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Full Length Research Article

Effects of *Azadirachta indica* Leaf Powder on some Biochemical Parameters of the African Catfish (*Clarias gariepinus*)

***K.M. Adamu, M. Aliyu-Paiko, F. Abdullahi and A.Y. Mustapha**
Department of Biology, Ibrahim Badamasi Babangida University, P.M.B. 11, Lapai, Niger State, Nigeria
[*Corresponding Author: E-mail: kabrmoh@yahoo.com; ☎: +2348035826075]

ABSTRACT
The study investigated the alterations in biochemical parameters of African catfish (*Clarias* gariepinus) exposed to neem *(Azadirachta indica*) leaf powder during the short term experimental period in static renewable bioassay system. The fish with mean length and weight of 18.35 ± 0.60cm and 22.38 ± 1.25g respectively were exposed to 0.192, 0.096, 0.048 and 0.00mg/L concentrations of neem leaf powder for 14-day period. During the experimental period, some physicochemical parameters such as temperature, pH, dissolved oxygen and total alkalinity were monitored weekly. At the end of the experimental period, the fish were sacrificed for the test of biochemical parameters that include: total protein, glucose, triglycerides, cholesterol, uric acid and creatinine in the serum, liver and kidney of the test fish in order to ascertain the effect of the plant on these biomarkers. As the concentrations of the plant leaf powder increased, the activities of serum total protein, triglycerides, cholesterol, creatinine and glucose either significantly ($p<0.05$) or insignificantly ($p>0.05$) decreased. An increase in liver glucose and total protein were recorded while kidney total protein, cholesterol and triglycerides were also either significantly ($p<0.05$) or insignificantly ($p>0.05$) increased. However, kidney glucose decreased significantly ($p<0.05$) while liver cholesterol, creatinine and triglycerides insignificantly decreased. Therefore, it is an indication that the plant leaf powder has effect on the physiological activities of the test fish during the study period.
Keywords: Health status, Neem leaf powder, *Clarias gariepinus,* Toxicity.

INTRODUCTION
One of the challenges in aquaculture production is the presence of pests, predators and competitors of cultivated fishes (Chiayvareesajja, *et al.,* 1997). The Neem (*Azadirachta indica*) plant called "*Dogonyaro*" in Northern Nigeria belong to the Family *Milliaceae.* The plant adapts favorably to the sub-Sahelian Nigeria with severe drought, poor, shallow and even saline soils (Schmutterer, 1990). The Biological active compounds isolated from different parts of the plant include azadirachtin, meliacin, gedunin, salanin, nimbin, valassin, margosine, eriterperenoid, azatin, rotinine and quinine among other active ingredients (Ade-Serrano, 1982; Adewole, *et al.,* 2002). The plant has been recommended in ancient medical texts for gastrointestinal upsets, diarrhea and intestinal infections, skin ulcers malaria and infectious diseases (Schmutterer, 1990; Van Der Nat *et al.,* 1991). Every part of the neem tree have been known to possess a wide range of pharmacological properties especially as antibacterial, antifugal, antiulcer, antifeedant, repellent, pesticidal, molluscidal, ecdysone inhibitor and sterilant (Biswas, *et al.,* 2002; Das, *et al.,* 2002). As natural insecticide it contains tetranitroterpenoid compounds known as meliatoxins that are highly toxic to insects and mammals (Ascher, *et al.,* 1992).

Similarly, aqueous extract of neem leaves and other neem-based products have been extensively used in fish-farms as alternative for the control of fish parasites and fish fry predators such as dragon-fly larvae (Martinez, 2002). The use of this plant or its products directly or indirectly enters into water bodies (streams, river and lakes), which may affect non-target organisms (Schroder, 1992). These non target organisms could be fishes (Omoregie. and Okpanachi, 1997; Winkaler *et al.,* 2007; Khedkar

and Gyananath, 2008; Kumar, *et al.,* 2010; Mousa, *et al.,* 2008), mosquitoes (Arunpandiyan, 2011) and zooplankton (Mousa, *et al.,* 2008).

One of the fishes of interest for this study is the African Catfish (*Clarias gariepinus*, Family Clariidae) which is widely cultured in many tropical and subtropical regions of the World. This fish constitutes the largest group of cultured species, it grows well under various culture systems. In studying the effect of Neem (*A. indica*) on non-target organism such as fish, there are biomarkers that revealed these effects long before the death of the organism (Adamu, 2009). These biomarkers include total protein, glucose, cholesterol, uric acid, creatinine, triglycerides levels amongst others (Patti and Kulkarni, 1993 and Adamu, and Idris, 2014). Therefore, the study was aimed at determining the effect of neem leaf powder on the selected biochemical parameters of African catfish after the 14 days exposure period.

MATERIALS AND METHODS

Plant Leaf Sampling

The leaves of *A. indica* were collected from the Main Campus of Ibrahim Badamasi Babangida University, Lapai, identified at the Department of Biology herbarium unit. The collected leaves were air dried for 21 days, ground into fine powder using blender and stored in a plastic container for use.

Experimental Fish Sampling

Juveniles of African catfish (*C. gariepinus*) of mixed sex with mean length and weight of 18.35 ± 0.60cm and 22.38 ± 1.25g respectively were purchased from Adamu fish farms Kpakungu Km 10, Minna/Bida Road, Minna, Niger State, Nigeria. They were transported in aerated aquaria to the Department of Biology Laboratory.

Experimental Procedure

The fishes were acclimatized in 80L capacity aquarium for 14 days and fed with 3.00mm commercial fish pellet (Coppens) twice daily. The aquaria were cleaned every 48hrs and uneaten feed and feacal matters were siphoned while dead fish were promptly removed to avoid contamination. After the 14 days of acclimatization period, ten (10) fishes were introduced into four sets of 30L plastic aquaria each with two replicate. The fishes were exposed to 0.00 (control), 0.048, 0.096 and 0.0192mg/L concentration of *A. indica* leaf powder after preliminary investigation to ascertain the used concentrations.

The leaf powder concentrations were mixed and dissolved into its equivalent volume of water in the test aquaria. Bore-hole water was used during the acclimatization and exposure period. In order to maintain the effect of toxicant strength, reduce the effect of evaporation and stress during experimentation, the test media were replaced by 50% prepared concentration of the same quality, after removing its equivalent along with the undigested food and defecation every 48 hours to maintain the requisite level and potency of the concentration (Adamu and Ademuyiwa, 2014). In the static renewable bioassay system of the exposure during the 14 days test period some physico-chemical parameters such as water temperature, pH, total alkalinity, dissolved oxygen, conductivity and free carbon (IV) oxide were monitored in accordance to the standard procedures of APHA (1998).

At the end of the 14-day experimental period, one hundred and twenty (120) fishes were sacrificed, for their blood, liver and kidney. The liver and kidney were each pulverized in a laboratory mortar and pestle. Extractions were prepared by adding 2ml of 10% sucrose solution before been centrifuged. The supernatant was collected and stored in another test tube in the refrigerator. The procedure was in accordance to the report in Adamu and Nwadukwe (2013).

The procedures for the selected biochemical parameters were based on the instructions of the commercial manual reagent kits used such that total protein was determined based on Biuret method (Tietz, 1995), triglycerides - enzymatic colorimetric endpoint method (Buccolo and

David, 1973), cholesterol and glucose - enzymatic endpoint method (Trinder, 1969), uric acid - enzymatic calorimeter method (Fassati, *et al.,* 1980) and creatinine - calorimeter method (Bartels and Bohmer,1972) with the use of spectrophotometer (20D PEC Medicals, U.S.A) for absorbance reading.

Data Analyses

All data were presented as means ± standard error, the data from the 14-day test were analyzed using analysis of variance, after which individual means were compared using Turkey multi-sample correction/test. In all cases, differences were considered statistically significant at either $p<0.01$ or $p<0.05$. All statistical analyses were performed using the software package (graphpad prism @ software version 6.0 San Diego, C.A).

RESULTS

The monitored physico-chemical parameters of the experimental set-up are presented in Table1. The mean values of temperature, free carbon, pH and dissolved oxygen were not significantly different (*p>0.05*) in the set-up with fish exposed to neem leaf powder compared to the control

Table 1: Physico-chemical parameters of *Clarias gariepinus* exposed to sub lethal concentrations of neem leaf powder

Concentrations of neem leaf powder (mg/L)	Temperature (^{0}C)	FCO (mg/L)	TAL (mg/L)	pH	DO (mg//L)
0.00	26.00±0.00 (26.00-26.00)	4.25±0.25 (3.50-5.5)	28.80±0.73 (25.50-30.00)	6.50±0.00 (6.50-6.50)	6.62±0.11 (6.00-7.00)
0.048	26.13±0.08 (26.00-26.50)	4.38±0.25 (3.00-5.00)	29.88±0.13 (29.00-30.00)	6.50±0.00 (6.50-6.50)	6.27±0.24 (5.00-6.83)
0.096	26.06±0.06 (26.00-26.50)	4.19±0.25 (3.00-5.00)	29.98±0.02 (29.85-30.00)	6.34±0.44 (6.00-6.45)	6.34±0.29 (4.50-6.92)
0.192	26.13±0.08 (26.00-26.50)	4.06±0.36 (3.00-5.00)	29.50±0.21 (28.50-30.00)	6.50±0.00 (6.50-6.50)	6.39±0.21 (5.00-6.90)

The mean total protein (g/dl) presented in Figure 1 revealed that as the concentrations of the leave powder increased, the serum total protein increased while the liver and kidney values decreased. The significant decrease and increase in the serum and kidney total protein respectively of the test fish exposed to 0.096 and 0.192 mg/l of the neem leaf powder compared to the control test. The value of serum total protein was directly proportional to the concentrations of the plant leaf powder while that of the liver and kidney were inversely proportional. The highest total protein of 2.39±0.32g/dl, 1.74±0.12g/dl and 1.30±0.04g/dl were recorded in test fish exposed to 0.00mg/l and 0.192mg/l respectively.

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The mean glucose value presented in Figure 2 revealed that as the concentrations of the plant leaf powder increased, the values of serum and kidney glucose decreased significantly (*p<0.05*) while that of the kidney was not significantly *(p>0.05)* increased. Fish in the control group recorded the highest serum and kidney glucose levels (93.48±8.25 and 115.21±4.97 mg/dL) respectively compared to fish exposed to 0.192 mg/L of the leaf powder which were the least recorded (51.37±7.45 and 43.61±6.61 mg/dL) respectively

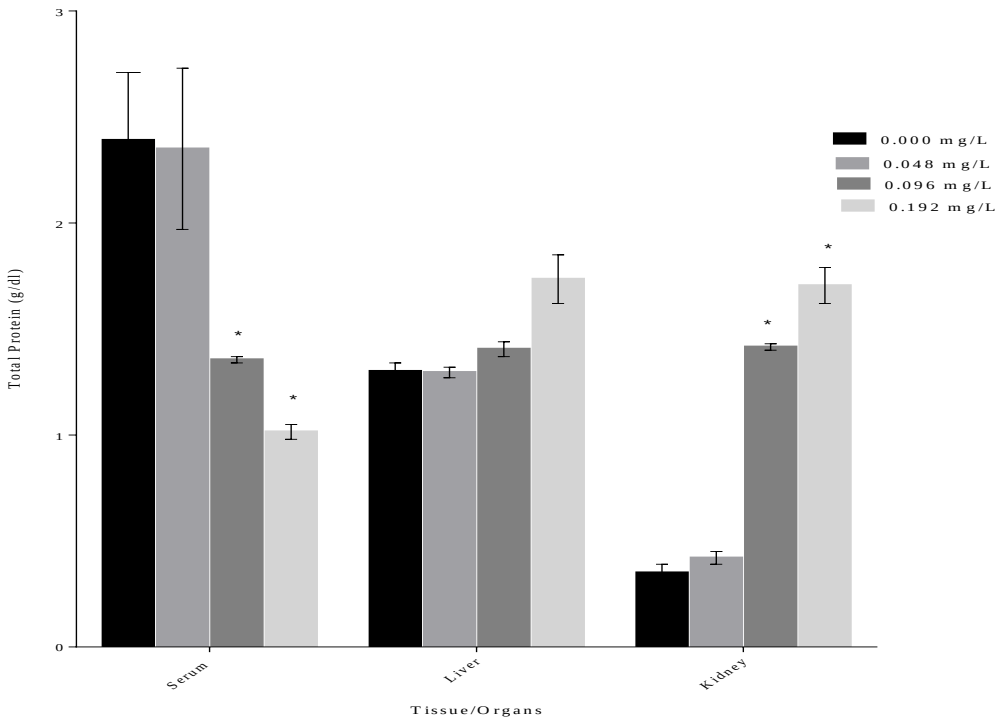

Figure 1: Mean value of total protein (g/dl) in *Clarias gariepinus* exposed to concentrations of neem leaf powder. Each vertical bar represents standard error. *- p<0.05.

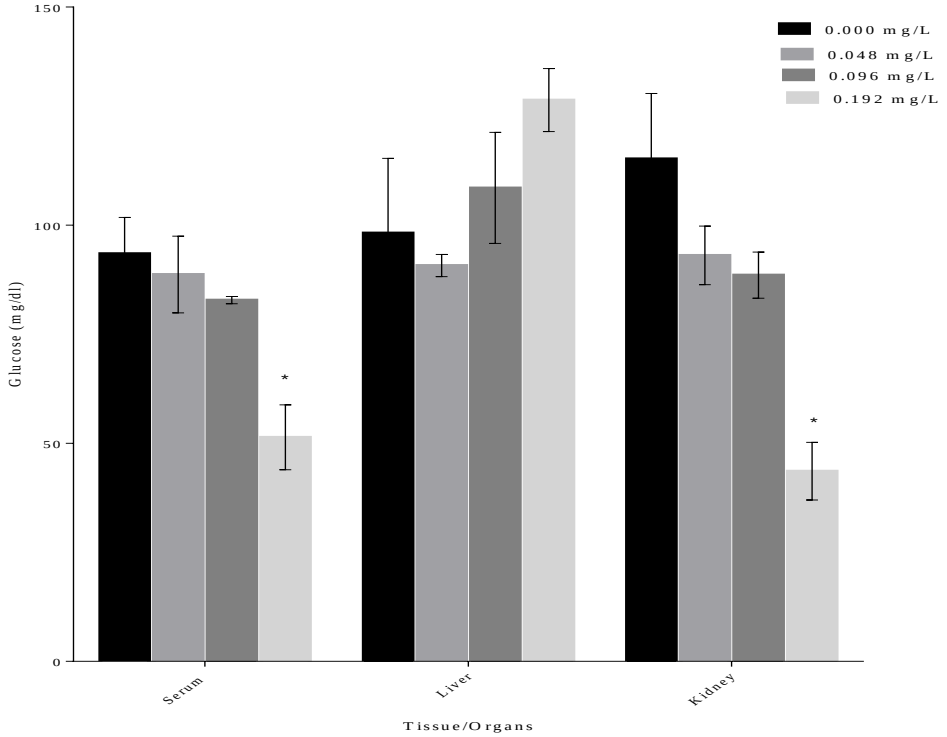

Figure 2: Glucose Levels (mg/dl) in *Clarias gariepinus* exposed to concentrations of neem leaf. Each vertical bar represents standard error*- p<0.05.

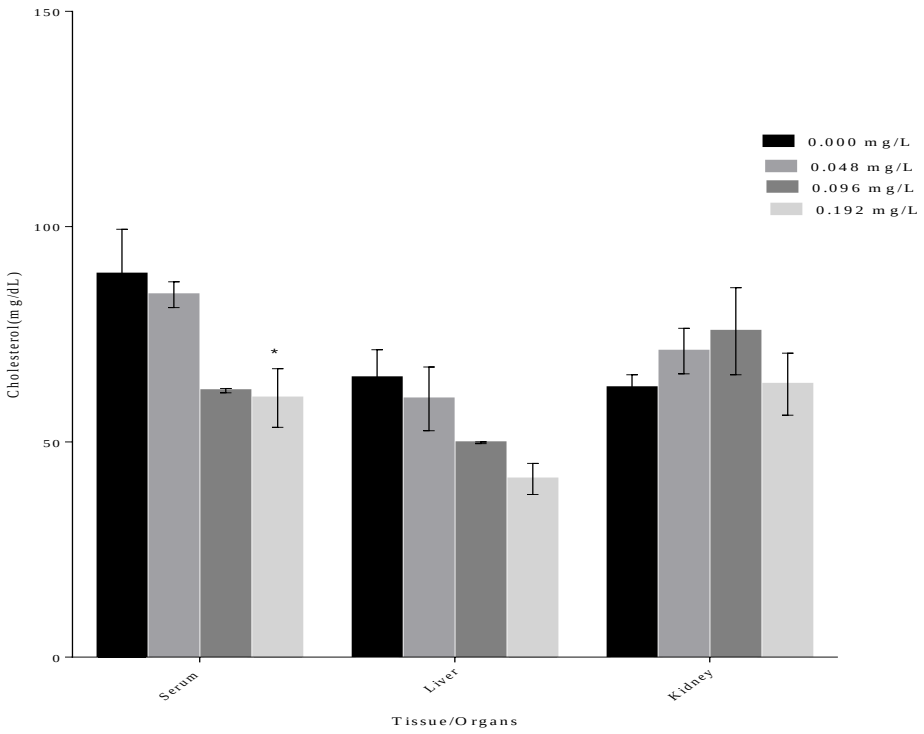

Figure 3: Mean value of Cholesterol (mg/dl) in *Clarias gariepinus* exposed to concentrations of neem leaf powder after 14- day period. Each vertical bar represents standard error. *- p<0.05.

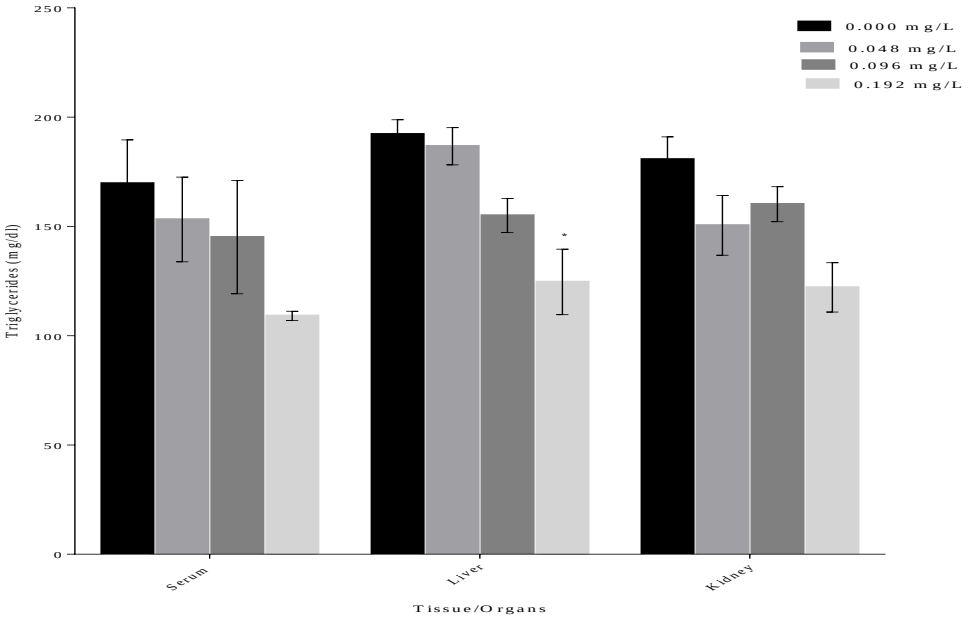

Figure 4: Mean value of triglyceride in *Clarias gariepinus* exposed to concentrations of neem leaf powder after 14- day period. Each vertical bar represents standard error. *- p<0.05.

The mean cholesterol level presented in Fig 3 revealed significant ($p<0.05$) while the values of serum and liver cholesterol were not significantly *(p>0.05)* different in fish exposed to 0.192mg/l; however, the kidney recorded fluctuated values that were not significantly *(p>0.05)* different. The

mean triglycerides values were presented in Figure 4. The value of cholesterol in the serum and kidney of fish exposed to the plant leaf powder were not significant ($p>0.05$) compared to the control. The fish exposed to 0.192 mg/l of the leaf powder recorded the only significant ($p<0.05$) difference in liver triglycerides

The determined mean uric acid (mg/dl) levels are presented in Fig 5. With the exception of kidney uric acid that was lesser in fish exposed to the control, the serum and liver uric acid level revealed a fluctuated pattern with fish exposed to 0.192 and 0.048 mg/l showing higher serum and liver uric acid level respectively. The determined mean creatinine (mg/dl) levels are presented in Fig 6. Generally, the values of creatinine in serum, liver and kidney of fish exposed to the plant leaf powder were not significantly lower (*p>0.05*) than the control.

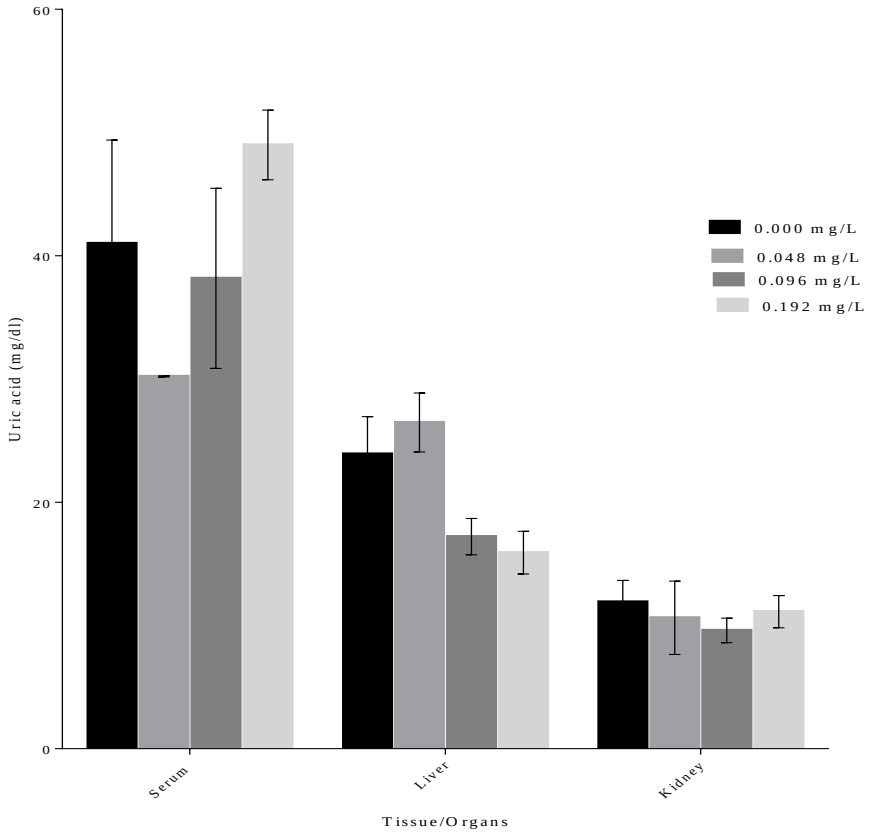

Figure 5: Mean value of uric acid (mg/dl) in *Clarias gariepinus* exposed to concentrations of neem leaf powder after 14- day period. Each vertical bar represents standard error.

DISCUSSION

Physico-chemical parameters such as temperature, dissolved oxygen, free carbon (iv) oxide, pH and total alkalinity are parameters that are important to the many factors that affect fish health, growth and reproduction (Hill, 1995)'. However, Richards (1977) reported that the main cause of mortality in aquarium fish could be the inadequate maintenance of the water environment. In this study the monitored parameters were noted not to be significantly different (*p>0.05)* from the control test after the 14 days exposure period. They were all within the recommended acceptable limits (Adamu and Kori-Siakpere, 2011).

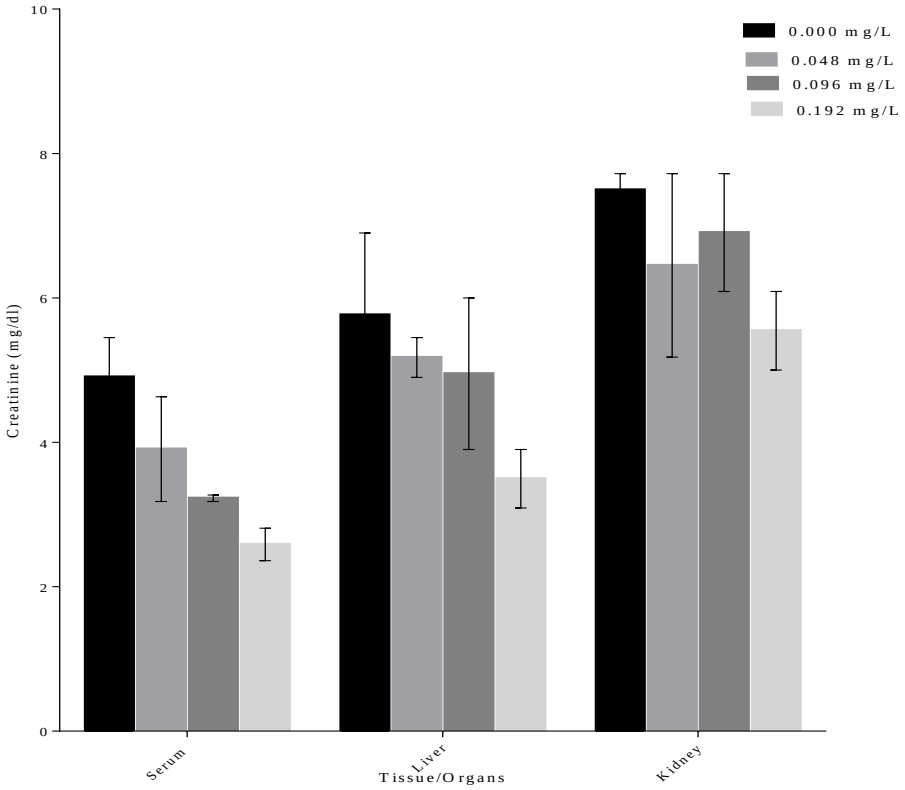

Figure 6: Mean value of creatinine (mg/dl) in *Clarias gariepinus* exposed to concentrations of neem leaf powder after 14- day period. Each vertical bar represents standard error.

Proteins are mainly involved in the architecture of the cell. Proteins are the chief source of nitrogenous metabolism (Adamu and Kori-Siakpere, 2011) thus the decrease in serum total protein reported in this study was supported by earlier study (Adamu and Kori-Siakpere, 2011) where it was suggested that the decreased value may be due to protein hydrolytic activity as a consequent of the elevation of protease activity. Therefore, this plant powder revealed similar mode of action of the test fish as reported in tobacco leaf powder Adamu and Kori-Siakpere, (2011) causing a stress induced effect on protein synthesis revealing lower serum total protein and higher liver total protein. This finding is similar to earlier report by Mousa, *et al.,* (2008) in African Catfish and Saravanan, *et al.,* (2010) in *Labeo rohita.*

The reduced serum glucose reported in this study is in accord of the earlier reports of Mousa, *et al.,* (2008) and Dunkel and Ricilards, (1998). The high liver glucose in fish exposed to the plant leaf powder may be an indication of the liver inability to effectively conduct its function in glycolysis thereby lowering its value recorded in the serum and kidney. It may therefore, be inferred that as the concentrations of the plant powder increased, the liver was unable to effectively utilize the glucose produced thus increasing its liver concentration with subsequent decrease values in the serum and kidney; thus implying that the plant affects glucose metabolism.

The decrease in serum and liver cholesterol in this study was also reported earlier by Garg *et al.,* (1994). The effect of the plant leaf powder reported in this study is also similar to that

reported by Adamu and Kori-Siakpere (2011) in tobacco leaf dust. This may be an indication that the fish exposed to the plant leaf powder used lipid metabolism to generate their energy. During exposure periods, the role of glyceride as glucose precursor becomes more important as observed in this study.

It could be that in order to generate its metabolic energy in the presence of the plant leaf powder, the fish utilize an alternate form of energy. This may have been responsible for the high liver glucose and lower liver triglycerides and cholesterol recorded in this study. Thus the fish utilized the lipid as source of metabolism as the ketone utilization to necessitate the energy being supplied via oxidation of fatty acid. The decrease in serum triglyceride was supported by the earlier report (Adamu, 2009). The reduction in serum triglyceride may be as a result of lipolysis proceeding during exposure period as the major source of energy (Adamu, 2009). Similarly, the decrease in liver triglyceride reported with corresponding increase in liver glucose may be an indication that glyceride was used as glucose precursor.

Uric acid is the major product of the catabolism of purine nucleoside (adenosine and guanosine) from the purine metabolism pathway. The report in this study is similar to that earlier report by Adamu and Ademuyiwa, (2014). The decrease in serum and liver creatinine in this study was also reported by Adamu and Kori-Siakpere (2011). Therefore, the high value in the kidney may confirm the earlier possibility that the neem leaf powder may have prevented the kidney from performing properly its function of filtration.

CONCLUSION

In conclusion, neem (*Azadirachta indica*) leaf powder in the concentrations used in this study appear to have negative effect on the experimental fish (*Clarias gariepinus*) affecting glucose metabolism and proper function of the kidney cells. Thus the effect was noticed to be concentration dependent.

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