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Toxicological Studies on the Short Term Exposure of *Clarias albopunctatus* (Lamonte and Nichole 1927) to Sub-lethal Concentrations of Roundup

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Abstract: The short term toxicity in *Clarias albopunctatus* (Lamonte and Nicole, 1927) exposed to sub-lethal concentrations of Roundup was studied. A total of 36 fish were divided into 3 groups in a static bioassay model. Fish in 2 test groups were exposed to 5 and 15 ppm, respectively, of Roundup while fish in the control group were not exposed to the toxicant. Six fish per group were bled exhaustively (each) at 48 h and at 96 h while the livers and gills were harvested at 96 h for histological studies. The results showed that serum concentrations of total and conjugated bilirubin increased significantly ($p<0.05$) in a dose-and time-dependent manner. Serum concentrations of alanine and aspartate aminotransferases and alkaline phosphatase were also significantly ($p<0.05$) elevated in the test fish. Similarly, serum concentrations of creatinine and urea were significantly ($p<0.05$) elevated in a dose-dependent manner. Histomorphologic studies of the liver and gills showed marked destruction of their architecture in the test fish, thus corroborating the data from the biochemical analyses. Short term exposure of *Clarias albupunctatus* to sub-lethal concentrations of Roundup was found to be toxic to the fish.

Key words: Catfish, *Clarias albopunctatus,* herbicide, roundup, static bioassay, toxicity

INTRODUCTION

The need to feed the world's growing population has prompted the use of agrochemicals meant to increase food production. Such agrochemicals include several formulations of inorganic fertilizers, herbicides like 2,4-diphenoxyacetic acid, (2,4-D) and glyphosate [N-(phosphonomethyl) glycine], etc. Roundup-a mixture of isopropylamine salt of glyphosate and surfactants like Polyoxyethylamine (POEA) in water-is a non-selective, broad-spectrum, post-emergent systemic herbicide. Its herbicidal activity is expressed by direct contact with leaves with subsequent translocation throughout the plant (Piesova, 2005). Glyphosate inhibits plant growth through the inhibition of enolpyruvylshikimate phosphate synthase (EC: 2.5.1.19) responsible for the biosynthesis of chorismate (an intermediary in the phenylalanine, tyrosine and tryptophan biosynthesis), by acting as an analog of the second substrate, phosphoenolpyruvate (Williams *et al*., 2000). Glyphosate could also exercise its herbicidal action by acting as an energy drain on the organism (Orcaray *et al*., 2012) or as an inhibitor of Cytochrome P-450 complex (Green, 2009).

Farmers in most agrarian (especially rice farming) communities in Nigeria employ Roundup extensively in managing weeds. Roundup used in the fields may leach into fresh water ponds as aerial drift or as run-offs. The toxicological implications of such fresh water contamination with respect to the fresh water fish population are still unresolved. Contrary to the description of Roundup as a pesticide of low toxicity and environmental friendliness by its manufacturer, some studies have shown that it might pose a variety of health and environmental hazards (Cox, 2000) probably caused by glyphosate itself or other inert ingredients known as surfactants (Singh *et al*., 2011).

The chronic effects of sub-lethal doses of Roundup on fresh water fish have been studied (Jiraungkoorskul *et al*., 2003). However, the short term effects of such sub-lethal doses of Roundup on fresh water fish, particularly the catfish, have not been properly studied. This study aims to ascertain the short term toxicity in the common catfish, *Clarias albopunctatus* exposed to sub-lethal concentrations of Roundup, in an attempt to mimic what obtains in fresh water bodies that surround (rice) farms.

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MATERIALS AND METHODS

Fish: *Clarias albopunctatus* (Lamonte and Nichole, 1927) mean weight 42.5±1.7 g, were purchased from a commercial farm in Agbor, Delta State, Nigeria. The fish were transported to the Wet Laboratory of the Fisheries and Hydrobiology Researches Unit, University of Nigeria, Nsukka in FAO fish transit tanks. Potassium tetraoxomanganate (VI) ($KMnO_4$) (1%) was used as flush prophylactic treatment for the fish, upon arrival. Two minutes thereafter, the fish were rinsed in tap water and acclimatized to the wet laboratory for 7 days. Water and feed were changed daily at 08:00 h throughout the duration of the study.

Experimental design: A total of 36 fish were used for the study. The fish were divided into 3 groups of 12 fish each and treated as follows: Group 1 (control) was exposed to tap water only; while Groups 2 and 3 were exposed to 5 ppm and 15 ppm Roundup, respectively. In order to maintain the toxicant level and avoid accumulation of waste products during the study period, the static bioassay method was adopted. The study lasted for 96 h and sampling was done at the 48th and 96th h. Six fish were sampled per group, per time. Blood from the fish was collected by cardiac puncture, using disposable hypodermic syringes and placed in clean sample containers. Sera were separated by centrifugation (after allowing the blood samples to clot at ambient temperature) and used for the biochemical analysis. Portions of the livers and gills of the fish were removed immediately after blood collection at the 96th hour and prepared for histological studies.

Assays and determinations

Total and conjugated bilirubin determinations in serum: The method described by Jendrassik and Grof (1938) was used for these determinations. The method is based on the principle that conjugated bilirubin reacts with diazonitized sulphonic acid in an alkaline medium to form a blue colored complex. Total bilirubin is determined in the presence of caffeine which releases albumin-bound bilirubibin in the reaction with diazonitized sulphonic acid.

Serum alkaline phosphatase (ALP) Assay: Serum ALP was assayed by the method of Rec (1972). The principle for assay is that serum alkaline phosphatase hydrolyzes a colorless substrate of phenolphthalein monophosphate giving rise to phosphoric acid and phenolphthalein which, at alkaline pH, turns pink and can be spectrophotometrically measured.

Serum aspartate aminotransferase (AST) Assay: Serum AST was assayed by the method of Reitman and Frankel (1957). This assay's principle is that AST catalyzes the reaction between α-oxoglutarate and L-aspartate to give L-glutamate and oxaloacetate. The AST in the sample is measured by monitoring the concentration of oxaloacetate hydrazone formed with 2,4-dinitrophenylhydrazine.

Serum alanine aminotransferase (ALT) assay: Serum ALT was assayed by the method of Reitman and Frankel (1957). The principle for this assay is that ALT catalyses the reaction between α-oxoglutarate and L-alanine to give L-glutamate and pyruvate. The concentration of ALT is measured by monitoring the concentration of pyruvate hydrazone formed with 2,4-dinitrophenylhydrazine.

Serum urea determination: Urea in serum was estimated by the method of Searcy *et al.* (1967). The principle is based on the hydrolysis of urea by urease, to give ammonia and carbon dioxide. The ammonia so formed reacts with alkaline hypochloride and sodium salicylate in the presence of nitroprusside to form a coloured chromophore, the intensity (measured spectrophotometrically) of which is proportional to the concentration of urea in the sample.

Serum creatinine determination: Serum creatinine was determined using the method of Newman and Price (1999). The principle is based on the production of a red colored complex by the reaction between creatinine and picric acid in alkaline solution to form a red colored complex. The intensity of the colour is spectrophotometrically measured and is proportional to the concentration of creatinine in the sample.

Histology: Liver and gill tissues were fixed in 10% formal saline for 24 h. Thereafter, they were washed and dehydrated in grades of ethanol, cleared in xylene and then infiltrated and embedded in paraffin. Sections of the embedded tissues (4-5 μm each) were cut using an ultramicrotome, stained with hematoxylin and eosin and their photomicrographs taken at ×10 magnification.

Statistical analysis: Descriptive statistics was carried out and the results presented as Mean±Standard deviation. Differences between means were separated using one way ANOVA with the significant threshold employed at p = 0.05. Data analyses were done using SPSS version 16.0 (SPSS Inc., Chicago IL).

RESULTS

Figure 1 shows that mean total bilirubin concentration in serum increased significantly ($p<0.05$) in a dose-dependent and time-dependent fashion, relative to the control group. Mean conjugated bilirubin concentrations in serum was not altered significantly ($p<0.05$) after 48 hours in the fish in group 2, relative to the control, but was significantly ($p<0.05$) higher at 96 h in the fish in group 2 and irrespective of duration in group 3.

From Fig. 2, it is seen that mean serum concentrations of ALP increased significantly ($p<0.05$) in a dose-dependent manner, relative to the control group. Mean serum concentrations of AST also increased significantly ($p<0.05$) relative to the control group, but more in a time-dependent manner than in a dose-dependent manner. Mean serum concentrations of ALT were statistically comparable ($p>0.05$) between the control group and group 2 at 48 h. However, at 96 h (in group 2) and irrespective of duration in group 3, mean serum concentrations of ALT were significantly ($p<0.05$) higher than that of the control group.

Mean serum concentrations of urea and creatinine were all significantly ($p<0.05$) higher in the test groups, relative to their respective controls, in a dose-dependent and time-dependent manner (Fig. 3).

Figure 4a shows a section of the liver from fish in the control group. Normal liver histology, characterized by inconspicuous lobulation and cords of hepatocytes separated by sinusoids, making a centrifugal spread from the central blood vessels to the portal area, is seen. The polygonal shaped hepatocytes have centrally located vesicular nuclei. Figure 4b shows slight distortion of the lobular architecture of the liver of fish in group 2. The hepatocytes in the centrilobular areas have mild granular cytoplasm and vesicular nuclei. Only few focally located hepatocytes are seen to have pyknotic nuclei. From Figure 4c, severe distortion of the lobular architecture of the hepatocytes of fish in group 3 is seen. Nuclei condensation (pyknosis) in the centrolobular area and severe vacuolation in the cytoplasm are also noticed.

Figure 5a shows a section of fish gill from the control group. The transverse section shows normal appearance of primary lamellar epithelium, choride cells and pillar cells. Figure 5b shows a section of fish gills from group 2. Abnormal and irregular thickening of the primary lamellar epithelium is noticed. Finally, Fig. 5c shows a section of

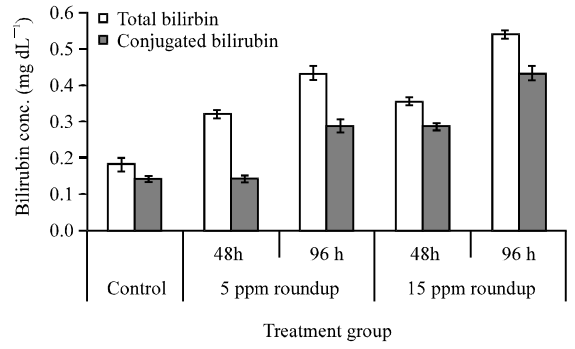

Fig. 1: Concentrations of total and conjugated bilirubin in the sera of fish exposed to sub-lethal concentrations of roundup

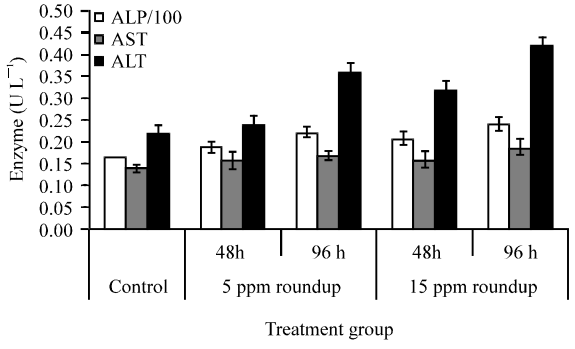

Fig. 2: Concentrations of some liver enzymes-ALP, AST and ALT-in the sera of fish exposed to sub-lethal concentrations of roundup

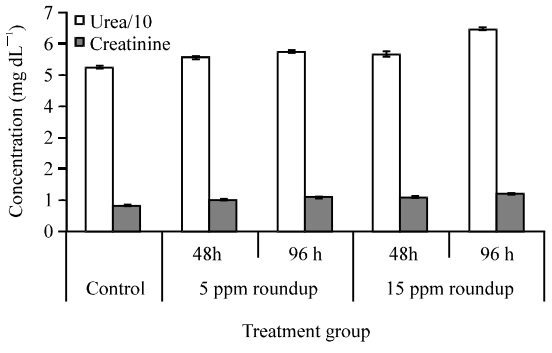

Fig. 3: Concentrations of urea and creatinine in the sera of fish exposed to sub-lethal concentrations of roundup

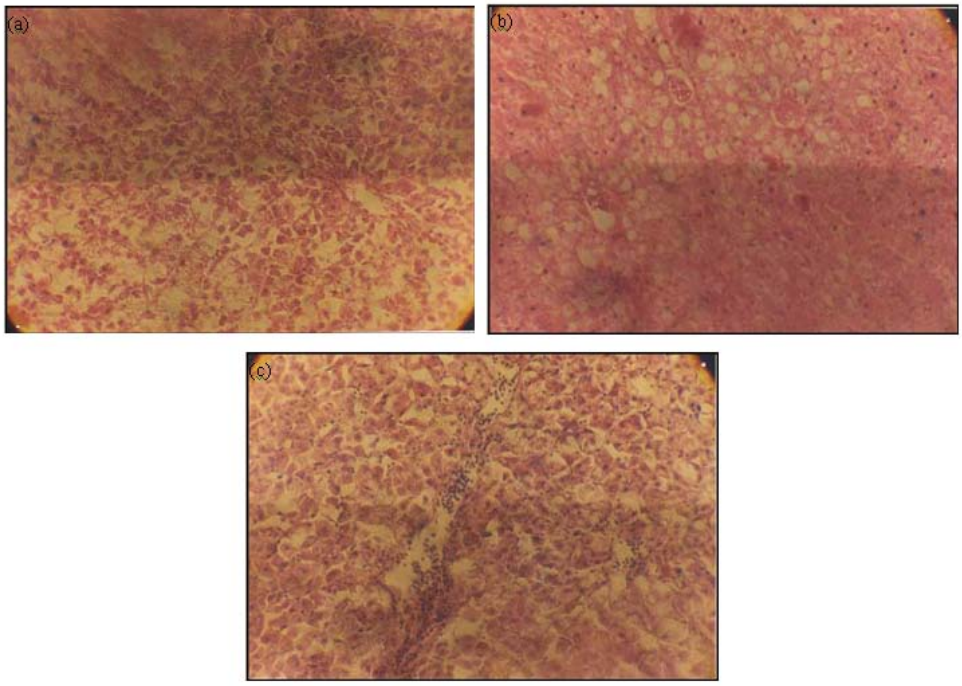

Fig. 4(a-c): Sections of the liver of fish from the (a) control group, (b) Exposed to 5ppm of Roundup and (c) Exposed to 15ppm of roundup

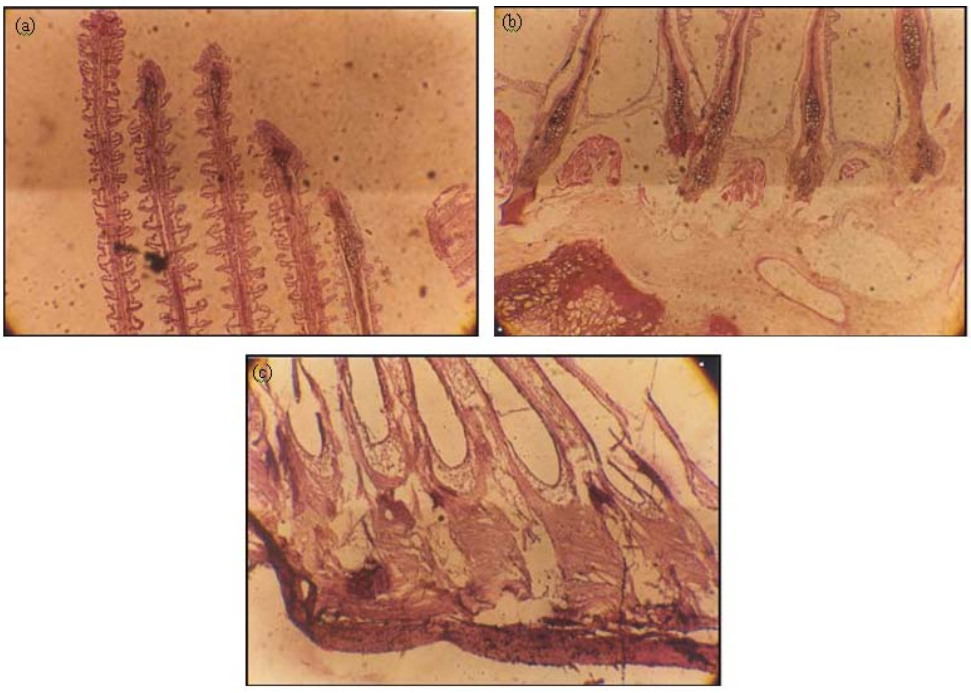

Fig. 5(a-c): Section of the gills of fish from the (a) control group, (b) Exposed to 5 ppm of roundup and (c) Exposed to 15 ppm of roundup

the gills of fish from group 3. Severe distortions in the appearance of the gills and severe tissue damage are glaring in the section.

DISCUSSION

The liver is subject to potential damage by a wide array of pharmaceutical and environmental chemicals because it is the organ responsible for xenobiotic transformations. Thus, the assessment of toxicity in animals is usually done through such surrogates as the liver function tests. Elevations in serum total and conjugated bilirubin are attributable to liver and/or biliary tract disease (Vitek and Ostrow, 2009) while elevations in ALT and AST are usually due to disruption of hepatic cells as a result of necrosis or altered membrane permeability and elevations in ALP is often elevated due to cholestasis (Ejike *et al*., 2008).

Clearly, the exposure of fish to sub-lethal concentrations of roundup even for a short period of time resulted in significant toxicity as shown by the results of the liver function tests. Similar elevations in liver enzymes have been reported in fish exposed to cypermethrin insecticide (Kumar *et al*., 2011). The authors suggested that such increase may be as a result of stepped-up transamination, where amino acids are used to generate intermediates for the tricarboxylic acid cycle, in attempt to cope with the energy crisis during cypermethrin stress. Liver damage in *Clarias batrachus* exposed to phorate and carbaryl has been reported and thought to be as a result of the production of lipolytic mitochondrial enzymes that dissolve cell membranes, lysosomal membranes and other hepatocellular organelles, thereby releasing liver enzymes into the blood (Karami-Mohajeri and Abdollahi, 2011).

The elevations in serum levels of bilirubin and conjugated bilirubin in a time-and dose-dependent manner may be subsequent to the disruption of the hepatic architecture by the toxicant (roundup) such that the conjugation of bilirubin and excretion of bilirubin is altered. It is such a disruption of hepatocytes and thus in liver function, that would result in the assayed liver enzymes having significantly elevated concentrations in the serum of the test fish especially at 96 h for the fish exposed to 5 ppm of Roundup or as early as 48 h in fish exposed to 15 ppm of Roundup.

The data from the liver function tests are corroborated by the histomorphologic changes observed in the liver sections of the test fish, relative to the control fish. Interestingly, the sections show a clear dose-dependent disruption of the lobular architecture, distortion, vacuolation and condensation in the liver.

Urea is a product of the deamination of glucogenic amino acids in the liver usually under conditions that the system requires energy generation to overcome any physiological stressor. Creatinine in serum is a metabolite of muscle creatinine. The concentrations of urea and creatinine in serum are usually constant because both are easily excreted by the kidneys. Elevated levels of both urea and creatinine therefore, indicate diminished renal function (Jayasundera and Macnab, 2012). The dose-dependent elevations in the concentrations of urea and creatinine in the serum of fish exposed to Roundup are therefore indicative of diminished renal function as a result of kidney damage.

In fish, the gills are the first organs to get in contact with xenobiotics because they provide a large interface between the external and internal environments of the fish (Jiraungkoorskul *et al*., 2003). The gills perform such vital functions as gas exchange, ion osmoregulation and nitrogen excretion and are particularly sensitive to changes in environmental conditions. Changes in gill epithelia are considered a good indicator of tissue lesions caused by chemical pollutants (Authman, 2011). This study shows a severe dose-dependent destruction of the gills by the toxicant, despite the fact that the doses used are known to be sub-lethal. The histologic sections of the gills corroborate the liver toxicity observed in this study. The exposure of *Clarias albopunctatus* to short-term sub-lethal concentrations of Roundup was found to be toxic to the fish. This is worrisome as the sustained use of this agent, over time, may have very severe negative consequences for the fish population in the water bodies surrounding farmlands and in turn affect the source of animal proteins for human populations inhabiting such areas and farming such lands. Further studies are required to show whether the constituents of Roundup bio-accumulate in fish and if the consumption of such fish by man could have any deleterious effects.

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