

Restorative prospective of powdered seeds extract of *G. kola* in *Chrysichthys furcatus* induced with *Glyphosate* formulation

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Abstract: Responses of *Chrysichthys furcatus* to glyphosate formulation and *Garcinia kola* seeds extracts was investigated using organization for Economic Cooperation and Development #203 and #407 recommended toxicity bioassay. The fish were divided into five groups and exposed to different treatments of glyphosate formulation and *Garcinia kola* seed extract, with control serving as a reference. The fish were divided into five groups of five juveniles per test concentration in three replicates. Group A was given de-chlorinated water. Group B was treated with 0.16mg/L glyphosate formulation only while other groups were exposed to the same concentration as in group A, but with different concentrations of *Garcinia kola* extract. Group C, D and E received 150, 250, and 350mg/L of extract of *Garcinia kola* seeds respectively. There was no significant different ($p > 0.05$) between fish treated with glyphosate formulation alone and other treatments except dissolve oxygen which was highly significantly ($p < 0.01$) between fish treated with glyphosate formulation alone and other treatments and the control. All the blood parameters were significantly ($p < 0.05$) affected by glyphosate formulation when compared with the control. The changes observed in glyphosate formulation alone were reversible when *G. kola* seeds extract were added, and was dose dependent. The plant's extract has shown to be a good remedy to pollutants, and formulations of the seed extract into tablets or capsules could serve as antidote to ameliorate the effects of pollutants. This finding can reduce the risk of biomagnifications of poisons along the food chain.

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Introduction

In the last few years there has been an exponential growth in the field of herbal medicine and these drugs are gaining popularity both in developing and developed countries because of their natural origin and less side effects (Grover et al., 2002). Herbal products cause few adverse effects, but have beneficial pharmacological and therapeutic uses in a number of illnesses, including HIV where they have been examined for their capacity to reduce symptoms and improve quality of life (Weber et al., 1999). Literature review had shown that oral administration of an aqueous *T. cordifolia* root extract to alloxan diabetic rats caused a significant reduction in blood glucose and brain lipids. Though the aqueous extract at a dose of 400 mg/kg could elicit significant anti-hyperglycemic effect in different animal models, its effect was equivalent to only one unit/kg of insulin (Dhaliwal, 1999). It is reported that the daily administration of either alcoholic or aqueous extract of *T. cordifolia* decreases the blood glucose level and increases glucose tolerance in rodents (Gupta et al., 1967). Also, oral administration of 2 and 8 g/kg of plant extract of fenugreek seeds produced dose dependent decrease in the blood glucose levels in both normal as well as diabetic rats (Ravikumar and Anuradha, 1999). The

plant's seeds extract also improved glucose metabolism and normalized creatinine kinase activity in heart, skeletal muscle and liver of diabetic rat (Khosla et al., 1995). Similarly, methanolic extract of *Phyllanthus amarus* was found to have potent antioxidant activity and reduced the blood sugar in alloxanized diabetic rats (Raphael et al., 2002).

Garcinia kola (commonly called bitter kola) is an economic and highly valued tree available in large quantity in West Africa. The fruit, seeds, nuts and bark of the plant has been used extensively in African traditional medicine for the treatment of various diseases (Okoli, 1991). The active constituent of the plant is a dimeric flavonoid molecules fused together biflavonoid. Other constituents include xanthenes and benzophenones (Ebong and Korubo, 1996). The dry powdered seeds contain 0.003% of flavonoids while the crude extract contained 0.007% of flavonoids based on rutin used as the standard (Onunkwo, 2004). The seeds when chewed have a bitter astringent taste. *Garcinia spp* are known to elaborate a complex mixture of phenolic compounds including biflavonoids, xanthenes and benzophenones.

Bitter kola has been used as an antidote for cases of poisons or suspected poisons. When food is suspected to be contaminated by bacteria, bitter kola is

chewed to prevent the development of any infection or poisoning. The plant products also contains chemical compounds that help to breakdown glycogen in the liver. This is because the phenolic compounds present in bitter kola possess anti-inflammatory, anti-microbial, anti-diabetic and antiviral properties (Tita *et al.*, 2001).

Several works done on *G. kola* have confirmed its hypolipidemic (Oluyemi *et al.*, 2007) anti histanic (Nakatani *et al.*, 2000) and antimicrobial (Iwu *et al.*, 1990). The presence of biflavonoids and xanthone in *G. kola* seeds have been confirmed (Olaleye and Farombi, 2000). These compounds are potent antioxidants (Oluyemi *et al.*, 2007). Administration of *G. kola* seed extracts caused an increase in testosterone production in sprague –dawley rats (Braide *et al.*, 2003; Akpantah *et al.*, 2005). The seed extract and dry powdered seed of *G. kola* plants have been made into various forms including tablets, cream, and toothpaste (Iwu, 1990). These ensure dosage precision, since herbal medicines have been widely criticized due to lack of standardization. Also formulation of *G. kola* into a modern pharmaceutical conventional tablet dosage form would confer into it many of the good properties of tablets. Some examples include ease of administration, greater acceptance due to presentation, prolonged shelf life, quality assurance, greater accuracy in dispensing and reduction in transportation cost arising perhaps from formulation into less bulky dosage form (Gunsel, 1996).

When the pesticide enter aquatic systems either through accidental spraying, spray drift, or surface runoff, it dissipates rapidly from the water column as a result of adsorption and possibly biodegradation. Glyphosate is a broad-spectrum non-selective systemic herbicide, registered for use on many food and non-food crops as well as non-crop areas where total vegetation control is desired. When applied at lower rates, it serves as a plant growth regulator (Grave, 2011). The herbicide has been approved by regulatory bodies worldwide and is effective in killing a wide variety of plants, including grasses, broadleaf, and woody plants. It is marketed with the following trade names: Roundup, Rondo, Sting, Rodeo, Spasor, Muster, Tumbleweed, Sonic, Glifonox and Glycel. Sediment is the primary sink for glyphosate and after spraying its levels in sediment rise and then decline to low levels in a few months (Relyea, 2005). It is very toxic to earthworms and beneficial insects (Springett and Gray, 1992, Hassan *et al.*, 1991). Frog embryos treated with glyphosate formulation and pure glyphosate alone suffered diminution of body size, alterations of brain morphology, reduction of the eyes, alterations of the branchial arches and optic placodes, alterations of the neural plate, and other abnormalities of the nervous system (Paganelli *et al.*, 2010). When absorbed into

plants, it delays soil-degradation, and can increase glyphosate persistence in soil from two to six times (Doublet *et al.*, 2009). Because of this known toxicity, only some formulations of glyphosate are registered for use in aquatic applications (Chivian, *et al.*, 2008).

By volume, the herbicide is one of the most widely use herbicides (Grave, 2011). The pesticide formulations do contain surfactants (detergents) to help the active ingredient penetrate the waxy cuticle of the plant and is very toxic to aquatic organisms than glyphosate alone (Giesy *et al.*, 2000, Salbego *et al.*, 2010). The pesticide is readily available as formulated compound. This research therefore, is to examine the prospect of using *G. kola* seed extracts to ameliorate the water quality and to restore the blood chemistry of fish induced with glyphosate formulation.

Materials and Methods

Chemical Analysis

Glyphosate (99.5 % purity) and methanol (analytical grade) for high-performance liquid chromatography (HPLC) were obtained from Chemical Service (West Chester, PA, USA). Na₂SO₄ (99% purity), petroleum ether (analytical grade), acetonitrile (analytical grade), Ethyl 3-aminobenzoate methanesulfonate salt (Sigma-Aldrich, USA), Bovine serum albumin (BSA) used for the determination of protein quantity were supplied by Sigma Chemical Company St Louis, MO, USA. High purity pesticide grade solvents (hexane, dichloromethane and the surrogate standard solution) were obtained from Merck (Darmstadt, Germany), and helium (purity 99.999 %) by Messer Technogas (Czech Republic).

Equipment

Equipment included heparinized syringe, glassware, Cecil HPLC system comprised of CE 1200 high performance variable wavelength monitor and CEII00 liquid chromatography pump, high-resolution gas chromatography (HRGC), UV detector with variable wavelength and stainless steel column (C18 Reverse phase) packed with Octasilica, vacuum pump, and ultrasonic check.

Collection and Acclimatization of test Organisms

One hundred and fifty post- juveniles stage of *C. furcatus* of mean weight (30.00 + 0.13g) and length (13.09 + 0.2cm) from fresh water environment were collected from a private farm (Patiby Agro Industrial Enterprise) from Erawa Owhe, Delta State, Nigeria. They were acclimatized to laboratory conditions in holding glass tanks containing deionized water for two weeks before they were used for the experiments. The holding tanks were aerated with the help of air pump, cleaned and water renewed daily. Fish were fed on 30% protein pellets, unconsumed feed and faecal wastes were removed and water replenished regularly as recommended by (Oyelese and Faturoti, 1995).

Collection and Processing of Seeds. Matured *G. kola* seeds were obtained from a private farm at Walode, Osun State Nigeria. Brown coated seeds were manually removed from the pod and air dried for five days. The dried brown coat was hand peeled, and the seeds cut into pieces and re-dried at room temperature (22 + 0.15)°C for three months. The seeds sample were grounded using Nakai blender (dry mill) and filter through a 40-mesh screen and extracted for 7 hours using the Soxhlet apparatus as described by Onunkwo et al., (2004) with slight modification.

Extraction of the Powdered Seeds. One hundred gram (100g) of the powder of *G. kola* was spiked with a solution of surrogate standard (d8-naphthalene, d10-acenaphthene, d12-chrysene and d12-perylene) and extracted with a mixture of dichloromethane and n – hexane in a ratio 2: 3, having been subjected to a vigorous shaking in a sonication bath for 5 hours. The solvent was separated, concentrated using a rotatory evaporator and eluted with methanol. The elute was transferred into an open 250 ml conical flask in a placid environment for 48 hours to evaporate the methanol.

Experimental Design

The concentrations of glyphosate formulation for the test were prepared from the stock solution via serial dilution. Stock solution, test water concentrations and *G. kola* seed extract concentration were verified by Cecil HPLC system comprised of CE 1200 high performance variable wavelength monitor and CEII00 liquid chromatography pump and high-resolution gas chromatography (HRGC), using a Hewlett-Packard 5890 capillary gas chromatograph (Hewlett-Packard, Avondale, PA, USA) equipped with an electron capture detector (Hewlett-Packard).

Acute Test

The fish were determined to be free of external parasites prior to the exposure. The test was conducted under OECD Guideline No. 203 for static-renewal test conditions (OECD, 1992) and APHA, AWWA, WEF, (1998) with some modification. Fifteen glass aquaria were used for this study, with three replicates per treatment. Each aquarium contains different concentrations of the toxicant. All experiments conducted at room temperature and the tanks properly aerated. Fish were not fed during the experiment (Reish and Oshida, 1987). Thirty minutes after the preparation of test solution, 5 post - juveniles were carefully placed into each tank with their replicate tanks of five different concentrations including the control (0.00, 1.50, 3.00, 4.50 and 6.0)mg/L. Seventy-five percent of the test solution was renewed each day and aerated with the aid of air pump. Fish and water quality parameters (pH, Temperature, Dissolved Oxygen, turbidity, alkalinity and total hardness) of the test solution were determined at 24hrs interval, using standard methods. Cumulative fish mortality was

recorded at 24-, 48-, 72-, and 96-h time intervals and the LC₅₀s of each period calculated using the lethal computer program developed by Finney (1971). Experiment lasted for 96hrs for the different concentrations of the pesticide.

Chronic Test

The chronic test was conducted under OECD test guideline 407 (OECD, 1997a). From the result of acute toxicity, sublethal concentrations (0.00, 0.08, 0.12, and 0.16 and 0.32) mg/L were prepared. Fifteen glass aquaria were also used with 3 replicates per treatment and with the same conditions as in acute toxicity. The fish were divided into five groups of ten post- juveniles per test concentration in three replicates. Group A was given distilled water as contained in the experimental doses, group B was treated with 0.16mg/L glyphosate formulation only while other groups were exposed to the same concentration as in group A, but with different concentrations of *G. kola* seed extract. Group C, D and E received 150, 250, and 350mg/L of extract of *G. kola* seeds extract respectively.

Fish and water quality parameters (pH, Temperature, Dissolved Oxygen, Turbidity, Alkalinity, and Hardness) of the test solution were monitored throughout the duration of the experiment. Signs of stress such as loss of co-ordination, unusual lethargy, erratic behaviour, and gasping of air were monitored throughout the period of experiment.

Blood indices; erythrocytes, leucocytes, haemoglobin, haematocrit, the metabolites; carbohydrates, protein and hydrocortisone, a steroid hormone were estimated in the experimental and control fishes at the end of 28 days exposure.

Water Quality

Total hardness and total alkalinity were measured by the titration method (Boyd and Tucker, 1992). Dissolved oxygen concentration was measured by the Winkler method (Boyd and Tucker, 1992). Water temperature and pH were determined with a glass electrode (Thermo Orion, Beverly, Massachusetts, USA).

Blood Collection

At the end of the 28 days, the fish were removed from aquaria and immediately anesthetized with MS222 (Ethyl 3-aminobenzoate methanesulfonate salt, Sigma). Blood samples were taken by puncturing the caudal vessels with a 20-gauge needle and aspirating 0.2- 0.4 ml sample of mixed arterial and venous blood into a heparinized syringe, a technique shown to minimize dilution by tissue fluids (Congleton and LaVoie, 2001). The blood samples were stored in heparinized blood collecting duct for the estimation of, total erythrocyte count (TEC), total leucocyte count (TLC), haemoglobin and carbohydrates level. Similarly, blood was collected in a plane bottles (without anticoagulants) and stored at -20°C for

protein, and hydrocortisone analysis. The blood was allowed to clot for 30min and centrifuged at 2000 g for 15 min for clear separation of the serum and stored at -80°C until the analysis.

Blood cells Determination

The whole blood was used for the estimation of the blood count. Erythrocytes and leucocytes were counted by method of Rusia and Sood (1992) as modified by Bomski (1995) using haemocytometer. Haemoglobin content was estimated by Cyanmethaemoglobin method (Drabkin, 1946) as modified by (Bomski, 1995), while haematocrit was estimated using microhaematocrit method (Goldenfarb et al., 1971).

Biochemical Parameters

Changes in Carbohydrates metabolism was determined by the method of Folin and Malmros micro procedure as modified by Murrel and Nace (1958) and verified by using the Enzymatic-Calorimetric method (Trinder, 1969). Bovine serum albumin (BSA) used for the determination of protein quantity was purchased from Sigma Chemical Company St Louis, MO, USA. Protein determination was performed using the original Lowry method (Lowry et al., 1951). Electrochemiluminometric assay was used in the determination of the hydrocortisone levels. The test kit was prepared in accordance with the method described

by Barseghian et al.(1982) as modified by Chiu et al. (2003).

Statistical Analysis

The susceptibility of juveniles of *C. furcatus* to glyphosate formulation and responses to various treatments of *G. kola* seed extract was determined using the probit (Probit software) method for analysis (Finney, 1971) for LC_{50} at 96 hrs. Student's t test and one- way analysis of variance (George and William, 1989; Petrie and Watson, 1999) was used to test for significant differences in the values of the parameters in fish control fish and various treatments, p values of 0.05 or less were considered statistically significant (Fisher, 1950).

Results

Physico-chemical properties of the test media

The water quality parameters (pH, Temperature, Dissolved Oxygen, Turbidity, Alkalinity and Total hardness) monitored during the exposure periods were not significantly different between fish treated with glyphosate formulation alone and other treatments ($p > 0.05$, $F = 5.6$) except dissolve oxygen which was highly significantly ($p < 0.01$) between fish treated with glyphosate formulation alone and other treatments and the control (Table 1).

Table 1: Concentrations of physiochemical parameters of the test media

Parameters	pH	Temp ($^{\circ}\text{C}$)	DO (mg/l)	Turbidity (mg/L)	Alkalinity (mg/L)	Hardness (mg/L)
Treatments	Mean \pm SD	Mean \pm SD	Mean \pm SD	Mean \pm SD	Mean \pm SD	Mean \pm SD
A	7.32 \pm 0.02 ^a	25.67 \pm 0.16 ^a	7.10 \pm 0.22 ^a	0.23 \pm 0.04 ^a	17.40 \pm 0.72 ^a	31.23 \pm 1.15 ^a
B	7.36 \pm 0.16 ^a	25.00 \pm 0.30 ^a	5.12 \pm 0.19 ^b	0.23 \pm 0.06 ^a	17.63 \pm 0.42 ^a	31.33 \pm 1.15 ^a
C	7.25 \pm 0.10 ^a	27.33 \pm 0.68 ^a	7.30 \pm 0.31 ^a	0.24 \pm 0.02 ^a	17.40 \pm 0.36 ^a	31.20 \pm 1.02 ^a
D	7.52 \pm 0.25 ^a	27.00 \pm 1.20 ^a	7.03 \pm 0.22 ^a	0.25 \pm 0.04 ^a	17.13 \pm 1.20 ^a	30.60 \pm 0.50 ^a
E	7.32 \pm 0.20 ^a	26.33 \pm 0.48 ^a	7.16 \pm 0.02 ^a	0.26 \pm 0.03 ^a	17.23 \pm 0.12 ^a	30.60 \pm 0.16 ^a

Mean with different superscript in the column are significantly different * ($p < 0.05$)

Haemathological Indices

The responses of various haemathological indices in *C. furcatus* exposed to glyphosate formulation alone and other treatments is shown in table 2. All the treatments varies significantly ($p < 0.05$) between the control and various treatments except the glyphosate

formulation treated with 250mg/l *G. kola* seeds extract. However, erythrocyte sedimentation rate (ESR) showed no significant different between the control and glyphosate formulation treated with 250mg/l *G. kola* seeds extract ($p > 0.05$, $F=7.6$).

Table 2: Blood indices in *Chrysichthys furcatus* treated with glyphosate formulation and different concentrations of *Garcinia kola* seed extract

Blood indices	RBC(mill/cmm)	WBC(G.l^{-1})	Haemoglobin(g/L)	PCV (%)	ESR (mm / hr)
Treatments	Mean \pm SD	Mean \pm SD	Mean \pm SD	Mean \pm SD	Mean \pm SD
A	11.02 \pm 0.06 ^a	25.00 \pm 0.16 ^a	9.20 \pm 0.16 ^a	0.58 \pm 0.10 ^a	27.00 \pm 1.10 ^a
B	4.13 \pm 0.11 ^b	83.80 \pm 0.30 ^b	3.10 \pm 1.10 ^b	0.16 \pm 0.10 ^b	8.70 \pm 1.13 ^b
C	7.11 \pm 0.13 ^c	42.00 \pm 0.68 ^c	5.16 \pm 0.11 ^c	0.29 \pm 0.05 ^c	21.50 \pm 0.16 ^c
D	11.17 \pm 0.20 ^a	26.00 \pm 1.20 ^a	9.00 \pm 1.10 ^a	0.62 \pm 0.09 ^a	27.30 \pm 0.11 ^a
E	9.16 \pm 0.13 ^d	35.00 \pm 0.48 ^d	6.10 \pm 0.05 ^c	0.49 \pm 1.02 ^d	25.50 \pm 0.13 ^a

Mean with different superscript in the column are significantly different * ($p < 0.05$)

Metabolites**Carbohydrates**

Changes in carbohydrates metabolism in *C. furcatus* exposed to various treatments of *glyphosate* formulation alone and treatments with *G. kola* seeds extract is shown in figure 1. Carbohydrates metabolism

between the control and *glyphosate* formulation treated with various concentrations of *G. kola* seeds extract varies significantly ($p < 0.05$). However, carbohydrate metabolism in treatments D and E (250 mg/l and 350mg/l *G. kola* seeds extract) are comparable with the control.

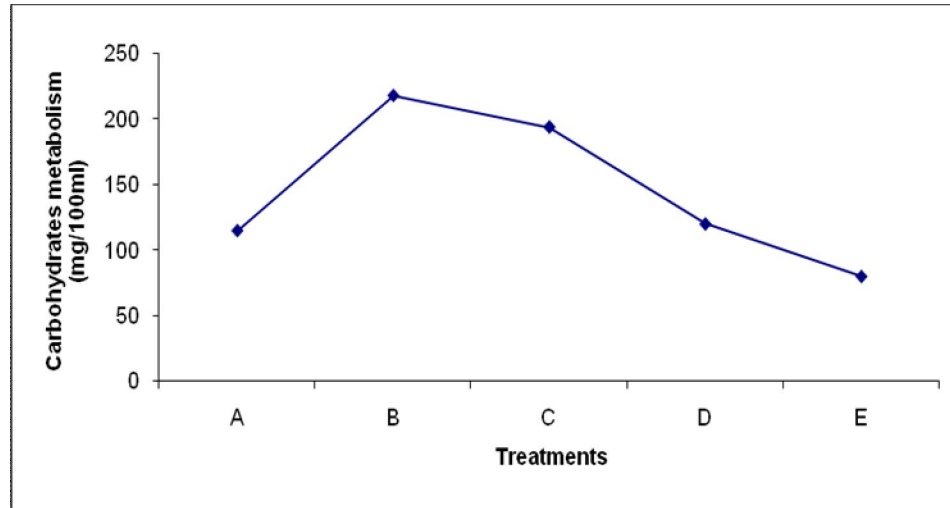


Figure 1: Changes in carbohydrates metabolism (mg/100ml) in *Chrysichthys furcatus* exposed to differential treatments of *glyphosate* formulation and *Garcinia kola* seeds extracts after 28 days.

Protein

Protein metabolism in fish exposed to various treatments of *glyphosate* and *G. kola* seeds extract are shown in figure 2. Protein metabolism in *glyphosate* formulation treated fish and the control fish and

treatment D and E was highly significant ($p < 0.01$, $F = 4.7$). Between *glyphosate* formulation and treatment C was significant ($p < 0.05$, $F = 6.0$). No significant different ($p > 0.05$) between the control and treatments C, D and E

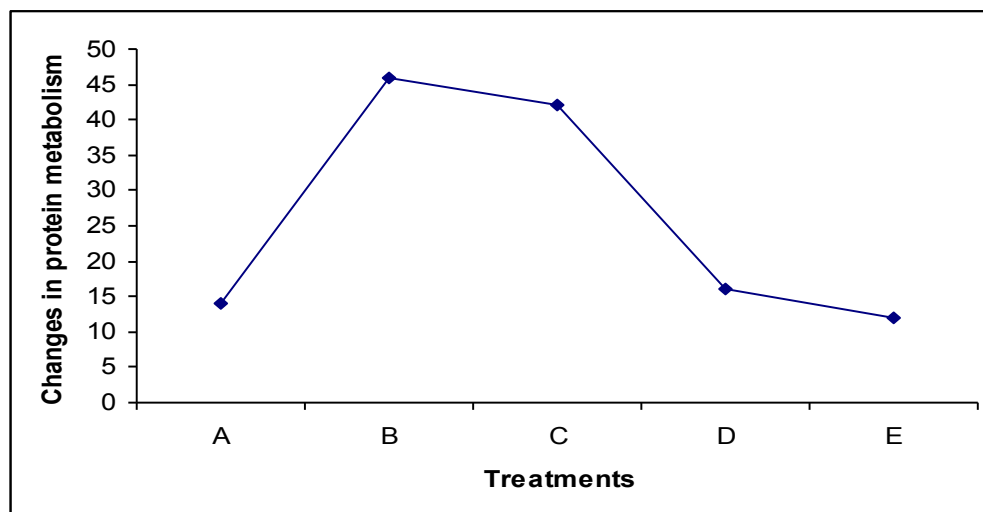


Figure 2: Variation in the serum protein (mg/100ml) in *Chrysichthys furcatus* exposed to differential treatments of *glyphosate* formulation and *Garcinia kola* seeds extracts after 28 days.

Steroid hormone Hydrocortisone

Hydrocortisone secretion in this investigation is shown in figure 3. Comparing the control and various treatments, the hormone secretion was significantly

affected ($p < 0.05$) between the control and glyphosate formulation treatment alone and C (glyphosate formulation and 150 mg/l *G. kola* seeds extract). Treatment D and E were comparable with the control.

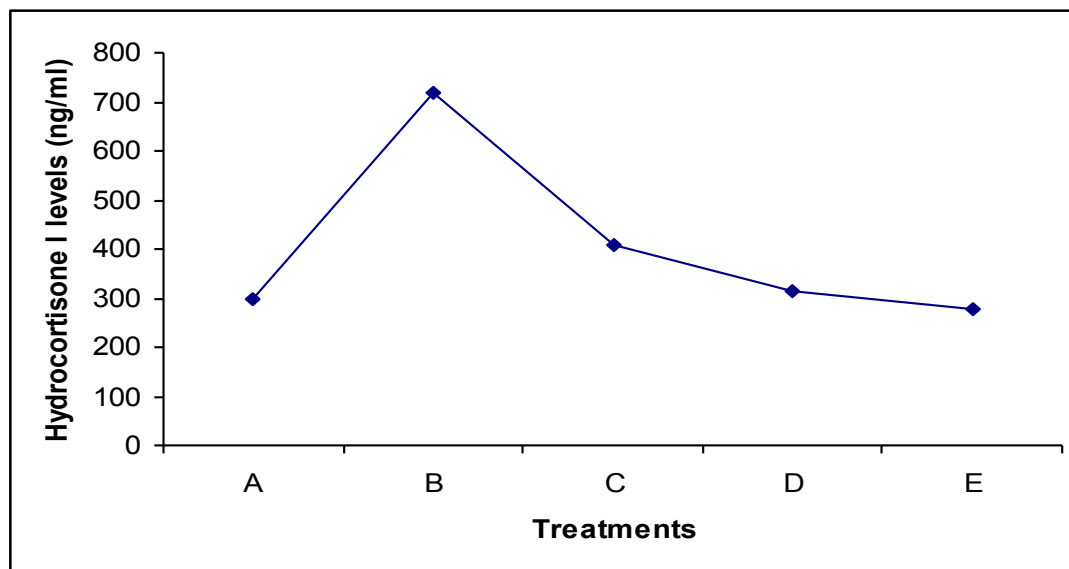


Figure 3: Hydrocortisone I levels (ng/ml) in the serum of *Chrysichthys furcatus* exposed to differential treatments of glyphosate formulation and *Garcinia kola* seeds extracts after 28 days

Discussion

Physicochemical parameters

The changes in water parameters and the responses of *C. furcatus* to the treatment of glyphosate formulation and various concentrations of the seed extract of *G. kola* showed no significant change when comparing the control group with the treated groups, except the dissolve oxygen which was significantly ($p < 0.05$) affected in glyphosate formulation treated alone. Also, only in treatment D (*G. kola* seed extract treated with 250 mg/l extract) that the dissolve oxygen is comparable to the control. An observation that showed that the plant extract cleansing property is dose dependent. Dissolve oxygen is very importance in aquatic ecosystem, it brings out various biochemical changes and it influence metabolic activities in organisms and a good quality water should have the solubility of oxygen 7.0 mg/L at 30°C (Sinha et al., 2000; Ajit et al., 2012), which is within the range observed in the control and *G. kola* seed extract treated with 250 mg/l seed's extract.

Haemathological Indices

Haematological indices are usually altered during diseases or malnutrition conditions and are very sensitive to various environmental factors and chemicals and can provide substantial diagnostic information (Haniffa and Vijayarani, 1989). On the basis of haematological studies, it would be possible to

predict the physiological state of fish in natural water bodies (Vosyliene and Kazlauskienė, 2004). Differences in blood parameters of fish in this investigation could therefore be attributed to glyphosate formulation which were significantly high ($p < 0.05$) when compared with the control and other treatments. All the measured blood parameters in *C. furcatus* were found to be affected by glyphosate formulation exposure during the study periods. The fish exposed to sublethal concentrations of glyphosate formulation had lower erythrocyte sedimentation rate, red blood cells, hematocrit and haemoglobin (Hb%) content compared with the control. Similar findings were reported when freshwater fish *C. gariepinus* and *O. niloticus* were exposed to endosulfan (Ikpesu, 2010). Reduction of TEC and Hb% may be suggestive of an appreciable decline in the hematopoiesis leading to various types of anemia (Jenkins and Smith, 2003, Seth and Sesena, 2003). Diseases and other environmental stressors can inhibit or elicit total leucocyte counts (TLC) and the degree of elevation often indicates the severity of the stress. Increase in the TLC in fish treated with glyphosate formulations might be due to the presence of toxic substances or may be associated with the pollutant induced tissue damage as was also stated by Solbe (1995). Generally, the haemathological indices obtained in fish treated with the mixture of glyphosate formulation and *G. kola*

seeds extract (group C-D) were within the normal ranges for fish (Svobodova et al, 1991). However, the *G. kola* seed extract treatment is dose dependent with the group D (*G. kola* seed extract treated with 250 mg/l extract) promising.

Metabolites

Carbohydrates

Changes in carbohydrates metabolism has been suggested as useful general indicator of stress in teleost (Luskova et al., 2002). Nemcsok and Bores (1982), also reported that blood glucose appeared to be a sensitive indicator of environmental stress and high levels of blood glucose are caused by disorders in carbohydrate metabolism appearing in the condition of physical and chemical stresses (Wedemeyer et al. 1981). The significant ($p < 0.05$) elevation of carbohydrates metabolism in *C. furcatus* exposed to glyphosate formulation alone may be due to mobilization of muscle and hepatic glycogen (Sing and Srivastava, 1981). Stress elicits rapid secretion of glucocorticoids and catecholamines from adrenal tissue of the fish. These hormones suppress insulin secretion from the pancreas, promote gluconeogenesis in the liver, and inhibit glucose uptake in peripheral tissues (Andrews and Walker, 1999). Therefore, hyperglycemia condition registered in the present study may be ascribed to glyphosate induced hyper-secretion of this hormone which cause glycolysis in the liver and muscle of fish. Omoregie et al. (1990) reported that tilapia showed marked hyperglycemic response to stressed environmental conditions as a result of incomplete metabolism of the blood sugar due to impaired osmoregulation. Carbohydrates metabolism in fish treated with the mixture of glyphosate formulation and *G. kola* seed extract (C-D) is dose dependent with the group D (glyphosate formulation treated with 250 mg/l *G. kola* seed extract) normalising the glucose levels after 28 days, an indication of antihyperglycemic property of the plant extract. Corroborating the therapeutic nature of herb in regulating carbohydrates metabolism is the observation reported when ethanolic extracts (50%) of *Caesalpinia bonducella* seeds normalized the blood sugar in streptozotocin (STZ)-diabetic rats (Sharma et al., 1997). In the same way administration of aqueous extract of *Aegle marmelos* leaves improves digestion and reduces blood sugar and urea, serum cholesterol in alloxanized rats as compared to control. Along with exhibiting hypoglycemic activity, this extract also prevented peak rise in blood sugar at 1 hour in oral glucose tolerance test (Karunanayake et al., 1984).

When the extracts was increased to 350mg / l, there was inhibition of glucose secretion, an indication that over dose could be detrimental to fish. Similar to this finding is the work of Aderibigbe et al., 1999, who reported hypoglycemic activity of aqueous extract of

Mangifera indica in either normoglycemic or streptozotocin induced diabetic in rats. In the same way, Ethanolic extracts of *M. charantia* (200 mg/kg) showed an antihyperglycemic and also hypoglycemic effect in normal and STZ diabetic rats (Shibib et al., 1993).

Protein

It is obvious that exposure of fish for a long time to most toxicants including pesticides interferes with protein metabolism. Increases protein level reported in glyphosate treated fish may be attributed to stress mediated immobilization of these compounds leading to an increase in energy demands by the fish to cope with environmental condition caused by the toxicant (Jenkins et al., 2003). Also, proteins are functional molecules, it is possible that any complications related to alteration of glucose level are related to defective synthesis of certain proteins since the amount of mitochondrial protein present is closely related to the rate of ATP production (Takahashi and Hood, 1997). The recovery mechanism to *Gracinia kola* is spontaneous. The result indicated that protein metabolism in the treated fish is dose dependent. Likewise Kamble et al., 1998 revealed how dried extracts of *Coccinia indica* (500 mg/kg body weight) regulates the protein and glucose metabolism in human. The extracts restored the activities of enzyme lipoprotein lipase (LPL) that was reduced and glucose-6-phosphatase and lactate dehydrogenase, which were raised in untreated diabetics. Also, Oral administration of 500 mg/kg of *C. indica* leaves showed significant hypoglycemia in alloxanized diabetic dogs and increased glucose tolerance in normal and diabetic dogs (Kamble et al., 1998).

Hydrocortisone

The high secretion of the steroid hormone in glyphosate formulation treated fish may be due to its response to stress caused by the herbicides (de Kloet et al., 2008). Stress heighten hydrocortisone secretion thereby enhancing the breakdown of proteins to provide the fuel to maintain body function and physiological antagonist to insulin by promoting breakdown of carbohydrates and lipids thereby mobilizing energy reserves (Du et al., 2009). The hormone also act as an anti-inflammatory agent by depresses immune reactions, and increases the vasoconstriction caused by epinephrine, a pivotal role in helping the hypothalamus-pituitary-adrenal axis adjust to stress (Losel and Wehling, 2003). It is importance to note that increase in hydrocortisone secretion in fish treated with glyphosate formulation can lead to a decrease in insulin sensitivity, increase insulin resistance, reduce kidney function, hypertension, suppress immune function, reduce growth hormone levels, and reduced connective tissue strength. This is detrimental to fish and animals in general including

human. This may affect the weight and size of the fish thereby reducing their market values. The hydrocortisone secretion normalized in *G. kola* extract treatments and was dose dependent with the treatments D and E most encouraging. Similar results were observed by Pathak et al., (2009) when he administered *Lycopodium* spores to rat. The rats first received carcinogens known to elevate cortisol and reduce testosterone. Subsequent administration of *Lycopodium* spores decreased cortisol and increased testosterone secretion. Also green tea pills reduced circulating cortisol relative to no treatment in obese men and women who received the pills for three months (Franseco Di Pierro, 2009).

Conclusion

Glyphosate formulation is a toxic chemical and its sublethal concentrations can alter fish blood chemistry. However, *Garcinia kola* a medicinal plant has provided raw material for innovative, useful and promising antidote to environmental glyphosate and other xenobiotics contamination. Therefore, in the area that is prone to pollution especially aquatic environment should be treated with right quantity of *Garcinia kola* seed extract to neutralize the contaminants effects. A further study is therefore required on how the extract of *Garcinia kola* seeds could be formulated into a tablet and capsules to ensure dosage precision that would increase its acceptability.

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