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EFFECT OF SUB-LETHAL CONCENTRATIONS OF ADHATODA BUCCHULZII PLANT EXTRACT ON SERUM BIOCHEMICAL COMPOSITION OF *PERIOPHTHALMUS BARBARUS*

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Abstract
Adhatoda bucchulzii is a piscicidal plant introduced into inland waterways by artisanal fishers in most water bodies in Rivers State, Nigeria, to stupefy, and kill fish for easy catch. This study was aimed at evaluating the sub-lethal toxicity of aqueous extracts of *A. bucchulzii* on serum biochemical compositions of *Periophthalmus barbarus* over a 96hrs exposure period. Results showed a decreasing values of urea (Blood Urea Nitrogen) and serum albumin as serum total protein, bilirubin, creatinine and globulin values were increasing. There was a significant difference between the various treatments for urea, Total Protein, Bilirubin, Albumin, Creatine and Globulin. But there was no significant difference between T1 and T2 values for bilirubin, T3 and T4 values for Albumin, T2 and T3 values for Creatine and T1 and T2 values for Globulin. All other values were significantly different as concentrations increased. The above parameters which are indices of the functionalities of kidneys and liver organs to a high extent revealed that high concentrations of the plant extract impaired liver and kidney functions. It was concluded that *Adhatoda bucchulzii* is a piscicidal plant and fishers should be careful with its use given its indiscriminate devastating tendencies in water bodies against the survival of living organisms.

Introduction
The use of piscicidal plants by African fishermen to enhance catches has been largely reported, (Adeogun*et al*., 2012). A wide range of active phytochemical compounds (alkaloids, flavonoids, saponins, glycosides, sugars) are present in these plants leading to growing concern on their potentials to cause adverse effects on the health of aquatic organism particularly non-target species (Wink, 2001; Sparg *et al*., 2004; Sun *et al*., 2005, Adeogun, *et al*., 2012). A number of plants have been identified for their ichthyotoxic properties (Tiwari and Singh, 2003, Sparg *et al*., 2004).
Considering the reliability of blood serum biochemistry as biomarkers of stress in fish urea (Blood urea nitrogen) test as it measures the amount of nitrogen in the blood that comes from the waste product urea, urea is made when the protein is broken down in the body (Ajeniyi and Solomon, 2014). When the kidneys are healthy, they remove the BUN, usually leaving a small amount of it in the blood. When the kidneys are not healthy, they have trouble removing BUN and leave more of it in the blood (Nayana, 2021). Any increase in protein introduced into the intestines to be digested (such as a very high protein diet of meat or blood proteins from a bleeding ulcer), increases the urea in the blood (Adejiniyi and Solomon, 2014). According to them dehydration also increase the urea value. Therefore, both the liver and the kidneys must be functioning properly for the body to maintain a normal level of urea in the blood. This is true given the fact that protein metabolism is chiefly the responsibility of the liver. The role of the kidney is to eliminate waste products of protein metabolism.
Measurement of creatinine according to Ajeniyi and Solomon, 2014 is an accurate estimation of how well the kidneys filtration processes are working. Anything that alters the ability of kidneys to filter efficiently (such as dehydration), can cause changes in creatinine levels in the blood (Ajeniyi and Solomon, 2014).
Studies of the hematology and blood biochemistry in different species of fish are of comparative physiological interest. They contribute to a greater understanding of habitat, food selection and mode of life (Kulkarni and Pruthvira, 2016). The availability of a particular fish species in the wild in large numbers reflects that the fish is thriving well in the environment which is most suitable for its survival, growth and breeding activities. In this context, it becomes necessary to identify the suitability of the use of *Adhatoda bucchulzii* extract in the catching of *Periophlthalmus barbarus* for normal functioning of specific organs or tissues that manifest in the states of the serum biochemical parameters of the fish.
Normal physiological processes are long affected before the death of an organism. Thereby creating the need for physiological and biochemical indicators of health and sub-lethal effects of toxicants (Abalaka, 2013).
Serum protein plays a key role in maintaining osmotic pressure and viscosity of fish blood (Effiong and Akpan, 2016). Of particular interest is the correlation of the albumin –globulin ratio which low level has been linked to nephoris in fishes (Sandnes *et al*., 1988). Moreover, it has been established that serum albumin plays the predominating role in exerting osmotic pressure of the protein (Effiong and Akpan, 2016). Different kinds of fish vary sharply in the total serum protein and in the distribution of the various fractions. The elasmobranchii,

according to Effiong and Akpan, 2016, are known to use urea which is present in their blood in very large amounts to maintain the osmotic pressure of their blood approximately equal to that of their environment. Serum proteins are the most important factors in blood and their clinical significance has been considered to be more in human and other mammals than in fish (Payghan, *et al*., 2014). Reportedly, Lepkovsky was the first to study fish blood serum protein (Rebulka, 1993). Serum protein plays an important role in transport of different substances, defense of the organism against pathological agents, osmotic regulation and some other functions (Rudneva and Kovyrshina, 2011). Rates of serium protein synthesis vary between tissues. The major tissues are liver, gill, gastrointestinal tract, kidney and white muscles. (Payghan *et al*., 2014). Liver has a central position in the synthesis and export of many proteins. Many factors that affect the liver function may have the potential to modify rates of protein synthesis and to differentially affect responses in different tissues (Wright and Anderson, 2001). Creatinine is a chemical waste molecule that is generated from muscle metabolism (Kulkarni and Pruthvira, 2016). According to Reimschuessel and Jones (2021), creatinine is a waste product from the muscles that is also filtered by the kidneys. Like BUN, high levels of creatinine could mean there is a lot of waste product that hasn't been removed by the kidneys (Reimschuessel and Jones 2021).

Materials and Methods

One hundred and fifty live adult *P. barbarus* were procured from fishers in Ogbogoro station of the New Calabar River, moved to the laboratory of NIOMR/ARAC in Buguma. The fish were acclimated for seven days prior to the commencement of the experiment in a netted happa with a mud at the bottom and floating materials which provide a resting terrestrial platform for the fish. The fish were fed with pieces of crabs. They were fed two times daily (9am and 4pm). Ten fish were placed in each of the fifteen aquarium tanks for the experiment. One aquarium served as control with only water, while the remaining four were treated with the plant extract and this was triplicated, the plant extract was obtained by adding 3.0 litre of water to 0.5kg of mashed plant. 0.0ml, 0.1ml, 0.2ml, 0.3ml and 0.4ml representing 0.0mg/l, 100mg/l, 200mg/l, 300mg/l and 400mg/l respectively were separately introduced in 20 litres of water in aquarium tanks. Ten live *P. barbarus* were introduced into each tank for 96hours. Water quality parameters were monitored before introduction of the experimental organisms into the aquarium tanks. The parameters that were measured are temperature, salinity, hydrogen ion concentration (pH) and dissolved Oxygen (DOs). This was done to make sure that the water quality standards are maintained. Water temperature was taken by using mercury in glass thermometer, and the hydrogen ion concentration (pH) by pH meter (Mettle 340). Dissolved Oxygen Concentration was determined using an Oxygen meter (Oxyguard Handy MK II type) and their means calculated.

The fish in each aquarium tank were randomly selected and bloods samples obtained from the caudal circulation with the aid of Non-Heparinized 2cm 3 disposable plastic syringes and a 2.1 Gauge disposable hypodermic needle. The site chosen for puncture was wiped dry with tissue paper to avoid contamination with mucus. The needle was inserted perpendicularly to the vertebral column of the fish and gently aspirated during penetration. It was then pushed down gently until blood started to enter as the needle punctured a caudal blood vessel.
Blood was takenunder gentle aspiration until $1cm^3$ was obtained, then the needle was withdrawn and content emptied into non-heparinized specimen bottles. The blood in the tubes were collected and then transferred into clean dry centrifuge tubes and centrifuged at 400rpm for 10minutes, followed by serum separation.
The serum was separated from the blood after centrifuging for 10minutes by using a Pasteur pipette and transferred into anticoagulant free test tubes and stored in a refrigerator until analysis. Serum biochemical analyses were determined using Medonic Hematology Auto Analyzer (a full automatic chemical analyzer at the chemical pathology laboratory of University of Port Harcourt Teaching Hospital). Analyses was run for Total protein (TP: mg/dl), Bilirubin, Albumin, Urea, Globulin and Creatinine.
Data obtained were analyzed, using one-way analysis of variance (ANOVA) and differences between means were separated with Duncan's Multiple Range Test. Test of significance was done at α=0.05. The above statistical test was carried out using SPSS version 23.

Results

Water quality parameters (Table 1) of experimental set up for all treatments were similar and within the optimum range recommended for a brackish water fish like *Periophthalmus barbarus.* The results of biochemical parameters of *P. barbarus* treated with sub-lethal doses of *A. buchulzii* aqueous extract (Table 2) showed that urea recorded 6.67 ± 0.32 for T1, 4.87 ± 0.15 for T2, 3.77 ± 0.14 for T3, 2.60 ± 0.52 for T4 and 1.72 ± 0.13 for T5. In that same vein, Albumin values also decreased from 29.00 ± 0.57 in T1 to 14.33 ± 0.88 in T5. Values for total protein increased from 33.00 ± 1.73 for T1 to 71.21 ± 1.57 for T5, while Bilirubin values also increased from 3.33 ± 0.08 for T1 to 6.53 ± 0.35 for T5. Creatinine values also increased as concentrations increased from T1-T5 significantly as globulin values. There was a significant difference between the various treatments for urea, Total Protein, Bilirubin, Albumin, Creatine and Globulin. But there was no significant difference between T1 and T2 values for bilirubin, T3 and T4 values for Albumin, T2 and T3 values for Creatine and T1 and T2 values for Globulin. All other values were significantly different as concentrations increased.

Table 1: Water quality parameters during the experiment

TREATMENTS	Salinity Ppt-	DO(O_2) (ppm)	pH	Temperature °c
T1 (0.0mg/ml)	8ppt	3.02ppm	7.0	26°c
T2 (100mg/ml)	8ppt	3.06ppm	6.5	26°c
T3 (200mg/ml)	8ppt	3.06ppm	6.5	26°c
T4(300mg/ml)	8ppt	3.06ppm	6.5	26°c
T5(400mg/ml)	8ppt	3.06pm	6.5	26°c

Table 2: Mean and Standard Deviations Of Blood Serum Parameters of *P. barbarus* used in the study

	Urea	Total Protein	Bilirubin	Albumin	Creatinine	Globulin
T1	6.67 ± **0.32** [a]	33.00 ± **1.73**[a]	3.33 ± **0.08** [a]	29.00 ± **0.57**[a]	14.33 ± **0.88** [a]	3.26 ± **0.37** [a]
T2	4.87 ± **0.15** [b]	42.33 ± **1.86** [b]	4.00 ± **0.15** [a]	25.67 ± **1.20**[b]	28.66 ± **2.18** [b]	4.57 ± **0.47** [a]
T3	3.77 ± **0.14**[c]	55.33 ± **3.51** [c]	4.80 ± **0.15** [b]	21.00 ± **0.57** [c]	36.33 ± **3.71** [b]	4.67 ± **0.33**[ab]
T4	2.60 ± **0.52**[d]	59.67 ± **1.45** [c]	5.63 ± **0.23** [c]	19.33 ± **0.88** [c]	56.33 ± **4.33** [c]	6.00 ± **0.57**[bc]
T5	1.72 ± **0.13**[e]	71.21 ± **1.57** [d]	6.53 ± **0.35**[d]	14.33 ± **0.88** [d]	71.33 ± **1.85** [d]	7.33 ± **0.33** [c]
P-value	0.000	0.000	0.000	0.000	0.000	0.000

Values with different subscripts are significantly different.

Discussion

Serum blood biochemical values are not commonly used as a diagnostic tool in fish medicine, partly because of the lack of reference intervals for various fish species and because changes in blood analysis associated with specific diseases and metabolic disorders are not well characterized (Ajeniyi and Solomon, 2016). With sufficient background data, according to them, clinical biochemical analysis could be developed to detect metabolic disorders and sub-lethal disease states affecting production efficiency. In the present study, the mean values for urea (BUN) decreased from 6.67 ± 0.32 to 1.72 ± 0.13 as concentrations increased from 0.0mg/ml – 400mg/ml. there was a marked significant difference between the various concentrations as concentrations increased from 0.0mg/ml (T1), 100mg/ml (T2), 200mg/ml (T3), 300mg/ml (T4) and 400mg/ml (T5) yet the organisms survived. This is in agreement with Joshi *et al*., (2002) who reported that survival of fish can be correlated with increase in anti-body production which helps in the survival and recovery. There was a significant difference between the levels of creatinine values recorded in T1 (14.33 ± 0.88), T2 (28.66 ± 2.18), T3 (36.33 ± 3.71), T4 (56.33 ± 4.33) and T5 (71.33 ± 1.85) representing 0.0mg/ml, 100mg/ml, 200mg/ml, 300mg/ml and 400mg/ml respectively.

Both urea and creatinine levels were significantly different in the present investigation. These compounds are the most abundant non-protein constituents in the body and their determination are the most commonly ordered test of the kidney ability to excrete metabolic wastes (Triesseles, 1988). The result showed significant increase in the creatinine level and a significant decrease in urea level. The presence of increasing creatinine concentration in the blood suggests a decrease in Glomerular Filtration Rate (GFR). It has been reported that creatinine is a more accurate marker of kidney disease than urea. High creatinine level implied that many waste products in the fish blood stream would not be cleared, indicating that kidneys were not functioning properly (Ajeniyi and Solomon, 2014). The increase in globulin disagrees with the finding of Hammed *et al*., 2010 who reported that the reduction in globulin has been known to cause low immunity and also affects immunological action. According to Tietz, (1986) globulins are important for immunologic responses. Globulins according to Hammed *et al*., 2010 are larger protein and it has been reflected in the present result. The disparity in the albumin concentration indicates that the liver at a point, given the increase in concentrations of the extract, could not manufacture enough albumins to be circulated in the blood stream. Albumin and globulin are two indices used to access if the health status of the liver is in their right proportion. As reported by Omitoyin (2007), the alterations in albumin/globulin ratio (as seen in the present study) may result from the poorer liver function as well as proteinuria due to kidney damage characterized by degeneration in tubular epithelium as well as hyaline casts in the lumen. Since the albumin levels decreased, the increase in globulin levels may elevate the total protein as suggested by Uyanik *et al*. (2001). The increase in total protein and globulin levels as reported by Chapatwala *et al*., (1982a), Chapatwala *et al*. (1982b) and Uyanik *et al*., (2001) may depend on dehydration due

to the effect of the extract. The absence of total bilirubin is an indication of good liver function which means a case of no liver, dysfunction or liver damage in a fish (Hammed, *et al*., 2010). In the present study there was an increase in bilirubin values. This agrees with Cheeseborough (1992) who reported that a rise in the concentration of serum bilirubin indicates or suggests liver damage.

Conclusion

The present study investigated the effect of sub-lethal concentrations of *A. bucchulzii* extract on serum biochemical composition of *P. barbarus*. It was evident that with increasing concentration, there were indications of liver and kidney failure arising from the manifestations of the various parameters that were examined. This confirms that *A. bucchulzii*is piscicidal. The reduction in liver and kidney performance confirms that normal physiological processes are long affected before the death of an organism.

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