Global Veterinaria 4 (2): 198-206, 2010
ISSN 1992-6197

Biochemical Effects of Aqueous and Ethanolic Extracts of *Parkia biglobosa* Pods on *Clarias gariepinus* Juveniles

[1]S.E. Abalaka, [1]K.A.N. Esievo and [2]S.V.O. Shoyinka

[1]Department of Veterinary Pathology and Microbiology, Ahmadu Bello University, Zaria, Nigeria
[2]Department of Veterinary Pathology and Microbiology, University of Nigeria, Nsukka, Nigeria

Abstract: The biochemical effects of aqueous and ethanolic extracts of *Parkia biglobosa* pods (55, 65, 75, 85 and 95 mg L^{-1}) were investigated in *Clarias gariepinus* juveniles. The activities of Aspartate aminotransferase and Alanine aminotransferase as well as serum glucose concentrations increased significantly ($P<0.05$) with increasing concentrations of both extracts. However, there were insignificant changes in Alkaline phosphotase activity including cholesterol and total protein concentrations with increasing concentrations of both extracts even though there were actual variations in their values. It therefore, means that both extracts which elicited strong stress responses in exposed fish are highly hepatotoxic in nature and with the exception of their cholesterol concentrations ($P<0.05$), there were insignificant differences in the biochemical parameters of exposed fish induced by either extract. It was concluded that both extracts are toxic to exposed fish.

Key words: *Clarias gariepinus* · *Parkia biglobosa* pods · Juveniles · Extracts · Toxicity · Biochemical parameters

INTRODUCTION

Fish is a very important valuable source of high grade and easily digestible protein [1-3]. Even though the conventional means of fishing have always been through nettings and traps, some fishermen have deliberately used piscicides to crop fishes from water bodies [4-6] either for human consumption [6, 7] or to eradicate unwanted fishes from ponds before the stocking of desirable ones [8, 9] to save time, cost and efforts. Omnivorous *Clarias gariepinus* which grows quickly and are desirable food species worldwide has become an ecologically important and commercially valuable fish for the Nigerian fish industry [10-12]. However, *Parkia biglobosa* which is a widely distributed tree plant within the Sudanian and Guinean savannahs that greatly abounds in Nigeria has been recognized as a piscicide [4, 6, 13].

Blood biochemical changes are amongst the indices that are used in determining the toxicity of pollutants [14] especially as they indicate the status of the internal environment of the fish [15]. The aim of this study therefore, was to determine possible biochemical changes associated with the exposure of C. gariepinus juveniles to both aqueous and ethanolic extracts of P. biglobosa pods.

MATERIALS AND METHODS

Preparation of Aqueous and Ethanolic Extracts of P. Biglobosa Pods: Maceration method [16, 17] was used whereas a total of 1000.00g of the pulverized pods powder of P. biglobosa was soaked in 6L of distilled water over night prior to filtration which was later freeze-dried (Lyovac GT2, AMSCO/FINN-AQUA, Germany). This yielded 459.00g (45.90%w/w) of freeze-dried aqueous extracts. Similarly, same maceration method [16, 17] was used whereas 5L of absolute ethanol (99.80vol.%, Sigma-Aldrich Lab., Germany) was used to soak another 1260.00g of the fine pods powder of the same P. biglobosa in a separation funnel over a 48 hour (h) period at room temperature. This was filtered and concentrated to dryness in an evaporation dish within a 72 h period to yield 725.21g (57.56%w/w) of the ethanolic extracts.

Experimental Fish: Clarias gariepinus juveniles (mean weight 25.09 ± 0.52g and mean total lengths 15.38 ± 0.10cm) were purchased from Kune Integrated Farms Limited, Katsina, Katsina State. These were acclimatized in the laboratory for two weeks while being fed *ad libitum* twice daily with 0.8mm commercial pelleted catfish feed (Multi feed, Zemach feed mill,

Corresponding Author: S.E. Abalaka, Department of Veterinary Pathology and Microbiology, Ahmadu Bello University, Zaria, Nigeria

exclusive for O & T Group, Israel) containing 45% protein. The dechlorinated pond water was changed every other day with no form of prophylactic or therapeutic treatments given to fish within this period [18]. Mortality was less than 5% during this period and feeding was discontinued 48 h prior to and during the exposure period [19] in order to prevent the stomach content of exposed fish and their wastes in reconstituted extracts from interfering with the experimental results [20].

Toxicity Bioassay: A static bioassay was performed [21] after a range finding test was conducted [22, 23] to obtain toxicant concentrations of 55, 65, 75, 85 and 95mgL^{-1} by dissolving 1.1, 1.3, 1.5, 1.7 and 1.9g respectively of aqueous and ethanolic extracts of P. biglobosa pods powder in 20L of dechlorinated water in each aquarium, one at a time. These were then allowed to stand for 30 minutes [24] so as to ensure proper mixing before introducing 10 C. gariepinus juveniles into each of these aquaria at random. The first aquarium containing no toxicant acted as a control for each of the experimental group. Treatments had replicates while all observations and mortalities were recorded within the 96 h exposure period.

Biochemical Measurements: The caudal peduncles of surviving fish were cut and blood was collected in non-heparinized tubes [25, 26] after narcotizing them with 40% ethyl alcohol [27]. These were immediately centrifuged at 1,006xg for five minutes to obtain the serum [28]. Glucose oxidase method was used to determine serum glucose levels [29]. Autoanalyser (Bayer Express Plus, Model 15950, Germany) was used to determine serum total proteins level based on Biuret method [30] as well as for determining the Aspartate aminotransferase (AST) and Alanine aminotransferase (ALT) activities based on the reference method described in International Federation of Clinical Chemistry [31]. Alkaline phosphatase (ALP) activity was determined colourimetrically via enzymatic hydrolysis according to King and Armstrong [32] while serum cholesterol level was determined colourimetrically too using a commercially available reagent kits (Randox Laboratories Ltd, United Kingdom) based on the enzymatic method of Roeschlaw *et al.* [33].

Statistical Analysis: Histogram with error bars was plotted using Microsoft® Office Excel [34] while GraphPad Prism version [35] was used to run the means and standard error of means as well as one-way analysis of variance (ANOVA) for statistical significance ($P<0.05$).

RESULTS

There were significant increases ($P<0.05$) in both AST and ALT activities with insignificant changes in ALP activities in C. gariepinus juveniles exposed to increasing concentrations of both aqueous and ethanolic extracts of P. biglobosa pods. However, the significant increases ($P<0.05$) in AST and ALT activities with increasing extracts concentrations were marked at about 65 mgL^{-1} concentration for the ethanolic extracts. Exposed C. gariepinus juveniles showed significant increase ($P<0.05$) in serum glucose concentrations and insignificant changes in serum total proteins and cholesterol concentrations with increasing concentrations of both extracts respectively. However, the blood collected from C. gariepinus juveniles exposed to 95 mgL^{-1} of the ethanolic extract of P. biglobosa pods could not give enough serum for any biochemical analysis. All assessed biochemical parameters showed insignificant differences between the toxicities of both extracts with the exception of their cholesterol concentrations which showed significant differences ($P<0.05$) between the toxicity of aqueous and ethanolic extracts of P. biglobosa pods.

DISCUSSION

The significant increases ($P<0.05$) in AST and ALT activities with increasing extracts concentration may be due to severe hepatic necrosis resulting in their leakage into the blood [36, 37] even though increase in blood enzymatic activities may in addition, be due to their increased synthesis or enzymatic inductions [38]. The decline in AST and ALT activities after the highest activities obtained at extracts concentration of 65 mgL^{-1} might have occurred because these enzymes activities are usually elevated in acute liver toxicities which tend to decrease with prolonged intoxication due to prolonged hepatic damage [39]. These findings agree with the reports of Al-Attar [40] but disagree with the findings of Ogueji and Auta [26] and Oruç and Üner [41] who all reported decreased AST and ALT activities which may not be unconnected to sub-lethal doses of toxicants used in their works. Alkaline phosphatase, which is made in the liver, is membrane bound to the biliary

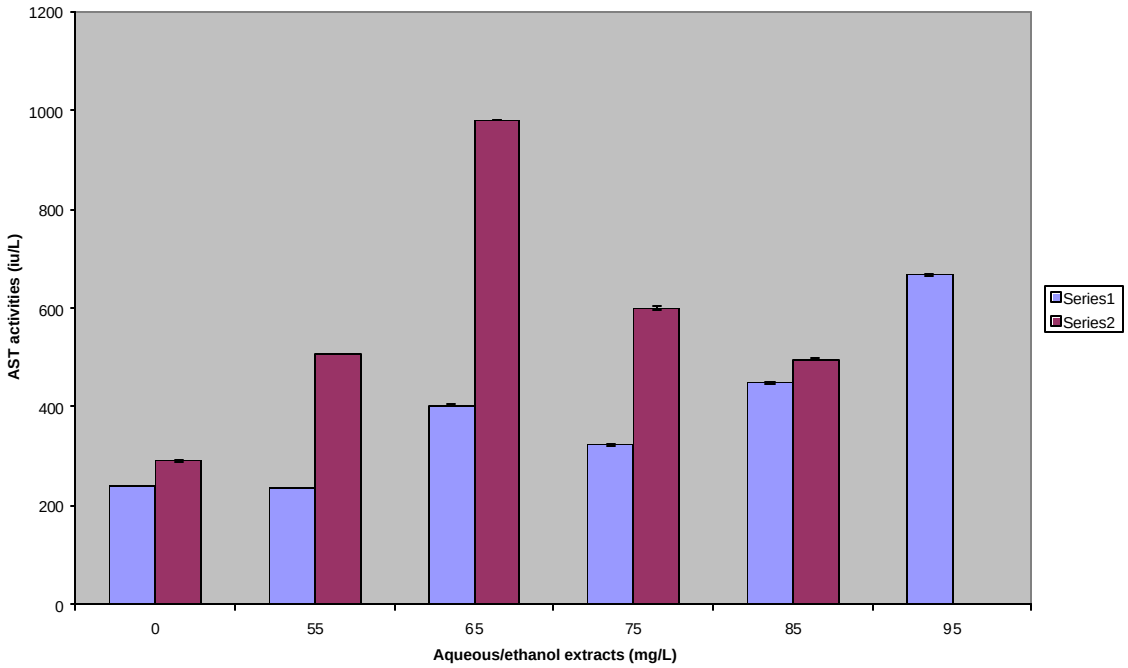

Fig. 1: AST activities of *C. gariepinus* juveniles exposed to aqueous extract (Series 1) and ethanolic extract (Series 2) of *P. biglobosa* pods

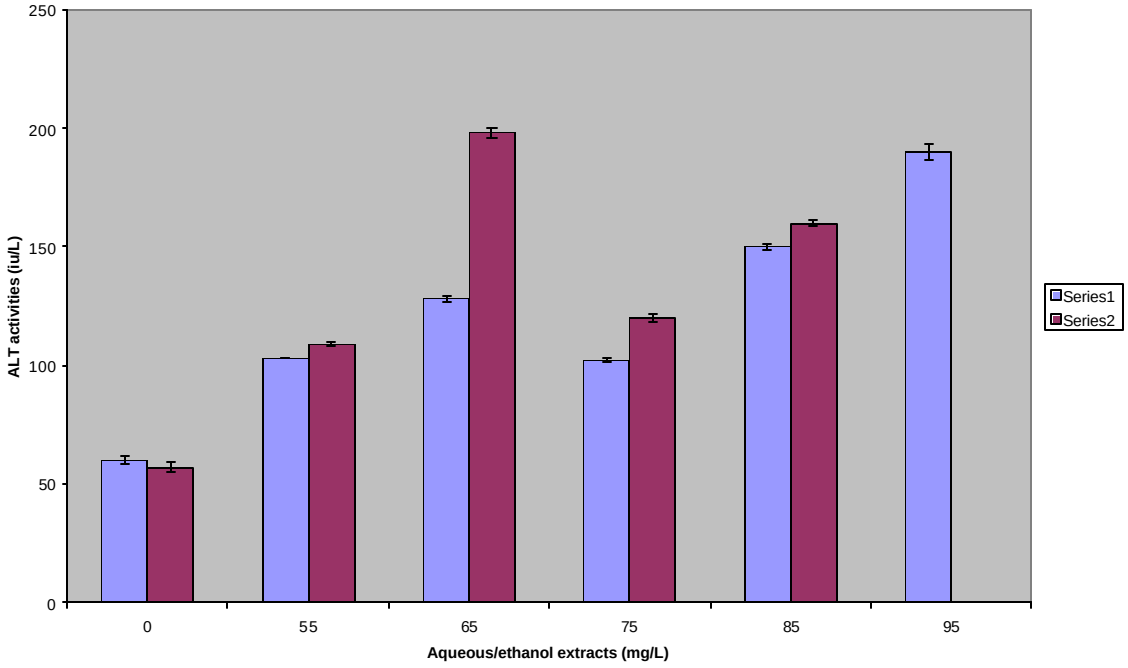

Fig. 2: ALT activities of *C. gariepinus* juveniles exposed to aqueous extract (Series 1) and ethanolic extract (Series 2) of *P. biglobosa* pods

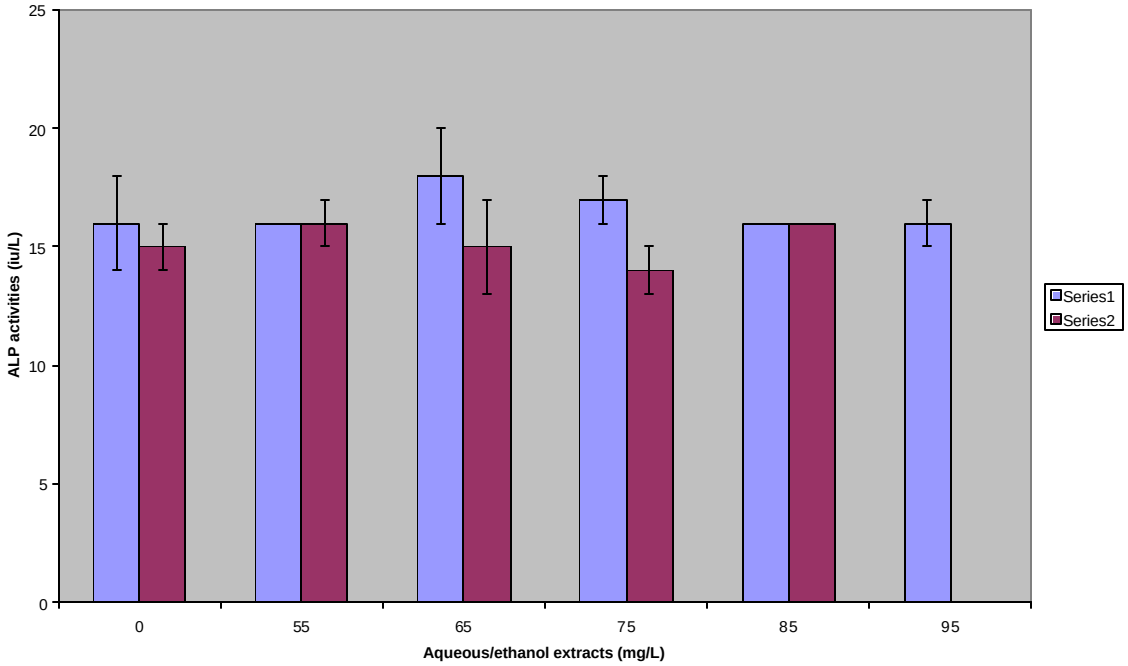

Fig. 3: ALP activities of *C. gariepinus* juveniles exposed to aqueous extract (Series 1) and ethanolic extract (Series 2) of *P. biglobosa* pods

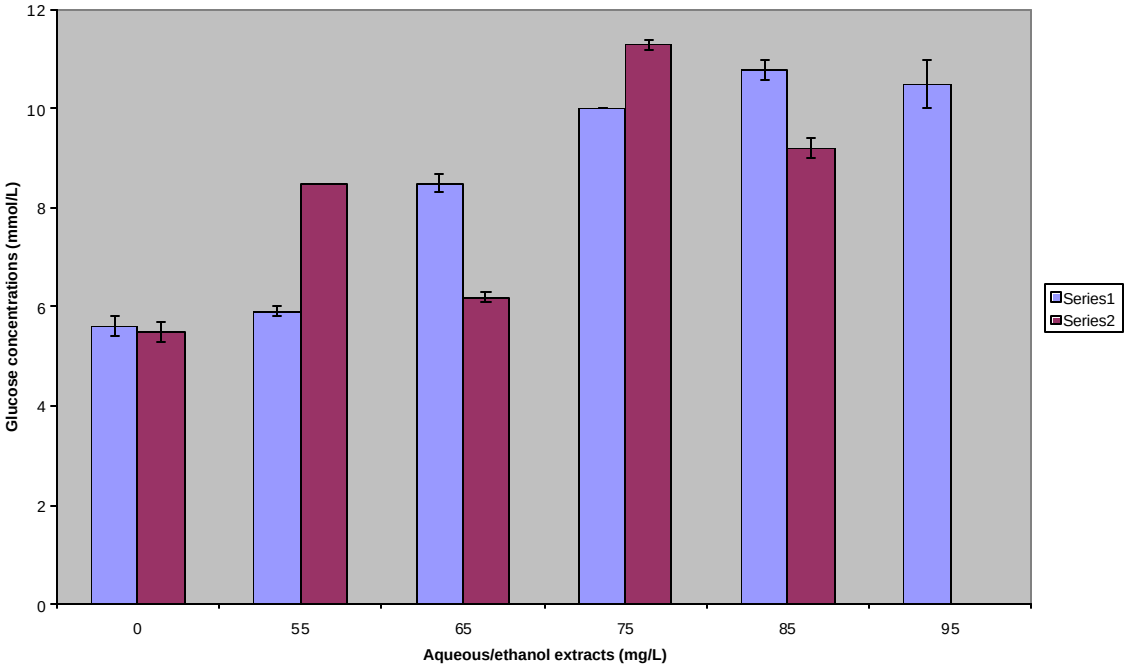

Fig. 4: Glucose concentrations of *C. gariepinus* juveniles exposed to aqueous extract (Series 1) and ethanolic extract (Series 2) of *P. biglobosa* pods

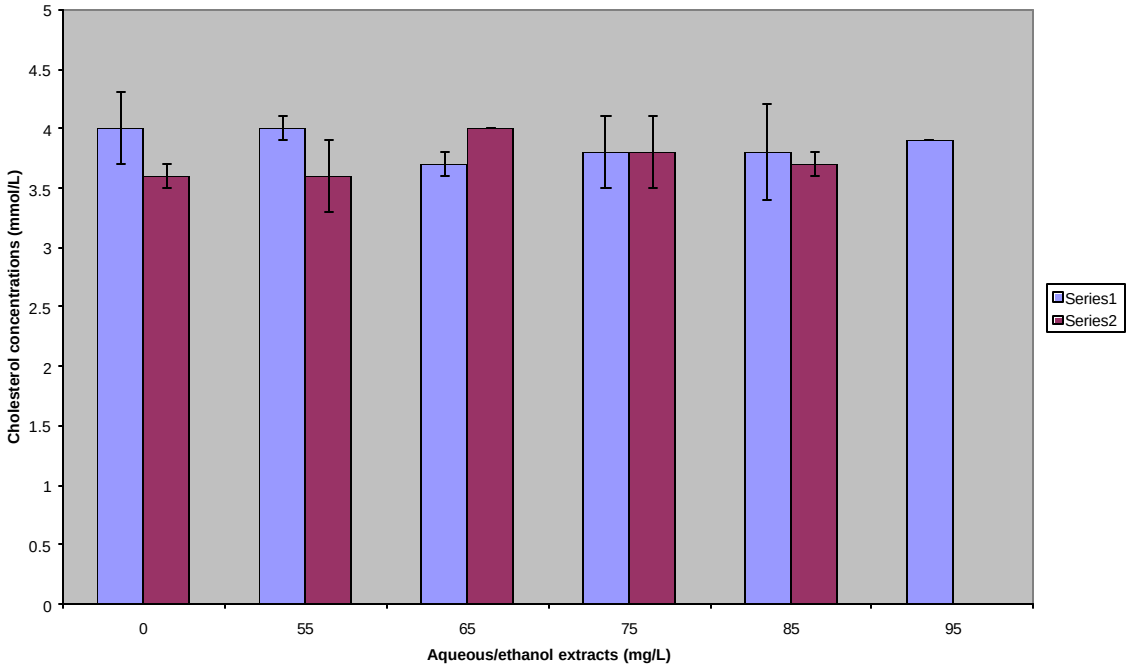

Fig. 5: Cholesterol concentrations of *C. gariepinus* juveniles exposed to aqueous extract (Series 1) and ethanolic extract (Series 2) of *P. biglobosa* pods.

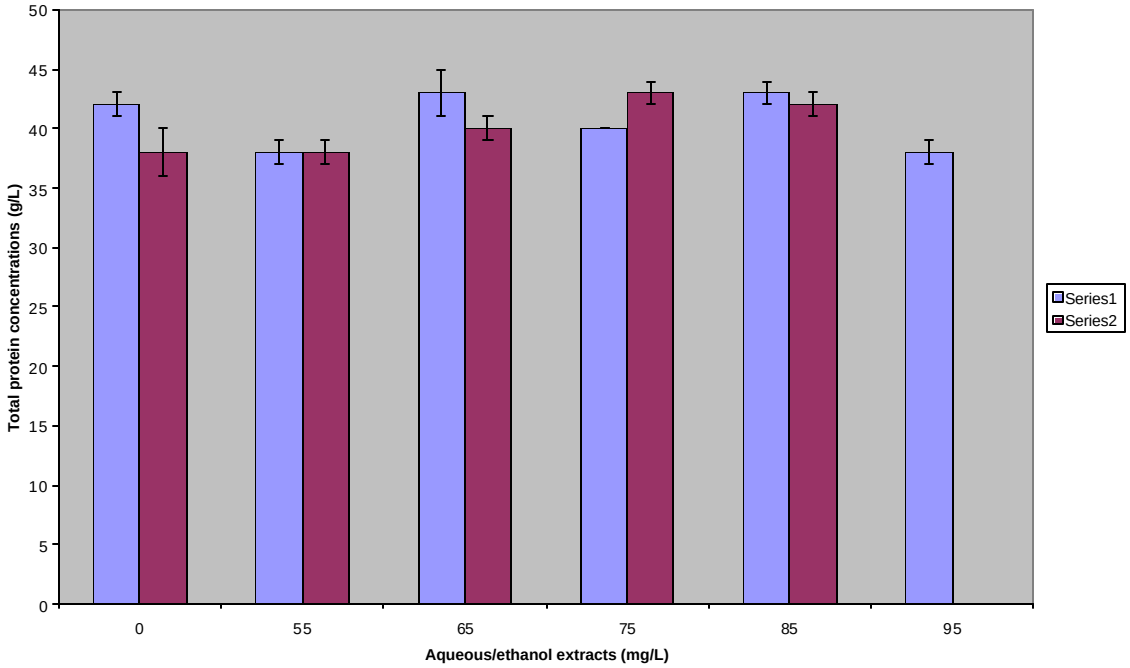

Fig. 6: Total protein concentrations of *C. gariepinus* juveniles exposed to aqueous extract (Series 1) and ethanolic extract (Series 2) of *P. biglobosa* pods

canaliculi from where it is secreted into the bile such that elevated activities of this enzyme signify cholestasis [42]. Even though direct hepatic damage may also cause moderate increase in these ALP activities [43], insignificant changes were observed in this study. This finding agrees with the report of Li *et al.* [44] but disagree with the report of Das and Mukherjee [45] who reported depleted ALP activity. However, toxicants concentrations and exposure duration may be responsible for the recorded variable changes in ALP activities in fishes exposed by these authors.

The toxic effects of both extracts on exposed C. gariepinus juveniles may have increased their need for more energy in order to combat the ensuing stress condition [46, 47] which can lead to carbohydrate metabolism disorders [48]. Such stressors are reported to stimulate the adrenal tissues resulting in increased levels of circulating glucocorticoids [49 - 51] and catecholamines [52, 53] with consequent hyperglycaemia. These findings agree with the work of Moussa *et al.* [54] but disagree with the findings of Omoniyi *et al.* [55] and Kori-Siakpere and Ubogu [56] who both reported hypoglycaemia with increasing toxicant concentrations. Such reported hypoglycaemia could have resulted from glucose loss through compromised kidneys indicating suppression of energy dependent glucose retention in kidney tubules [56].

The liver is the key organ for the synthesis and excretion of cholesterol such that any intra-hepatic and extra-hepatic obstructions will result in elevated concentrations within the serum [42] while chronic liver conditions like liver cirrhosis could result in decreased concentrations due to impaired synthesis [57]. The observed insignificant changes in the serum cholesterol concentrations meant none of these conditions actually prevailed. These findings are in agreement with the reports of Al-Attar [40] and Omitoyin [58] while disagreeing with the findings of Ogueji and Auta [26] and Okechukwu and Auta [42] who reported significant inhibition and elevated cholesterol concentrations respectively. Such elevated cholesterol concentrations could have resulted from the non-esterification of free cholesterol due to hepatic damages even though cholesterol concentrations will be more elevated in bile obstructions than in liver parenchyma damages [59].

Increased gluconeogenesis activities in order to meet up with increased energy demand under stress conditions leading to initial hyperproteinaemia have been reported in fishes [60, 61] even as water loss in the serum and elevated *de novo* synthesis could equally be responsible [40]. Liver necrosis with consequent impairment of protein synthesis machinery [62] or the impairment of amino acids incorporation into the polypeptide chains [63] can all result in hypoproteinaemia. However, the observed insignificant changes in total protein concentrations could possibly be due to the acute nature of these toxicities which never gave the liver the opportunity to compensate for any physiological compromise. The observed insignificant changes in serum total proteins concentration may also be due to the observed insignificant changes in ALP activities since ALP plays an important role in protein synthesis [62]. This report agrees with the findings of Omitoyin [58] but disagrees with the works of Hadi *et al.* [65] who reported hyperproteinaemia and the works of Omoniyi *et al.* [55] and Reeta *et al.* [66] who both reported hypoproteinaemia with increasing toxicant concentrations respectively. Excessive loss of serum total proteins through pathologically altered kidneys could lead to such hypoproteinaemia.

In conclusion, both aqueous and ethanolic extracts of *P. biglobosa* pods are highly hepatotoxic and even though there were insignificant differences in the biochemical parameters of exposed fish induced by either extract, there were significant difference ($P<0.05$) in the cholesterol concentrations of fish exposed to both extracts. These piscicidal properties can therefore, be exploited for obtaining fishes from water bodies. However, this should be with some caution as their effects on other aquatic organisms needs further investigations.

REFERENCES

1. Acton, J.C. and C.L. Rudd, 1987. Protein quality methods for sea foods. In: D.E. Kramer and J. Liston, (eds.), Seafood quality determination. Elsevier, Amsterdam, pp: 453.
2. Murray, J. and J.R. Burt, 1991. The composition of fish. Her Majesty Stationary Office (HMSO) Press, Edinburgh.
3. Choo, P.S. and M.J. Williams, 2003. Fisheries production in Asia: Its role in food security and nutrition. Paper presentation at the IX Asian Congress of nutrition, New Delhi, India, 23-27 February 2003, NAGA, WorldFish quarterly, 26: 11-16.
4. Jenness, J., 1967. The use of plants as fish poison within the Kainji basin. In: Fish and fisheries in Northern Nigeria. Ed., Reed, W. Gaskiya Corporation, Zaria, Nigeria, pp: 201-202.

5. Brummett, R.E. and R. Noble, 1995. Aquaculture for African smallholders. ICLARM contribution No.1135. Juta Print, Penang. Malaysia, pp: 32.
6. Fafioye, O.O., A.A Adebisi and S.O. Fagade, 2004. Toxicity of *Parkia biglobosa* and *Raphia vinifera* extracts on *Clarias gariepinus* juveniles. African J. Biotechnol., 3: 627-630.
7. Jothivel, N. and V.I. Paul, 2008. Evaluation of the acute toxicity of the seeds of *Anamirta cocculus* (Linn.) and its piscicidal effect on three species of freshwater fish. The Internet J. Toxicol., 5: 1559-3916. http://www.ispub.com/ostia/index.php?xmlprinter=true&xm/FilePath=journals/ijto/vol5n1/fish.xml. Retrieved: 01-09-08. 18: 44; 40 GMT.
8. Tiwari, S. and A. Singh, 2005. Possibility of using latex extracts of *Nerium indicum* plant for control of predatory fish, *Channa punctatus*. Asian Fisheries Sci., 18: 161-173.
9. Cagauan, A.G., M.C. Galaites and L.J. Fajardo, 2004. Evaluation of botanical piscicides on Nile Tilapia (*Oreochromis niloticus* L.) and mosquito fish (*Gambusia affinis* Baird and Girard). Proceedings of the 6th International symposium on tilapia aquaculture, Manila, Philippines, 12-16 September 2004, pp: 179-187.
10. Ita, E.O., 1980. A review of recent advance in warm water aquaculture resource and a proposed experiment design for maximizing fish production in Nigeria. Kainji Lake Research Institute Technical Report Series, 51: 30.
11. Hengsamat, K., F.T. Ward and P. Jururatjamorn, 1997. The effect of stocking density on yield, growth and mortality of African catfish (*Clarias gariepinus* Burchell 1822) cultured in cages. Aquaculture, 152: 67-76.
12. Omitoyin, B.O., E.K. Ajani, B.T. Adeshina and C.N.F. Okuagu, 2006. Toxicity of Lindane (Gamma Hexachloro - CycloHexane) to *Clarias gariepinus* (Burchell 1822). World J. Zoology, 1: 57-63.
13. Fafioye, O.O., 2005. Plants with piscicidal activities in Southwestern Nigeria. Turkish J. Fisheries and Aquatic Sci., 5: 91-97.
14. Wepener, W., 1997. Metal acotoxicology of the Ohtant River in the Kruger National park and the effect thereof in fish haematology. Ph.D thesis, Rand African University, South Africa.
15. Edsall, C.C., 1999. A blood chemistry profile for lake trout. J. Aquatic Animal Health, 11: 81-86.
16. Bentley, A.O., 1977. Bentley's textbook of pharmaceutics. 8th Ed. Bailliére Tindall, London, pp: 177-180.
17. Ghani A., 1990. Introduction to pharmacognosy. 1st Edn. Ahmadu Bello University Press Ltd., Zaria, Nigeria, pp: 198.
18. Oladimeji, A.A., A.A. Ayantoye and K.A.N. Esievo, 1988. Haematological differences between two tropical freshwater fishes, *Oreochromis niloticus* and *Clarias lazera*. J. African Zool., 102: 487-492.
19. Adeyemo, O.K., 2005. Haematological and histopathological effects of cassava mill effluent in Clarias gariepinus. African J. Biomedical Res., 8: 179-183.
20. Olufayo, M.O., 2009. Haematological characteristics of *Clarias gariepinus* (Burchell, 1822) juveniles exposed to Derris elliptica root powder. AJFAND ONLINE. 9: 920-933. http://www.ajol.info/index.php/ajfand/article/viewFile/43115/2663. Retrieved: 21-08-09. 6.42PM.
21. APHA (American Public Health Association), AWWA (American Water Works Association) and WPCF (Water Pollution Control Federation). (1981). Standard methods for examination of water and wastewater. 15th Edn., Washington D.C., USA, pp: 1134.
22. Omitoyin, B.O., A.O. Ogunsami and B.T. Adeshina, 1999. Studies on acute toxicity of piscicidal plant extracts (*Tetrapleura tetrapleura*) on Tilapia (*Sarotherodon gailaeus*) fingerlings. Tropical Journal of Animal Sci., 2: 191-197.
23. Fafioye, O.O., 2001. Lethal and sub-lethal effect of extracts of *Parkia biglobosa* and *Raphia vinifera* on some fresh water fauna. Ph.D thesis. University of Ibadan, Nigeria.
24. Usman, J.I., J. Auta, A.K. Adamu and M.S. Abubakar, 2005. Toxicity of methanol extract of *Euphorbia laterifolia* (Schum and Thann) to the juveniles of the African catfish (*Clarias gariepinus*) (Teugels). ChemClass J., 2: 59-61.
25. Al-Akel, A.S., 1996. Effects of asphyxiation on the haemoglobin and glycogen content in an African catfish *Clarias gariepinus*. J. King Abdulaziz University, Sci., 8: 45-50.
26. Ogueji, E.O. and J. Auta, 2007. Investigations of biochemical effects of acute concentrations of Lambda-cyhalothrin on African catfish, *Clarias gariepinus* - Teugels. J. Fisheries International, 2: 86-90.
27. Fafioye, O.O., S.O. Fagade and A.A. Adebisi, 2005. Toxicity of *Raphia vinifera*, P. beauv fruit extracts on biochemical composition of Nile Tilapia (*Oreochromis niloticus*, Trewavas). Biokemistri, 17: 137-142.

28. Ogbu, S.I. and F.I. Okechukwu, 2001. The effects of storage temperature prior to separation on plasma and serum potassium. J. Medical Laboratory of Sci., 10: 1-4.
29. Morgan, J.D. and G.K. Iwana, 1997. Measurement of stressed states in the field. In: Fish stress and health in aquaculture. Eds., G.K. Iwana, A.D. Pickering, J.P. Sumpter and C.B. Schreck, Society of Experimental Biology Seminar Series, 62: 247-268.
30. Henry, R., D.C. Canon and J.W. Winkelman, 1974. Clinical chemistry: Principles and techniques. Harper and Roe Publications, Maryland, USA, pp: 543.
31. Schwartz, M.K., N. de Cediel, D.H. Curnow, C.G. Fraser, C.J. Porter, H.G. Worth and O. Inder, 1985. International Federation of Clinical Chemistry, Education Committee and International Union of Pure and Applied Chemistry, division of Clinical chemistry: Definition of the terms certification, licensure and accreditation in clinical chemistry. J. Clinical Chemistry and Clinical Biochem., 23: 899- 901.
32. King, E.J. and A.R. Armstrong, 1934. A convenient method for determining serum and bile phosphatase activity. Canadian Medical Association J., 31: 376-381.
33. Roeschlaw, P., E. Bernt and J.W. Gruber, 1974. An investigation of the determination of serum cholesterol by an enzymatic way. J. Clinical Chemistry and Clinical Biochem., 12: 403.
34. Microsoft® Office Excel, 2002. Microsoft® Windows XP Professional. Microsoft ®Corporation. Redmond, Washington D. C., USA. http://www.microsoft.com.
35. GraphPad Prism version, 4.0, 2003. Grapgpad software, San Diego, USA. http://www.graphpad.com
36. Wroblewski, F. and J.S. La Due, 1955. Serum glutamic oxaloacetic transaminase activity as an index of liver cell injury: A preliminary report. Annals of Internal Medicine, 43: 345-360.
37. Saraswat, B., P.K Visen, G.K. Patnaik and B.N. Dhawn, 1993. Anticholestic picroliv, active hepatoprotective principle of *Picrorhiza kurrooa* against carbon tetrachloride induced cholestatis. Indian J. Experimental Biol., 31: 316-318.
38. Shakoori, A.R., J. Alam, F. Aziz and S.S. Ali, 1990. Biochemical effects of bifenthrin (talstar) administered orally for one month on the blood and liver of rabbits. Proceedings of Pakistani's Congress of Zool., 10: 61-81.
39. Cornellius, C.E., 1979. Biochemical evaluation of hepatic functions in dogs. J. American Hospital Association, 15: 25-29.
40. Al-Attar, A.M., 2005. Biochemical effects of short-term cadmium exposure on the freshwater fish, *Oreochromis niloticus*. J. Biological Sci., 5: 260-265.
41. Oruç, E.Ö. and N. Üner, 1999. Effects of 2,4-Diamin on some parameters of protein and carbohydrate metabolism in the serum, muscles and livers of *Cyprinus carpio*. Environmental Pollution, 105: 267-272.
42. Okechukwu, E.O. and J. Auta, 2007. The effects of sub-lethal doses of Lambda- cyhalothrin on some biochemical characteristics of the African catfish, *Clarias gariepinus*. J. Biological Sci., 7: 1473-1477.
43. Nduka, N., 1999. Clinical biochemistry for students of pathology. Longman Nig. Ltd., Lagos, Nigeria, pp: 64.
44. Li, X.L., I.K. Chung, J.I. Kim and J.A. Lee, 2004. Sub-chronic oral toxicity of microcystin in common carp (*Raphia vinifera* L.) exposed to microcystis under Laboratory conditions. Toxicon, 44: 821-827.
45. Das, B.K. and S.C. Mukherjee, 2003. Toxicity of cypermethrin in *Labeo rohito* fingerlings: Biochemical enzymatic and haematological consequences. Comparative Biochemistry and Physiology - Part C Toxicology and Pharmacol., 134: 109-121.
46. Shamsi, M.J.K and A.S. Al-Akel, 1986. Effects of mercuric chloride on the glycogen in various tissues of freshwater fish, *Oreochromis niloticus*. Proceedings of Saudi Biological Society, 9: 276-291.
47. Colombo, L., A.D. Pickering, P. Belvedere and C.B. Schreck, 1990. Stress inducing factors and stress reactions in aquaculture. In: Aquaculture Europe'89 - Business Science, Eds., N. De Pauw and R. Billard. European Aquacultural Society, Special Publication No. 12, Bredene, Belgium, pp: 93-121.
48. Wedemeyer, G.A. and D.J. McLeay, 1981. Methods for determining the tolerance of fishes to environmental stressors. In: Stress and Fish, Ed., Pickering, A.D. Academic Press, London, pp: 247-275.
49. Gluth, G. and W. Hanke, 1985. A comparism of physiological changes in carp, *Raphia vinifera* induced by several pollutants at sub-lethal concentrations I: The dependency on exposure time. Ecotoxicology and Environmental Safety, 9: 179-188.
50. Hontela, A., C. Daniel and A.C. Ricard, 1996. Effects of acute and sub-acute exposure to cadmium on inter renal and thyroid function in rainbow trouts, *Oncorhyncus mykiss*. Aquatic Toxicology, 35: 171-182.

51. Ricard, A.C., C. Daniel, P Anderson and A. Hontela, 1998. Effects of subchronic exposure to cadmium chloride on endocrine metabolic functions in rainbow trouts, Oncorhyncus mykiss. Archives of Environmental Contamination and Toxicol., 34: 377- 381.
52. Nakano, T. and N. Tomlinson, 1967. Catecholamines and carbohydrate concentrations in rainbow trouts, *Salmo gairdneri* in relation to physical disturbances. J. Fisheries Research Board of Canada, 24: 1701-1715.
53. Witters, H.E., S. Van Puymbroeck and O.C.J. Vanderborght, 1991. Adrenergic response to physiological disturbances in rainbow trout, *Oncorhyncus mykiss* exposed to luminum at acid pH. Canadian J. Fisheries and Aquatic Sci., 48: 414-420.
54. Mousa, M.M.A., A.M.M. El-Ashram and M. Hamed, 2008. Effects of Neem leaf extract on freshwater fishes and zooplankton community. The 8th International symposium on Tilapia in aquaculture. The Central Laboratory for aquaculture research, Cairo, Egypt, 12-14 October 2008, http: //www.ag.arizona.edu/ ISTAB/Abstract-Papers? Momdouh % 20 paper % 20 neem.doc. Retrieved: 05-04-09. 11.52 AM.
55. Omoniyi, I., A.O. Agbon and S.A. Sodunke, 2002. Effect of lethal and sub-lethal concentrations of Tobacco (*Nicotiana tobaccum*) leaf dust extract on weight and haematological changes in *Clarias gariepinus* (Burchell). J. Applied Sciences and Environmental Management, 6: 37- 41.
56. Kori-Siakpere, O. and E.O. Ubogu, 2008. Sub-lethal haematological effects of Zinc on the freshwater fish, *Heteroclarias* sp. (Osteichthyes: Clariidae). African J. Biotechnol., 7: 2068-2073.
57. Kamath, S.H., 1972. Clinical biochemistry for medical technologists. Churchill/ Livingstone, London, pp: 89.
58. Omitoyin, B.O., 2007. Plasma biochemical changes in *Clarias gariepinus* (Burchell, 1822) fed poultry litter. Asian J. Animal Sci., 1: 48-52.
59. Bodansky, O., 1957. Biochemistry of diseases. 2nd Edn. The Macmillan Company, New York, USA, pp: 394-472.
60. Alkahem, H.F., Z. Ahmed, A.S. Al-Akel and M.J.K. Shamusi, 1998. Toxicity bioassay and changes in haematological parameters of *Oreochromis niloticus* induced by Trichlorton. Arabian Gulf J. Scientific Res., 16: 581-593.
61. Martinez, C.B.R., M.Y. Nagae, C.T.B.V. Zaia and D.A.M. Zaia, 2004. Morphological and physiological acute effects of lead in the neotropical fish, *Prochildus lineatus*. Brazilian J. Biol., 64: 797-807.
62. Bradbury, S.P., D.M. Symonic, J.R. Coats and G.J. Atchison, 1987. Toxicology of fenvalerete and its constituent isomers to the fathead minnow (*Piephales promeos*) and blue gill (*Lepomis macrochirus*). Bulletin of Environmental Contamination and Toxicol., 38: 727-735.
63. Ram, P.Y., S. Digvijay, S.K. Singh and A. Singh, 2003. Metabolic changes in freshwater fish, *Channa punctatus* due to stem-bark extracts of *Croton tiglium*. Pakistani J. Biological Sci., 6: 1223-1228.
64. Pilo, B.M., M.V. Asnani and R.V. Shah, 1972. Studies on wound healing and repairs in pigeons III. Histochemical studies on acid/alkaline phosphatase activity during the process. J. Animal Physiology and Animal Nutrition, 19: 205-212.
65. Hadi, A.A., A.E. Shokr and S.F. Alman, 2009. Effects of Aluminium on the biochemical parameters of freshwater fish, *Tilapia zillii*. J. Sci. and Its Applications, 3: 33-41.
66. Reeta, P., S. Bhargava and D.K. Sarf, 1993. Toxic effects of some biocides on total serum protein in *Heteropneustes fossilis*. Indian J. Environmental Toxicol., 3: 5-6.