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Full Length Research Paper

Evaluation of biochemical changes in *Clarias gariepinus* adults exposed to aqueous and ethanolic extracts of *Parkia biglobosa* pods

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Clarias gariepinus **adults were exposed to aqueous and ethanolic extracts of** ***Parkia biglobosa*** **pods (85 mg/L, 100 mg/L, 115 mg/L, 130 mg/L and 145 mg/L), respectively, in order to evaluate their effects on the biochemical parameters of exposed fish. There were significant increases (P < 0.05) in aspartate aminotransferase and alanine aminotransferase activities as well as in plasma glucose concentrations respectively. The activity of alkaline phosphatase changed in a non significant (P > 0.05) manner with increasing concentrations of both extracts. However, plasma total proteins concentrations increased significantly (P < 0.05) with increasing extracts concentrations in fish exposed to ethanolic extracts while non significant changes (P > 0.05) occurred in those exposed to aqueous extracts. Similarly, there were significant decreases (P < 0.05) in cholesterol concentrations with increasing extract concentrations in fishes exposed to the ethanolic extract while there were no significant changes (P > 0.05) in those exposed to aqueous extracts. These biochemical changes in exposed fish revealed that both extracts of** ***Parkia biglobosa*** **pods are toxic to** ***Clarias gariepinus*** **adults with non significant differences (P > 0.05) between their levels of toxicities.**

Key words: *Clarias gariepinus*, *Parkia biglobosa* pods, toxicity, biochemical parameters.

INTRODUCTION

Cells naturally contain enzymes for their functions such that damages to cellular membrane lead to their escape into the blood where their presence or activities can easily be measured as an index of cell integrity (Coles, 1974; Coppo et al*.,* 2002). Certain serum chemistry could be used to identify tissue damage (Patti and Kulkarni, 1993). Aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphatase (ALP) are normally found within the cells of the liver, heart, gills, kidneys, muscles and organs (Shalaby, 2009) but their increase in the plasma indicate tissue injury or organ dysfunction (Wells et al*.,* 1986). However, changes in plasma glucose, total proteins and cholesterol concentrations can be indicative of a classical general adaptive response to stress in fishes exposed to pollutants (Martinez et al*.,* 2004). This is because fish blood is very sensitive to pollution-induced stress (Patti and Kulkarni, 1993).

Biochemical changes in fishes exposed to various pollutants have been documented (Attar, 2005; Ogueji and Auta, 2007; Kori-Siakpere and Ubogu, 2008; Mousa et al*.,* 2008; Shalaby, 2009). Despite the piscicidal use of *Parkia biglobosa* in Nigeria (Fafioye, 2005), its effects on biochemical changes has not been examined in *Clarias gariepinus* that is widely cultivated (FAO, 1977) and greatly abounds in our waters (Fagbenro, 1992). This study is therefore, aimed at evaluating biochemical changes in *C. gariepinus* adults exposed to both aqueous and ethanolic extracts of *P. biglobosa* pods.

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Abbreviations: AST, Aspartate aminotransferase; **ALT,** alanine aminotransferase; **ALP,** alkaline phosphatise.

MATERIALS AND METHODS

Aqueous and ethanolic extractions of *P. biglobosa* pods

Six liters of distilled water was used to soak 1000.00 g of the pulverized pods powder of *P. biglobosa* over night prior to filtration based on the maceration method (Bentley, 1977; Ghani, 1990) which yielded a total of 459 g (45.90%w/w) of aqueous extract after freeze drying (LYOVAC GT2, AMSCO/FINN-AQUA, CATNO. 204555B4-2, Germany). A total 725.21 g (57.56%w/w) of ethanolic extract was realized when 1260 g of the fine powder of *P. biglobosa* pods was soaked in 5 L of absolute ethanol (99.80vol% Lot No. 71930. SIGMA-ALDRICH LAB., GMbH. D-30926, Seezle, Germany) in a separation funnel over a 48 h period at room temperature based on the same maceration method prior to con-centrating it to dryness in an evaporation dish over a 72 h period.

Experimental fish

Adult *C. gariepinus* (163.70 ± 3.71 g mean weight and 27.23 ± 0.19 cm mean total lengths) purchased from Kune Integrated Farms Limited, Katsina, Katsina State, Nigeria were acclimatized in the laboratory for two weeks while being fed *ad libitum* with 4 mm commercial pelleted catfish feed (Multi feed, Zemach feed mill, Israel). Dechlori-nated pond water was changed every other day and mortality was less than 5% within this period. There was no form of prophylactic or therapeutic treatments within this period (Oladimeji et al*.,* 1988) while feeding was discontinued 48 h before and during the exposure period (Adeyemo, 2005) so as to prevent possible interference from stomach contents of exposed fish and their wastes in reconstituted extracts (Olufayo, 2009).

Toxicity bioassay

A pilot study was conducted to determine five graded concentrations (Omitoyin et al*.,* 1999; Fafioye, 2001) prior to the actual experiment that was based on static bioassay (APHA, 1981). Known weights of 3.4, 4.0, 4.6, 5.2 and 5.8 g of the aqueous and ethanolic extract were each dissolved in 40 L of dechlorinated water per aquarium per experimental set-up to give extracts concentrations of 85, 100, 115, 130 and 145 mg/L, respectively. Ten adult *C. gariepinus* were introduced at random into each of these aquaria after allowing the reconstituted extracts to stand for 30 min (Usman et al*.,* 2005) for proper mixing with the first aquarium per experimental set-up containing no extract and thus serving as control. A 96 h exposure period was observed with treatments having replicates.

Biochemical measurements

Surviving fish were subjected to 40% ethyl alcohol (Fafioye et al*.,* 2005) prior to cutting their caudal peduncle for blood which were collected in non-heparinized tubes (Al-Akel, 1996; Ogueji and Auta, 2007). Such blood was immediately centrifuged at 1,006 x g for five minutes to obtain the plasma (Ogbu and Okechukwu, 2001). Plasma total proteins concentration was determined based on Biuret method (Henry et al., 1974) while AST and ALT activities were determined using an autoanalyser (Bayer express plus, model: 15950, Germany. 2000) based on the reference method described in International Federation of Clinical Chemists (Schwartz et al*.,* 1985).

The colourimetric procedure was used to determine ALP activities (King and Armstrong, 1934) while the same procedure was used to determine serum cholesterol concen-trations using a commercial reagent kits (Randox Laboratories Ltd, United Kingdom) based on the method of Roesclaw et al. (1974).

Plasma glucose concentrations in exposed fish were estimated using glucose oxidase method (Morgan and Iwana, 1997).

Statistical analysis

The means and standard error of means as well as one-way analysis of variance (ANOVA) for statistical significance ($P < 0.05$) was determined using (GraphPad Prism, version 4, San Diego, CA) while histogram with error bars were created using Microsoft® Office Excel programme (Microsoft® Windows Professional, Redmond, Washington DC).

RESULTS AND DISCUSSION

There were significant increase ($P < 0.05$) in AST activities with increasing extract concentrations in fish exposed to both extracts which peaked at 100 and 130 mg/L concentrations for both aqueous and ethanolic extracts, even though there was a slight decrease in AST activities at 85 mg/L concentration in fish exposed to ethanolic extract (Figure 1). Similarly, there were significant increase ($P < 0.05$) in ALT activities with increasing extract concentrations in fish exposed to both extracts which peaked at 115 and 145 mg/L concentration for both aqueous and ethanolic extracts (Figure 2). There were insignificant wave-like changes ($P > 0.05$) in ALP activities with increasing extract concentrations in fish exposed to both aqueous and ethanolic extracts, which were characterized with an initial increase at 85 mg/L concentration that dropped to its lowest at 130 mg/L concentration before another increase at the highest extracts concentration of 145 mg/L for both extracts (Figure 3).

There were significant increase ($P < 0.05$) in plasma glucose concentrations with increasing extract concentrations in fish exposed to both extracts which peaked at 130 mg/L and at 115 mg/L concentrations for both aqueous and ethanolic extracts, even though there was a slight decrease in plasma glucose at 100 mg/L concentration in fish exposed to ethanolic extract (Figure 4). There were significant increases ($P < 0.05$) in plasma total proteins concentrations with increasing extract concentrations in fish exposed to ethanolic extracts which peaked at 145 mg/L concentration. However, despite an increase in plasma total proteins concentrations with increasing extracts concentrations in fish exposed to aqueous extracts, these were not significant ($P > 0.05$) as in Figure 5. There were significant decrease ($P < 0.05$) in cholesterol concentrations with increasing extract concentrations in fish exposed to ethanolic extracts even though there was no significant increase ($P > 0.05$) at 130 mg/L concentration while changes in cholesterol concentrations with increasing extracts concentrations in fish exposed to aqueous extracts were also not significant ($P > 0.05$) as in Figure 6. However, differences between changes in biochemical parameters induced in fish exposed to both extracts were not significant ($P > 0.05$).

Elevated AST and ALT activities in exposed fish are suggestive of hepatic cellular damages as observed in

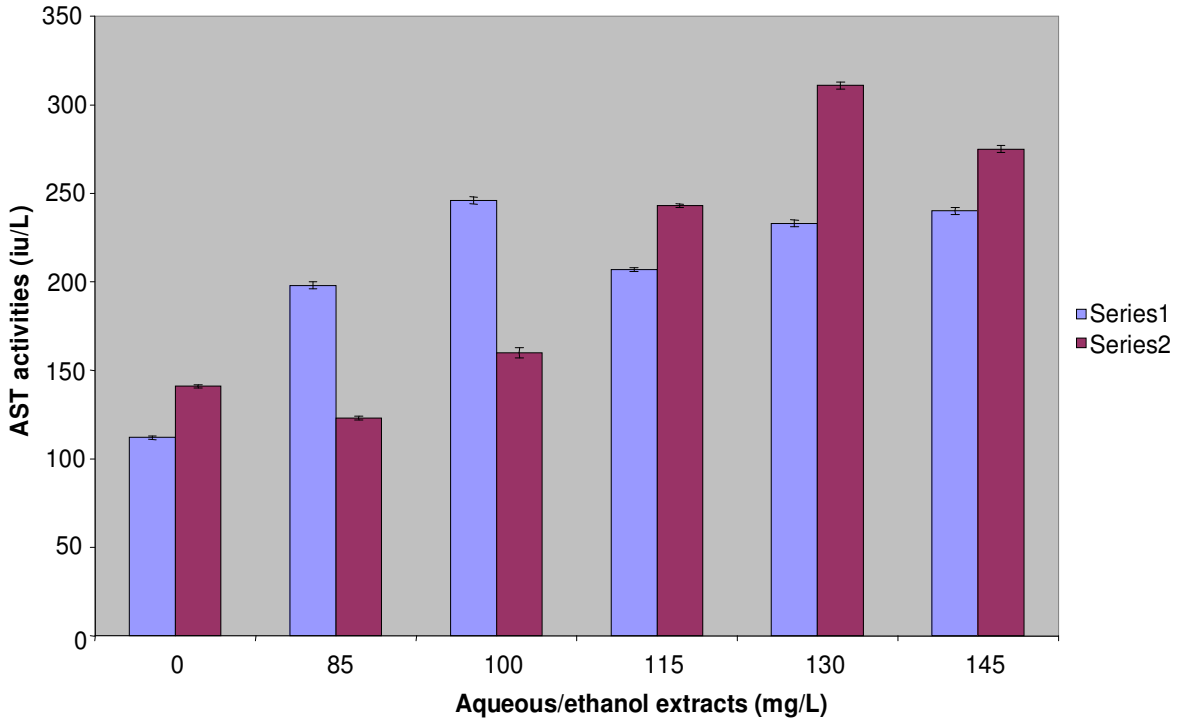

Figure 1. AST activities of adult *C. gariepinus* exposed to aqueous extract (series 1) and ethanol extract (series 2) of *P. biglobosa* pods.

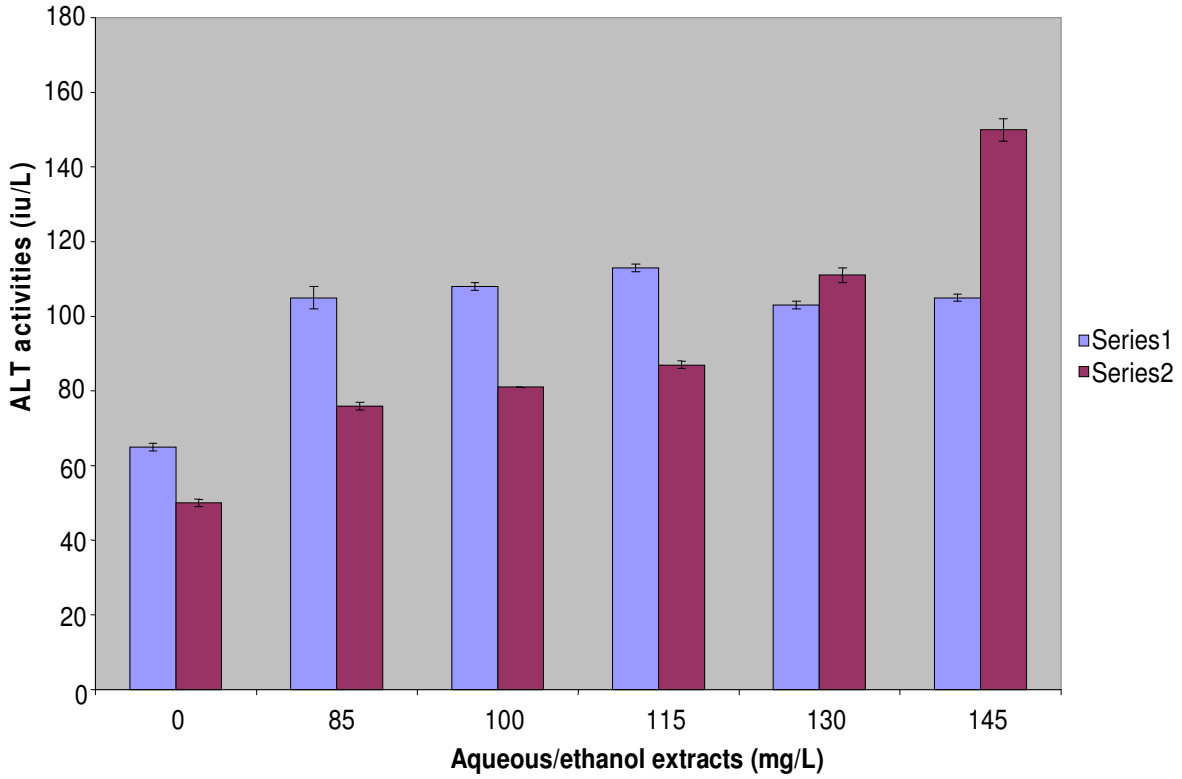

Figure 2. ALT activities of adult *C. gariepinus* exposed to aqueous extract (series 1) and ethanol extract (series 2) of *P. biglobosa* pods.

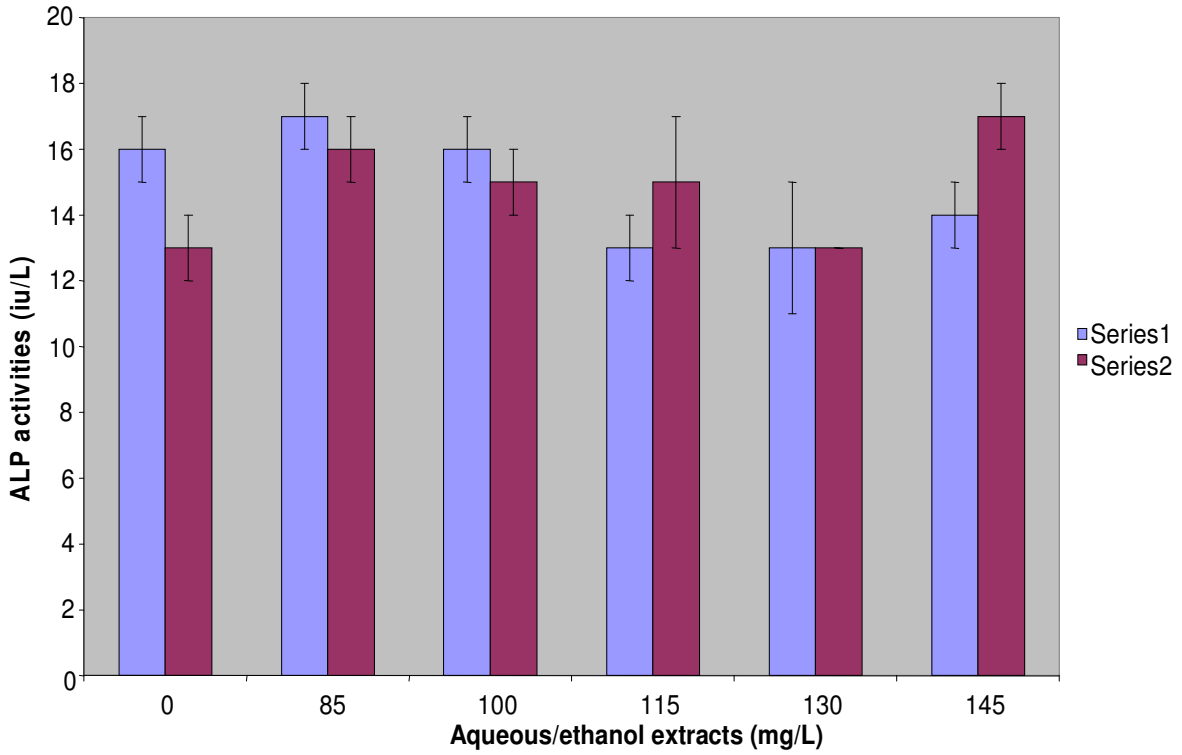

Figure 3. ALP activities of adult *C. gariepinus* exposed to aqueous extract (series 1) and ethanol extract (series 2) of *P. biglobosa* pods.

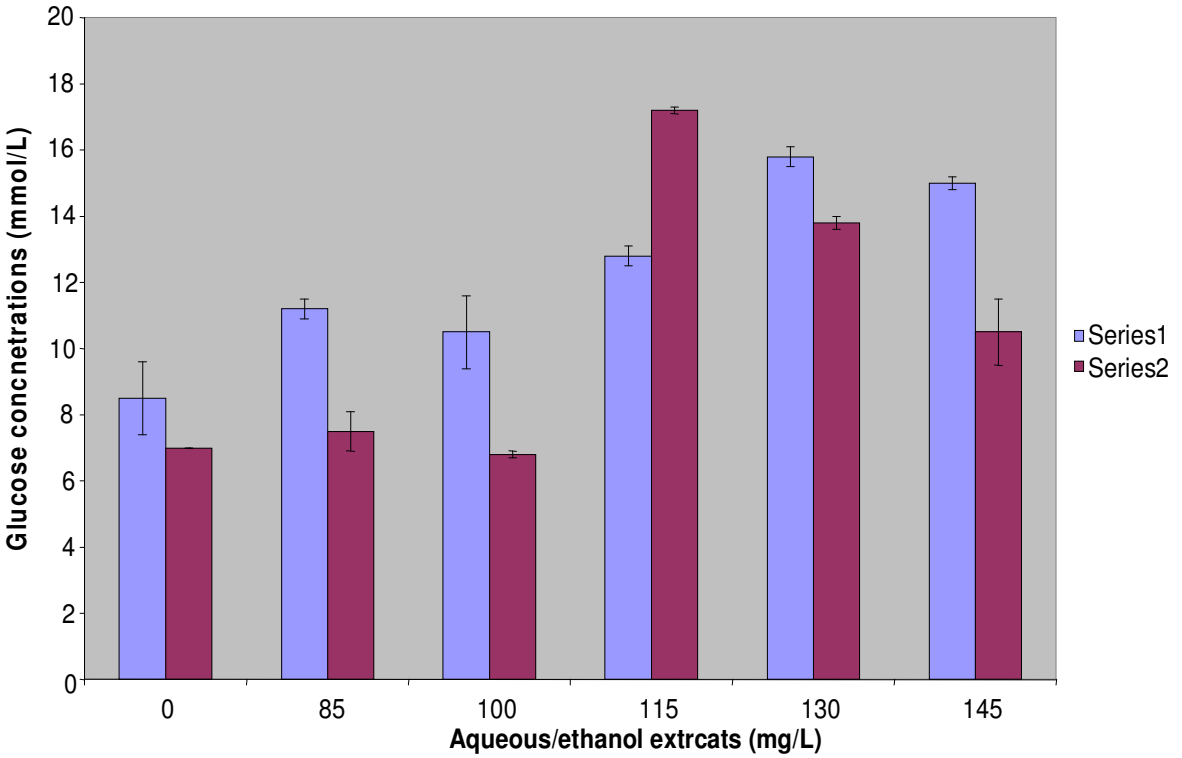

Figure 4. Glucose concentrations of adult *C. gariepinus* exposed to aqueous extract (series 1) and ethanol extract (series 2) of *P. biglobosa* pods.

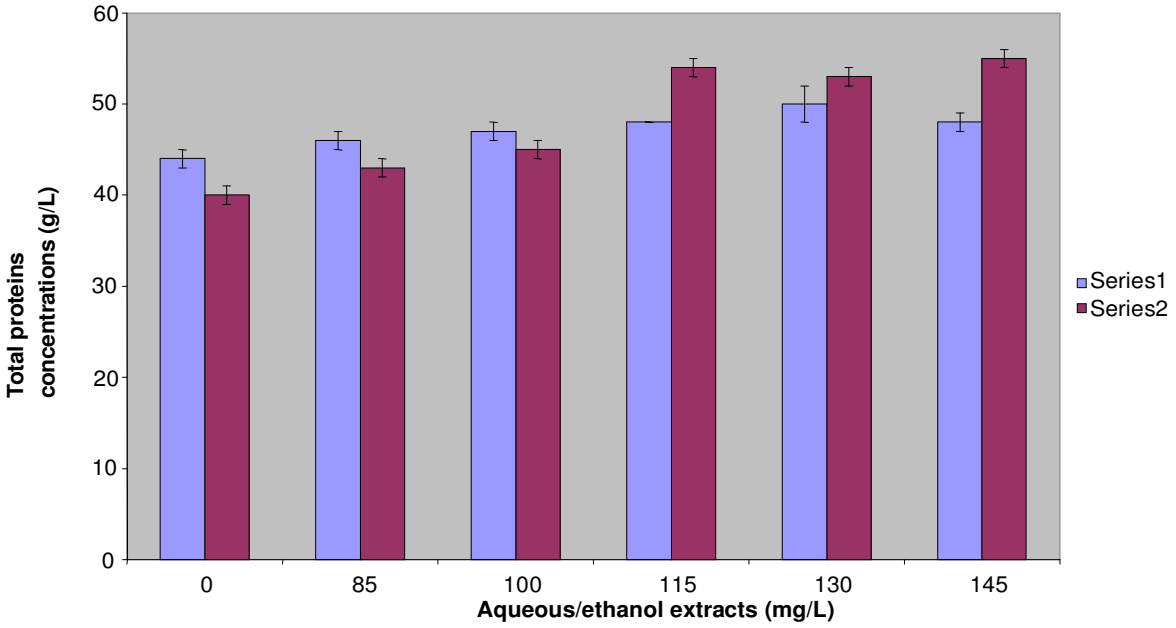

Figure 5. Total protein concentrations of adult *C. gariepinus* exposed to aqueous extract (series 1) and ethanol extract (series 2) of *P. biglobosa* pods.

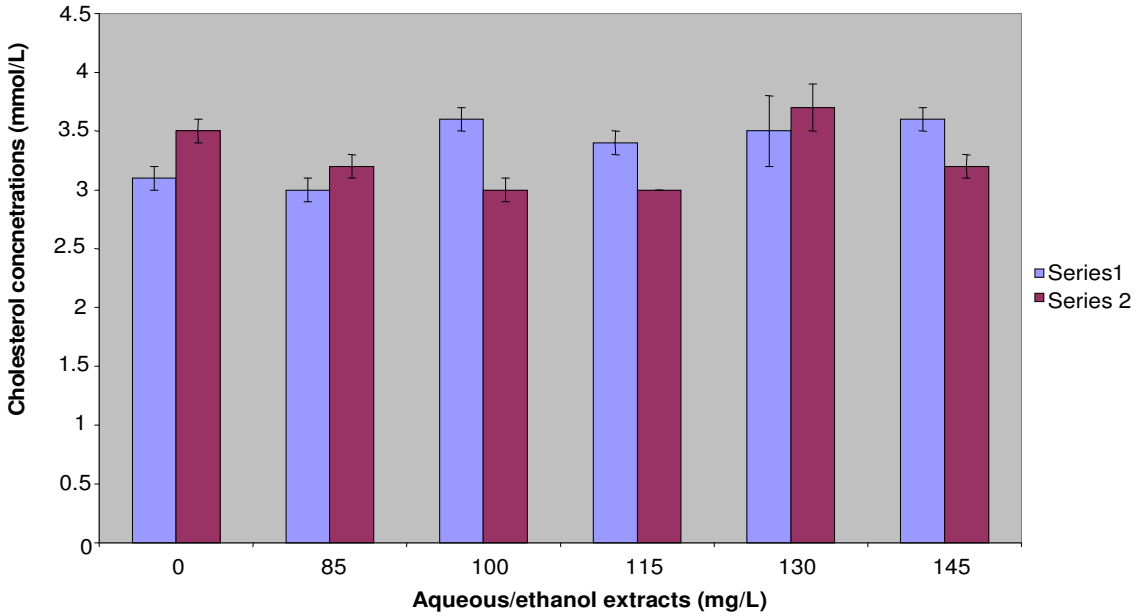

Figure 6. Cholesterol concentrations of adult *C. gariepinus* exposed to aqueous extract (series 1) and ethanol extract (series 2) of *P. biglobosa* pods.

this study leading to their leakage into circulation (Molander et al., 1957; Mousa et al., 2008). These findings are in agreement with the reports of Neskovic et al. (1996) and De Smet and Blust (2000). However, these findings disagreed with the reports of Sadhu et al. (1985) and Okechukwu and Auta (2007) who reported significant decrease ($P < 0.05$) in both AST and ALT activities which may have resulted from the use of sub-lethal doses of toxicants by both authors. The weak elevations of ALP activities in fish exposed to both extracts may be due to cholestasis and/or parenchymatous diseases of the liver even though such increases are usually higher in bile duct obstruction than in liver parenchymatous diseases (Bodansky, 1957). This is in addition to the

fact that moderate rise in ALP activities follow hepatic cellular damages (Nduka, 1999). These findings agreed with the report of Al-Attar (2005) but disagreed with the reports of Goel et al. (1982) and Das and Mukharjee (2003) who reported decrease in ALP activities, which may also not be unconnected to sub-lethal doses of malathion and cypermethrin used by them.

Marked elevations in plasma glucose concentrations may be due to increased demand for energy resulting in increased plasma catecholamines and corticosteroids (Pickeirng, 1981) that are known to induce excessive secretion of adrenalin, which stimulate breakdown of glycogen to glucose by inhibiting the neuroeffector sites in adrenal medulla (Gupta, 1974). Such elevations may also be due to enhanced gluconeogenesis response of stressed fish in their attempt to satisfy their new energy demand (Verma et al., 1983; Winkaler et al., 2007). These findings agreed with the reports of Benson et al. (1987) and Van Vuren et al. (1994) but disagreed with the works of Omoniyi et al. (2002) and Ajani et al. (2007) who reported hypoglycaemia, respectively. Ajani et al. (2007) attributed the decrease in plasma glucose concentrations after an initial increase to progressive depletion of energy due to the fact that the initial increased mobilization could not overcome the immediate threat of the toxicity. The observed hyperproteinaemia in fish exposed to ethanolic extract may be due to water loss in plasma, elevated *de novo* synthesis or relative changes in blood protein mobilization (Al-Attar, 2005). It may also be attempted by exposed fish to meet up increasing demand of detoxification of offending toxicants (Gill et al., 1990). Such observed hyperproteinaemia which can also be indicative of efficient immune response and body physiological reactions to these toxicants (Omitoyin, 2007), agreed with the findings of Gill et al. (1991) and Okechukwu and Auta (2007), respectively. However, it contrast with the findings of Omoniyi et al. (2002) and Shalaby (2009) who all reported significant hypoproteinaemia which may be due to the cellular destruction or necrosis with subsequent impairment of protein synthesis machineries (Bradbury et al., 1987) or due to pathological kidney alterations lead-ing to excessive loss of proteins (Salah El-Deen et al., 1996).

The significant hypocholestrolemia and non significant changes ($P > 0.05$) in cholesterol concentrations in fish exposed to ethanolic and aqueous extracts may be due to considerable damages to liver cells resulting in decrease synthesis within them (Kamath, 1972) or utilization of body lipid or fat as an energy supplier to meet the increased physiological demand (Salah El-Deen et al., 1996; Maruf Iftekhar et al., 2006). The finding of significant hypocho-lesterolemia agreed with the report of Okechukwu and Auta (2007) but disagreed with the findings of Krishna et al. (1994) who reported increased plasma cholesterol concentrations. Similarly, the finding of insignificant changes ($P > 0.05$) in cholesterol concentrations in fish exposed to aqueous extract agreed with the reports of Al-Attar (2005) and Omitoyin (2007), respectively.

In conclusion, the observed changes in biochemical parameters of exposed fish showed that aqueous and ethanolic extracts of *P. biglobosa* pods are both toxic to *C. gariepinus* adults and are also of almost equal toxicities to exposed fish. Therefore, both extracts can be exploited to either obtain fishes for human consumption and/or eradicate unwanted fishes from water bodies.

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