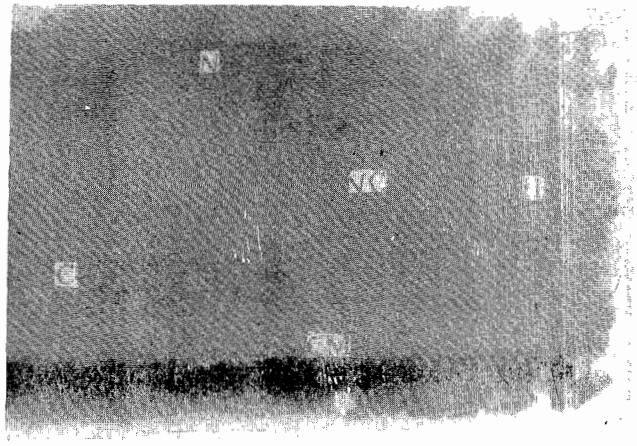

Fig. 1b: Treated liver tissue showing contracted nuclei (N) and chromatin (C) condensed into heavily stained clumps: breakdown of the chord-like arrangement of hepatocytes (H): vacuolation of the cytoplasm (VC) and rupture of the cell membrane (CM).

Test Procedure

Randomly selected fish were distributed in batches of ten among three aquaria containing test solution (3.0 mg of extract per litre) and a contro tank containing extract free-water only. Each set of experiment was replicated twice with a control. Temperature and Ph were determined at the start of experiment. The fish were exposed to 3.0 mg/l (96hr LC50) for 21 days (Okorie, Ugwumba

and Okon, 1992). During the duration of the experiment, water in the tank was replaced after every 48hr with freshly prepared extract solution. After the exposure period, fish from the experimental and control aquaria were dissected. The liver and kidney tissures were collected and fixed in Bouin,s fluid embedded in paraffin and sectioned (7 microns thickness) for staining with haematoxylin/Eosin stain. Histopathological changes due to treatment with the ethanolic extract of *P.guineense* were noted and photomicrographs taken.

RESULTS

Treatment of *Oreochromis niloticus* with 3.0mg/l of ethanolic extract of *Piper guineense* produced marked histological alterations in the different tissues (liver and kidney) examined. Figs 1a, 1b and 2a, 2b illustrate the changes in liver (control and treated) and kidney (control and treated) respectively .

The Liver Tissue

In the control liver tissue (Fig.1a), the hepatocytes are arranged in a definite chord-like pattern. The nucleolus of each hepatocyte is either spherical or lightly ovoid with regular surfaces scattered chromatin granules and one or more nucleoli.

The hepatic tissue from treated fish (Fig.1b) revealed cellular disorganization evidenced by a breakdown of the chord-like arrangement of hepatocytes (compared with Fig.1a) and a remarkable degree of necrobiosis, the degenerative process leading to cell death. There is vacuolation of the cytoplasm of hepatocytes, enlargement of the neclei, rupture of the cell membrane and thickening of connective tissue.

The Kidney Tissue

The control kidney tissue (Fig. 2a) shows the numerous renal corpuscles arranged in parallel rows, corresponding to the course of the inter tabular arteries from which they derive their blood supply. Conversely, in the treated tissue (Fig. 2b), the organized cellular pattern of the former tended to be distorted. There is a contraction of the nucleus and condensation of the tissue cells into one or more heavily stained clumps. This shows an evidence of tissue damage.

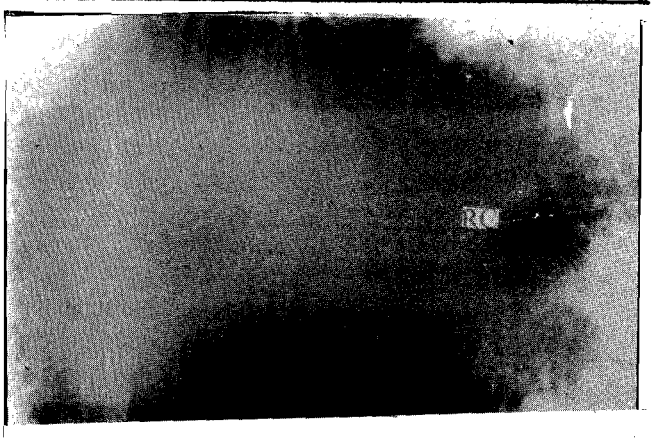

Fig. 2a: Control kidney tissue with numerous renal corpuscles (RC) arranged in parallel rows.

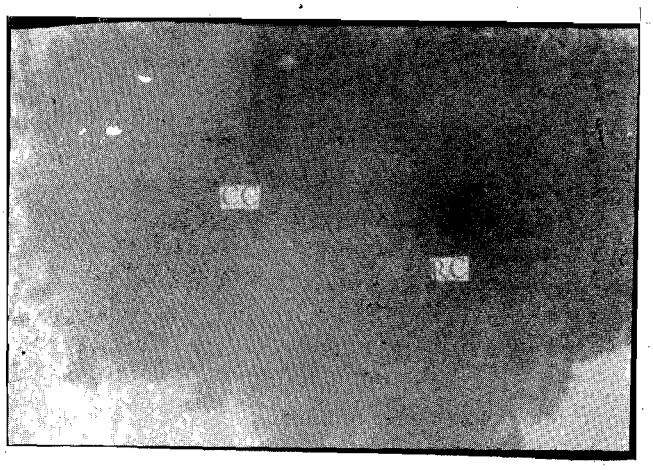

Fig. 2b: Treated kidney tissue with the organized cellular pattern of renal corpuscles (RC) distorted and condensation of tissue cells (CC) in heavily stained clumps

DISCUSSION

The histopathological changes observed in this study are not characteristic of *Piper guineense* but represent non specific changes induced by exposure to toxicant. Cytoplasmic vacuolation due to exposure to pesticides had been reported by a number of workers in different tissues of fish. King (1962) has given a detailed account of several histopathological symptoms of guppies and brown trout fry exposed to subiethal concentrations of DDT, mainly in the liver, intestine, and kidneys and with particular reference to cell vacuolation. Eller (1971) and Bhattacharya, Mukherjee, and Bhattacharya (1975) have also reported vacuolation in liver cells of fishes exposed to endrin. Dixon and Leuc (1981) revealed the histopathological examination of the liver as one of the most sensitive and significant indicators of chronic cyanide poisoning in rainbow trout.

Other conspicuous alterations in liver and kidney include cord disarray, connective tissue damage enlargement of liver cells and distortion of organized cellular pattern respectively. Similar changes have been observed by a number of workers (Mathur 1962, Eller, 1971, Bhattacharya, Mukherjee, 1975, Wood, Vasutake, Woodall and Halver 1957).

The liver is the site of synthesis for the protein portion of the yolk of eggs (Ho and Vanstone, 1961) and a Piper effected live may not be able to carry out these functions. (Lesniak, 1977) cited Kazuo Shinji, T. & Kentaro, K. (1979) has observed that the yolk deposition was the stage of oogenesis most drastically affected by exposure of rainbow trout to toxicants. Considering the numerous metabolic processes carried out by the liver, it is not surprising that *P. guineense* extract-toxified fish showed substantial damage. Not only would the ability of the liver to supply the products of intermediary metabolism necessary for growth be limited, but also a decrease in the capacity of the liver to eliminate waste products generated during periods of fish metabolic activity. These findings are in consonance with works of Eller, 1971; King, 1962; Sastry & Malik 1979. From the aforegoing, it is undoubtable that the use of toxicants for fishing is not sustainable. It must be discouraged along the lines of prevailing fisheries regulations.

REFERENCES

Bhattacharya, S. Mukherjee, and Bhattacharya, S., 1975. Toxic effect of endrin on hepatooancreas of the telecost fish, *Clarias batrachus* (Limn) Indian Journal of Experimental Biology, 13: 185.

BrockNeely, W., Branson, D. R. and Bau, G. E., 1974. Partition Coefficient to Measure Bioconcentration Potential of Organic Science & Technology, 187: 132.

Crossby, D. G., 1975. The Toxicant – Wildlife Complex. Pure & Applied Chemistry, 42: 233.

Dalzie, J. M.., 1948. The Useful Plant of West Tropical Africa. The Crown Agents for Colonies 612pp.

George, D. and Gerardi, L., 1981. Chronic Cyanide Poisoning of Rainbow Respiration and Liver Histopathology. Archives of Environmental Contamination & Toxicology 6: 200-215.

Eller, L. L., 1971. Histopathologic Lesions in cut throat trout. *Salmo* clarki exposed chronically to the insecticide endrin. American Journal of Pathology 64: 321.

F.A.O., 1986. Manual of Methods in Aquatic Environment Research Part 4. Bases for Selecting Biological Tests to Evaluate Marine Organisms. FAO, Rome, 56pp.

Hamelik, J. L. Waybrant, R. C. and Ball, R. C., 1977. A Proposal Exchange Equilibria control that degree Chlorinated hydrocarbons are Biologically manified in Lentic Environments. Transaction of American Fish Society, 100:207-214.

Ho, F. C. W. and Vanstone, W. E., 1961. Effect of estradiol. Monobenzoate on some serum constituent of maturing Sockeye Salmon (*Onchornchus merka*) Journal of Fisheries Resource Board Canada, 18: 859.

Ivbijaro, M. F. & Agbaje, M., 1986. Insecticidal activities of *Piper guineense*. Schum & Thonn, and *Capsicum* sp. On the Cowpea Bruchid, *Callosobrunchins maculates*, F. Insect Sci. Application 7(4): 521.

Kazuo, T. S., Shinji, T and Kentaro, K., 1979. Fate and Comparative. Toxicity of Metallothioneins and differing Cd/Zn ratios in Rat Kidney. Archives of Environmental Contamination $ Toxicity. 8: 85-95.

King, S. F., 1962. Spec. Scientific Report. U. S. Fish Wildlife. Serv., No. 399 cited in C. A. Edwards (ed): Environmental Pollution by Pesticides. London – New York.

Mathur, D. S., 1962. Histochemical changes in the liver of certain Fishes as induced by BHC and Lindane. Proc. Of National Academy of Science, India. 32: p. 429.

Mathiessen, P. and Brafield, A. E., 1973. The effects of dissolved Zinc on the gills of the stickle back, *Gasterosteus aculeatus* (L). Journal of Fish Biology; 5: 607-613.

Okorie, T. G; A. O. Ugwumba and Okon, A. O., 1992. Toxic and Sublethal effects of Ethanolic extract of *Piper guineense* on *Oreochromis niloticus* (Linn). (Pisces: Cichildae) Journal of Experimental & Applied Biology 4(1-4):34-52)

Sastry, K. V. and Malik , P. V., 1979. Studies on the effect of Dimecron on the Digestive System of a Fresh Water Fish, *Channa Punctatus*. Archives of Environmental Contamination & Toxicology. 8: 397-407.

Tobor, 1992. Fin and Shell Fish of Conservation interest in Nigeria. Nigeria Institute of Oceanography and Marine Research (NIOMR) Technical Paper No. 79.