TOXICOLOGICAL EFFECTS OF *Clarias gariepinus* EXPOSED TO AQUEOUS AND ETHANOIC EXTRACTS OF *Azadiractha indica*

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ABSTRACT

The use of biomarkers for monitoring both the condition of organisms and environmental quality especially fish has received increased attention in recent years. Exposure of aquatic organisms to bioassay test using various plant extracts have been shown to have detrimental effects on them thereby resulting in their death. Toxicological investigations were carried out to determine the lethal, sub-lethal concentration, the biochemical parameters and histopathological response of the exposed organisms to Aqueous and Ethanoic extracts of Azadiractha indica. One way ANOVA was used to determine levels of significance ($P < 0.05$).The physico-chemical parameters of Aqueous and Ethanoic extracts were carried out. The 96 hrs LC_{50} value for C. gariepinus exposed to Azadiractha indica were 9.930 g/L and 9.873 g/L for ethanol and aqueous extract respectively. Analysis of Variance (ANOVA) showed that there was significant difference ($P<0.05$) in the quantal response at 24, 48, 72, 96h of exposure. Alanine Aminotransaminase (ALT), Aspartate Aminotransaminase (AST), Alkaline Phosphatase (ALP) and Total Protein (TP) were significantly different, ($P < 0.05$) and increased compared to control group during the exposure to aqueous and ethanol extracts of Azadiractha indica. The results for the histopathology over a period of 28 days shows no inflammation or pathology on the gills, kidney and liver of C. gariepinus exposed to both aqueous and ethanol extract of Azadiractha indica at lower concentration (5, 5.5, and 6 g/L). At higher concentration (7.5 and 8 g/L), there was dense inflammatory infiltrate within the interstitium of the kidney, destruction of respiratory epithelium of the gills and vacuoles within the hepatocytes with areas of necrosis for the liver. This study showed that exposure to higher doses of both aqueous and ethanol extract of Azadiractha indica had negative effect on C. gariepinus, following the biochemical response and histopathology composition. This study is valuable for fish farmers to know the treatment concentration that can prove the use of Azadiractha indica as anti-stressors.

Keywords: Toxicity, Histopathological, *Clarias gariepinus*, Azadiractha indica

INTRODUCTION

Anthropogenic activities, natural disaster and pollution have affected aquatic organism and this has led to increased interest to research on fish health and the possibilities of utilizing these health parameters for assessment of the quality of aquatic environment (Henry *et al.*, 2004). The use of biomarkers for monitoring both environmental quality and the health of organisms inhabiting polluted ecosystems has received increased attention in recent years (Samecka-Cymerman *et al.*, 2003 and Gauthier *et al.*, 2004). Exposure of aquatic organisms to plant extracts have been shown to have detrimental effects on fish physiology, sometimes leading to deformation and end up in mortality (Barron *et al.*, 2003, Couillard *et al.*, 2005 and Liu *et al.*, 2006).

Antibiotics, hormones, vitamins and several other chemicals have been tested as growth promoters, antibacterial and other purposes in culture system (Jayaprakas and Sambhu1996). Although some of these chemicals have effects on organism especially fishes (Sambhu1996).They cannot be recommended in commercial culture operations due to their residual effects on the muscle of fishes and other organism. In fish hatcheries, the indiscriminate use of antibiotics in prophylactic treatment has led to the development of the resistant strains and the need to switch over to other antibiotics (Brown, 1989). The antibiotics also may reduce the larval growth and inhibit defence mechanisms of the fish larvae. Many of the antibiotics and other synthetic drugs have shown sensitization reaction and other undesirable side effects (Atal, 1982).

The use of antibiotics in the hatcheries most especially in aquaculture has led to bio-magnifications that in-turn leads to rejection of the total consignment during export (Atal, 1982).

Pollution of the aquatic environment by toxic substances is a worldwide problem, especially in developing countries. In recent years, the use of medicinal plants as effective alternatives to synthetic pesticides and fertilizers has gained importance especially to combat problem both in fish and aquatic environment. At present, attestations of scientist have been created towards development and usage of several botanical products due to availability, easy biodegradability, and reduced toxicity to human and environment. Such botanical products when used extensively may enter aquatic systems such as streams, rivers, and lakes, which may have adverse effect on non-target organisms.

Environmental problem started by man's concern to solve social problem, therefore rapid population growth, industrialization and concentration are the major contributions to environmental problems in both developing and developed countries (Ayoola, 2011). The length of the problem varies from country to country depending on various factors; including the degree of enforcement of environmental regulation and the stage of industrial development (Ayoola, 2011). Many sources of botanical fish toxicants in Nigeria are identified which are extremely toxic to a wide range of organisms including fish (Olufayo, 2009). Plants are source of structurally diverse biologically active substances (Baird, 1994).Some plants contain compounds of various classes that have insecticidal, piscicidal and molluscidal properties unlike synthetic chemical pesticides which leave harmful residues in the aquatic environment (Koesoemadinata, 1980). Botanical insecticides are believed to be more environmental friendly compared to synthetic chemicals because they are easily biodegraded and leave no residues in the environment. Since, some of the pesticidal compounds present in plants are also toxic to fishes, botanical pesticides have potential to be used as piscicide to eradicate unwanted fish in the pond. Fish farmers in Nigeria have persistently and indiscrimately abused these natural plant piscicides by using much higher concentrations than necessary, causing mass mortality of fish in ponds, contaminating the water body and affecting non target organisms (Fafioye, 2001). The physical and chemical changes in aquatic

environment often cause some physiological changes in fish. thus, the water quality of an aquatic body is very crucial because it determines the productivity and other parameters necessary for the fish survival (Fafioye, 2001). Many countries have legislated against the use of chemical poisons in aquatic systems and instead have policies favoring the use of natural biodegradable alternatives. Botanicals are natural biocides and their contamination of natural water has become inevitable in Nigeria because of recent wide use. Piscicidal plants *Blighiasapida, Kigelia Africana, Tetrapleura tetraptera, Raphia vinifera, Parkia biglobosa and Tephrosia vogelii are* frequently used by fishermen because they are highly potent (Fafioye, 2001).

Biochemical and histopathological 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 use of *Azadiractha indica,* their effect on biochemical and histopathological changes has not been examined in *Clarias gariepinus* that is widely cultivated (FAO,1977) and greatly a bounds in Nigerian waters (Fagbenro, 1992).

Azadiractha indica, (Neem leaf) has been used as insect repellent, anti-feedant, anti-hormonal and other various uses but majorly there is need to test if they can also serve as an anti-stress agents without any form of damage, disorder or deformity on aquatic organism majorly fishes using *Clarias gariepinus* as a test organisms, hence the need to carry out toxicology test, biochemical and histopathological analysis.

There is however little information on how *Azadiractha indica* (Neem leaf) affect *C. gariepinus.* Knowledge of the tolerance limits and growth development of *C. gariepinus* treated with *Azadiractha indica,* (Neem leaf) as an anti-stress would be very helpful in determining their suitability for use in aquaculture systems. This study therefore investigates the biochemical and histopathological effect *Azadiractha indica* (Neem leaf) on *Clarias gariepinus.*

MATERIALS AND METHODS

Experimental Procedure

Five hundred juveniles of *Clarias gariepinus* were bought from a fish farm in Ejigbo, Lagos State. The juveniles were transported in two aerated polythene bags to the Ecotoxicological laboratory of Department of Marine Sciences, University of Lagos in the early hours of the morning (8:00 am). The water to be used for stocking of the juveniles was dechlorinated by exposing it to sun for a period of 48 h. The *Clarias gariepinus* juveniles were kept in a rectangular glass tank and allowed to acclimatize to laboratory conditions for a period of 21 days in already dechlorinated tap water. The stock tank was fixed with cosmo 10,000 air pump with voltage 220-240v, to aerate the water. The juveniles were fed thrice daily using coppens commercial supplementary feed (42% protein content). The water was change daily to prevent accumulation of toxic waste. Experimentation was carried out under ambient laboratory conditions. Feeding of

the juveniles stopped a day before the bioassay test. The fresh leaves of *Azadiractha indica* were collected along the botanical garden and main gate of University of Lagos, Akoka, and Lagos State.

Extraction Process

The fresh leaves of *Azadiractha indica* (10 kg) were collected and washed well to remove any adhering foreign particles and soil materials. The washed leaves were weighed using Ohaustriple 700 to 800 series weighing balance and oven dried at 48°C for 36 hrs to prevent enzyme action. After drying, half portion of the dried leaves was coarsely powdered and later soaked in 10 Litres of clean water for 72 hrs. The solution was filtered through a muslin cloth to separate aqueous extract from residue, the aqueous extraction was collected in beakers and oven dried for a period of 72 hrs at 40°C to get a powdered substance.

Another portion of the dried leaf was coarsely powdered and soaked in 10 litres of ethanol for a period of 72 hrs, the solution was filtered through a muslin cloth to separate the ethanoic extract from the residue and the ethanoic extraction was collected in beakers and oven dried for a period of 72 hrs at 40°C to get a powdered substance.

Bioassay Procedures

The preliminary tests were carried out to determine suitable range of concentration for the bioassay experiment. The concentration used for the aqueous and ethanol extract of *Azadiractha indica* after preliminary test were: 10, 15, 20, 25 g/L, respectively for the definitive test. These concentrations were carefully measured out to make up 10 litres of solution in 5 bioassays containers in triplicate. Another bioassay container with 10 litres of water, free of the extract was used as control. In each of the container, 10 juveniles (8.7 ± 0.3) cm were introduced. Care was taken to minimize the stress on the fish by using a hand net to collect and drop the fish carefully into the rectangular plastic tanks. The sub-lethal test was conducted using the following concentration 5, 5.5, 6, 7.5 and 8 g/L. The *Clarias gariepinus* exposed to different concentration of ethanoic and aqueous extract of *Azadiractha indica* were monitored for mortality at 24, 48, 72 and 96 hrs for acute toxicity and 28days chronic toxicity testing and the histopathology parameters and enzymatic biomarkers was examined.

Physico-Chemical Analysis

Water temperature was determined by mercury in glass thermometer, calibrated in centigrade (°C). The thermometer was inserted into water for five minutes and temperature measured in degree Celsius (^{0}C).The pH or Hydrogen ion concentrations were determined using Hanna instrument pH 211-microprocessor pH meters. After every measurement, the instrument was standardized using buffer solution and then washed with distilled water.

The Dissolved Oxygen (DO) was measured with DO meter (model EUTECH DO 600). The measurement is carried out by inserting the probe into the test bioassay tanks.

Enzymatic Biomakers

Aspartate Aminotransferase (AST), Alanine Aminotransferase (ALT) and Alkaline Phosphatase (ALP) were determined using randox reagent, method described by Reitman and Frankel (1957).

DETERMINATION OF HISTOPATHOLOGICAL PROTOCOLS

PREPARATION OF TISSUE FOR MICROROMY:

The tissues were fixed in Bouin's fluid for 24 hours, this is the process involved in the preparation of sectioning (microtomy) which are: Grossing or cutting up, Tissue processing and Embedding(Tietz, 1999).

Statistical Analysis

Data analysis was carried out by one way-analysis of variance (ANOVA). The One-way analysis of variance, ANOVA and Student Newman-Keul's, (SNK) test were used to test for significant difference (5% level) in the mean mortality response of *C. gariepinus* to different concentrations of the toxicants for 96hrs, followed by Duncan's Multiple Range Test (DMRT) were used. Values were considered significant when $P < 0.05$. The data from the physico-chemical parameters were also analyzed using graphical representatives. Analysis was performed using SPSS 18 windows.

The indices of toxicity measurement derived from the analysis were:

- LC_{50} = The concentration that kills 50% of the test population
- LC_{95} = The concentration that kills 95% of the test population
- TF = Toxicity factor for relative potency measurement. The values are:

$$TF_1 \text{ (Ethanoic)} = \text{Toxicity factor} = \frac{LC_{50} \text{ of test compound at 24 hrs}}{LC_{50} \text{ of test compound (48, 72, 96 hrs)}}$$

$$TF_1 \text{ (Aqueous)} = \text{Toxicity factor} = \frac{LC_{50} \text{ of test compound at 24 hrs}}{LC_{50} \text{ of test compound (48, 72, 96 hrs)}}$$

$$TF_2 = \text{Toxicity factor} = \frac{LC_{50} \text{ of test compound (Aqueous) at 48 hrs}}{LC_{50} \text{ of test compound (Ethanoic at 24, 48, 72, 96 hrs)}}$$

SE= Standard error, DF= Degree of freedom, CL= Confidence limit, LC= Lethal concentration

RESULTS

Physico-chemical Parameters of the Test Media During Toxicity Testing

The Physico-chemical measurements of test media are as presented in Table 1.

Relative Toxicity of Ethanoic and Aqueous Extract Exposed to *C.gariepinus*

The results of the acute toxicity of ethanoic and aqueous on *C. gariepinus* at 24, 48, 72, and 96 hrs of exposure are shown in Table 2. The analysis of concentration- mortality data of Ethanoic and Aqueous Extract when tested against *C. gariepinus* revealed that the derived toxicity indices (LC_{50}) ranged from 9.930 (96 hrs LC_{50}) to 20.936 (24 hrs LC_{50}) for Ethanoic and 9.873 (96 hrs LC_{50}) to 22.664 (24 hrs LC_{50}) for Aqueous (Table 2). On

the basis of computed toxicity factor (TF_2) using 96 hrs LC_{50} the treatment was found to be more toxic against *C. gariepinus* at the 48 hrs LC_{50} of aqueous with 1.61 compared with others. In this study, the acute toxicity level based on the 96 hrs LC_{50} value of Ethanoic and Aqueous concentration was found to be 9.930 and 9.873 ml/L when tested against the *C. gariepinus*. Analysis of Variance (ANOVA) showed that there was significant difference ($P < 0.05$) in the quantal response at 24, 48, 72, 96 hrs of exposure (Table 2). The Probit analysis showing the Log concentration plotted against the probit percentage mortality of *C. gariepinus* exposed to ethanoic and aqueous extract of Neem leave was represented in Tables 2 and 3.

No adverse behavioural change or any mortality was recorded in the control fish throughout these periods of 96 hrs. Symptoms of toxicosis observed in fish behavior with Ethanoic and aqueous extract includes agitated or erratic swimming, sudden quick movements were observed. The fish became weak, settled at the bottom and died.

Table 1: Physico-chemical characteristics of the water treatments for the Ethanoic and Aqueous extract media.

PARAMETERS	ETHANOIC					
	Day 1	Day 2	Day 3	Day 4	Mean	FEPA Limit
DO	5.60 ± 0.36	5.47 ± 0.38	5.38 ± 0.48	5.18 ± 0.45	5.41	NS
pH	6.55 ± 0.26	6.27 ± 0.42	6.25 ± 0.40	6.20 ± 0.41	6.32	6 - 9*
TEMP (^{0}C)	27.85 ± 0.11	27.5 ± 0.45	27.33 ± 0.43	27.27 ± 0.44	27.49	< 40
PARAMETERS	AQUEOUS					
DO	5.68 ± 0.26	5.33 ± 0.36	5.23 ± 0.40	5.24 ± 0.42	5.37	NS
pH	6.65 ± 0.21	6.40 ± 0.33	6.23 ± 0.40	6.22 ± 0.43	6.38	6 - 9*
TEMP (^{0}C)	27.78 ± 0.17	27.48 ± 0.35	27.28 ± 0.43	27.37 ± 0.40	27.48	< 40

Table 2: Relative Toxicity of Ethanoic and Aqueous extraction C. *gariepinus*

Exposure Time (Hrs) Ethanoic	LC_{50} (95ml/L CL ml/L)	LC_5 (95ml/L CL ml/L)	LC_{95} (95ml/L CL ml/L)	Slope ± S.E	Probit line equation	DF	TF_1	TF_2
24	20.936 (0)	19.225 (0)	22.800 (0)	44.41 ± 6.12	Y = -53.66+ 44.41x	2	1	2.3
48	14.112 (0)	8.810 (0)	22.605 (0)	8.04 ± 1.44	Y = -4.24 + 8.04x	2	1.48	1.55
72	10.459 (0.917 – 1.076)	6.112 (0.505-0.897)	17.898 (1.186 – 1.399)	7.05 ± 1.65	Y = -2.19 – 7.05x	2	2.00	1.15
96	9.930 (0)	8.176 (0)	12.060 (0)	19.49 ± 22.16	Y = -14.43 – 19.49x	2	2.11	1.09
Exposure Time (Hrs) Aqueous								
24	22.664 (1.33 – 1.38)	20.035 (1.26 – 1.33)	25.637 (1.39 – 1.45)	30.73 ± 6.21	Y = -36.64 + 30.73x	2	1	2.3
48	15.812 (1.14 – 1.25)	8.955 (0.79 – 1.04)	27.917 (1.45 – 1.25)	6.66 ± 1.24	Y = -2.99 + 6.66x	2	1.43	1.61
72	10.24 (0.89 – 1.07)	5.609 (0.41 – 0.88)	18.68 (1.20 – 1.44)	6.30 ± 1.53	Y = -1.36 + 6.30x	2	2.21	1.04
96	9.873 (0.89 – 1.04)	6.62 (0.50 – 0.91)	14.73 (1.11 – 1.35)	9.47 ± 2.80	Y = -4.413 + 9.47x	2	2.30	1

Table 3: Percentage mortality of *C. gariepinus* exposed to Ethanoic and Aqueous Extract

Concentration	No of Test Organisms	ml/L Mortality/Time (Hours)			
		24	48	72	96
Ethanoic extract					
Control	21	0.0^{a}	0.0^{a}	0.0^{a}	0.0^{a}
10g	21	0.0^{a}	9.5^{a}	42.9^{d}	52.4^{a}
15g	21	0.0^{a}	66.7^{b}	90.5^{c}	100^{a}
20g	21	19.1^{b}	80.9^{b}	95.2^{b}	100^{a}
25g	21	100^{c}	100^{a}	100^{a}	100^{a}
Aqueous extract					
Control	21	0.0^{a}	0.0^{a}	0.0^{a}	0.0^{a}
10g	21	0.0^{a}	9.5^{a}	38.1^{c}	42.9^{a}
15g	21	0.0^{a}	42.9^{b}	85.7^{c}	95.2^{a}
20g	21	4.8^{a}	71.4^{c}	100^{bc}	100^{a}
25g	21	85.7^{b}	95.2^{a}	100^{ab}	100^{a}

Mean frequencies with the same superscript letter in a row are not significantly different in the DMRT (p = 0.05)

$$\text{Survival (\%)} = \frac{\text{Number of fish their survived after exposure}}{\text{Initial number of fish stocked}} \times 100$$

Biochemical Parameters

The results of the biochemical changes observed in the blood of *C. gariepinus* exposed to varying concentrations of Ethanoic and aqueous extract is shown in Table 4.

Aspartate Aminotransaminase (AST)

The mean AST in the blood of *C. gariepinus* exposed to varying concentrations of Ethanoic extract (E.E) ranged from 94.52±1.18 to 742.50 ± 4.73 (Table 4). The lowest value (94.52 U/I) was recorded at the concentration of 7.5grams of E.E and the highest (742.50 U/I) was at 8grams The analysis of variance (ANOVA) of the AST levels in the blood for the Ethanoic extract showed a significant difference ($P < 0.05$). Furthermore, Post-hoc test using Duncan Multiple Ranged Test (DMRT) revealed that there was a significant difference ($P<0.05$) in the AST levels in the blood of *C. gariepinus* exposed to varying Ethanoic extract concentrations (Table 5).The mean of AST levels in the blood of *C. gariepinus* exposed to varying concentrations of Aqueous extract ranged from 14.40 ± 0.50 to 1124.75 ± 2.57 (Table 5). The lowest value 14.40 U/I was recorded at the concentration of 6grams and the highest 1124.75 U/I was at 5.5grams. The analysis of variance (ANOVA) of the AST levels in the blood for the aqueous extract showed a significant difference ($P < 0.05$). Furthermore, Post-hoc to using Duncan Multiple Ranged Test (DMRT) revealed that there was a significant difference ($P<0.05$) in the AST levels in the blood of *C. gariepinus* exposed to varying aqueous extract concentrations (Table 5)

Total Bilirubin (TBL)

The mean TBL in the blood of *C. gariepinus* exposed to varying concentrations of Ethanoic extract ranged from 321.25± 19.30 to 1726.66 ± 12.86 (Table 4). The lowest value (321.25 U/l) was recorded at the concentration of 5grams and the highest 1726.66 U/l was at 8grams. The analysis of variance (ANOVA) of the TBL levels in the blood for the Ethanoic extract showed a significant difference ($P < 0.05$). Furthermore, Post-hoc using Duncan Multiple Ranged Test (DMRT) revealed that there was a significant difference ($P<0.05$) in the TBL levels in the blood of *C. gariepinus* exposed to varying Ethanoic extract concentrations (Table 4).While the mean of TBL levels in the blood of *C. gariepinus* exposed to varying concentrations of Aqueous extract ranged from 81.26 ±2.07 to 1505.37 ± 3.25 (Table 5). The lowest value (81.26 U/l) was recorded at the concentration of 6grams and the highest 1505.37 U/l was at 5grams. The analysis of variance (ANOVA) of the TBL levels in the blood for the aqueous extract showed a significant difference ($P < 0.05$). Furthermore, Post-hoc using Duncan Multiple Ranged Test (DMRT) revealed that there was a significant difference ($P<0.05$) in the TBL levels in the blood of *C. gariepinus* exposed to varying aqueous extract concentrations (Table 5).

Creatine

The mean of Creatine in the blood of *C. gariepinus* exposed to varying concentrations of Ethanoic extract ranged from 44.17±2.86 to 100.71 ± 2.73 (Table 4). The lowest mean 44.17 U/l was recorded at the control and the highest 100.71 U/l was at 8grams. The analysis of variance (ANOVA) of the Creatine levels in the blood for the Ethanoic extract showed a significant difference ($P < 0.05$). Furthermore, Post-hoc using Duncan Multiple Ranged Test (DMRT) revealed that there was a significant difference ($P<0.05$) in the Creatine levels in the blood of *C. gariepinus* exposed to varying Ethanoic extract concentrations (Table 4).

While the mean of Creatine levels in the blood of *C. gariepinus* exposed to varying concentrations of Aqueous extract ranged from 44.17 ± 2.86 to 96.09 ± 1.30 (Table 5). The lowest value (44.17 U/l) was recorded at the control and the highest value (96.09 U/l) was at 5grams. The analysis of variance (ANOVA) of the Creatine levels in the blood for the aqueous extract showed a significant difference ($P \leq 0.05$). Furthermore, Post-hoc using Duncan Multiple Ranged Test (DMRT) revealed that there was a significant difference ($P<0.05$) in the Creatine levels in the blood of *C. gariepinus* exposed to varying aqueous extract concentrations (Table 5).

Alanine Aminotransaminase (ALT)

The mean ALT in the blood of *C. gariepinus* exposed to varying concentrations of Ethanoic extract ranged from 21.31±2.65 to 53.18 ± 1.77 (Table 4). The lowest mean 21.31 U/l was recorded at the control and the highest 53.18 U/l was at 5grams. The analysis of variance (ANOVA) of the ALT levels in the blood for the Ethanoic extract showed a significant difference ($P < 0.05$). Furthermore, Post-hoc using Duncan Multiple Ranged Test (DMRT) revealed that there was a significant difference ($P<0.05$)

in the ALT levels in the blood of *C. gariepinus* exposed to varying Ethanoic extract concentrations (Table 4).

While the mean of ALT levels in the blood of *C. gariepinus* exposed to varying concentrations of Aqueous extract ranged from 21.16±1.84 to 96.98 ± 4.72 (Table 5). The lowest mean 21.16 U/I was recorded at the concentration of 8grams and the highest 96.98 U/I was at 6grams. The analysis of variance (ANOVA) of the ALT levels in the blood for the aqueous extract showed a significant difference ($P < 0.05$). Furthermore, Post-hoc using Duncan Multiple Ranged Test (DMRT) revealed that there was a significant difference ($P<0.05$) in the ALT levels in the blood of *C. gariepinus* exposed to varying aqueous extract concentrations (Table 5).

Urea

The mean Urea in the blood of *C. gariepinus* exposed to varying concentrations of Ethanoic extract ranged from 4.20±0.21to 9.62 ± 0.44 (Table 4). The lowest mean 4.20 U/I was recorded at the concentration of 8grams and the highest 9.62 U/I was at 5grams. The analysis of variance (ANOVA) of the Urea levels in the blood for the Ethanoic extract showed a significant difference ($P < 0.05$). Furthermore, Post-hoc using Duncan Multiple Ranged Test (DMRT) revealed that there was a significant difference ($P<0.05$) in the Urea levels in the blood of *C. gariepinus* exposed to varying Ethanoic extract concentrations (Table 4).

While the mean of Urea levels in the blood of *C. gariepinus* exposed to varying concentrations of Aqueous extract ranged from 5.17±0.29 to 24.72 ± 1.13 (Table 5). The lowest mean 5.17 U/I was recorded at the concentration of 5grams and the highest 24.72 U/I was at 6grams. The analysis of variance (ANOVA) of the Urea levels in the blood for the aqueous extract showed a significant difference ($P < 0.05$). Furthermore, Post-hoc using Duncan Multiple Ranged Test (DMRT) revealed that there was a significant difference ($P<0.05$) in the Urea levels in the blood of *C. gariepinus* exposed to varying aqueous extract concentrations (Table 5).

Albumine (ALB)

The mean ALB in the blood of *C. gariepinus* exposed to varying concentrations of Ethanoic extract ranged from 2.93 ± 0.17 to 4.99 ± 0.11 (Table 4). The lowest mean 2.93 U/I was recorded at the concentration of 5grams and the highest 4.99 U/I was at 6grams. The analysis of variance (ANOVA) of the ALB levels in the blood for the Ethanoic extract showed a significant difference ($P < 0.05$). Furthermore, Post-hoc using Duncan Multiple Ranged Test (DMRT) revealed that there was a significant difference ($P<0.05$) in the Urea levels in the blood of *C. gariepinus* exposed to varying Ethanoic extract concentrations (Table 4).

While the mean of ALB levels in the blood of *C. gariepinus* exposed to varying concentrations of Aqueous extract ranged from 2.77 ± 0.94 to 8.91 ± 0.51 (Table 5). The lowest mean 2.77 U/I was recorded at the concentration of 5grams and the highest 8.91

U/I was at 8grams. The analysis of variance (ANOVA) of the ALB levels in the blood for the aqueous extract showed a significant difference ($P < 0.05$). Furthermore, Post-hoc using Duncan Multiple Ranged Test (DMRT) revealed that there was a significant difference ($P<0.05$) in the ALB levels in the blood of *C. gariepinus* exposed to varying aqueous extract concentrations (Table 5).

Table 4: The Mean levels of Biochemical contents in *C. gariepinus* exposed to sublethal concentrations of Ethanoic extracts.

CONCENTRATIONS OF EXTRACT	MEAN ± S.E											
							TOTAL PROTEIN					
	AST	TBL	CREATINE	ALT	UREA	ALB		HDL	LDL	CHOL	TG	ALP
CONTROL	415.3±5.9d	622.8 ± 3.9c	44.2 ±2.9a	21.31 ±2.65 a	9.2 ±0.2cd	4.1± 0.9a b	12.1 ±0.3 ab	0.7 ±0. 1ab	2.9 ±0. 4a	3.1 ±0. 4b	1.1 ±0. 3ab	138. 1±1. 3c
5g	136.0±1.9b	321.3 ±19.3 a	81.3 ±3.4c	53.18 ±1.77 d	9.6 ±0.4d	2.9± 0.2a	12.29±0.4ab	1.2 ±0. 2b	4.9 ±0. 2b	2.1 ±0. 2a	1.9 ±0: 2b	145. 4±2. 6cd
5.5g	421.6±4.4d	631.2 ±6.9c	60.7 ±1.2 b	35.79 ±2.88 c	8.3 ±0.2c	4.8± 0.2b	15.60±0.4c	2.2 ±0. 2c	4.26 ±0. 4b	4.6 ±0. 3c	1.5 ±0. 3ab	121. 2±1. 6b
6g	301.1±2.6c	533.6 ±5.5b	52.8 ±1.6 b	32.20 ±2.35 bc	6.7 ±0.5b	4.9± 0.1b	11.31±0.09a	3.7 ±0. 4e	1.9 ±0. 2a	3.6 ±0. 3b	0.9 ±0. 2a	146. 6± 3.3d
7.5g	94.5 ±1.2 a	708.9 ±10.2 d	58.9 ±2.3 b	23.51 ±2.11 a	7.1 ±0.4b	3.3± 0.2a	13.14±0.22b	2.9 ±0. 1d	4.9 ±0. 2b	5.1 ±0. 3c	1.2 ±0. 3ab	251. 0±3. 5c
8g	742.5±4.7e	1726.7±12.9e	100.7 ±2.7 d	25.14 ±2.87 ab	4.2 ±0.2a	4.3± 0.2a b	23.63±0.61d	0.3 ±0. 2a	2.8 ±0. 6a	2.7 ±0. 2ab	0.9 ±0. 2a	13.6 ±0.6 a

Mean frequencies with the same superscript letter in a column are not significantly different in the DMRT (p = 0.05)

Table 5: The Mean levels of Biochemical contents in *C. gariepinus* exposed to sublethal concentrations of Aqueous extracts.

CONCENTRATIONS OF EXTRACT	MEAN ± S.E TOTAL											
	AST	T.BL	CREATINE	ALT	UREA	ALB	PROTEIN	HDL	LDL	CHOL	TG	ALP
CONTROL	415.3 ±5.9c	622.7 ±3.9c	44.1± 2.8a	21.3 ±2.6 a	9.2± 0.2b	4.0± 0.9a b	12.1± 0.2a	0.7± 0.1a	2.9± 0.4a b	3.1 ±0. 4a	1.1 ±0. 3a	138.1 ±1.3c
5g	525.7 ±2.7d	1505. 3±3.2 c	96.0± 1.3d	31.2 ±2.3 b	5.1± 0.2a	2.7± 0.5a	14.5± 0.3bc d	0.5± 0.2a	2.3± 0.4a	2.0 ±0. 4a	0.9 ±0. 2a	146.5 ±3.6d
5.5g	1124. 7±2.5 f	644.7 ±6.1d	48.2± 3.4a	58.3 ±1.4 d	5.2± 0.2a	4.0± 0.1a b	15.3± 0.4d	0.8± 0.1a	3.3± 0.2a b	3.2 ±0. 2a	1.1 ±0. 2a	55.6± 0.9a
6g	14.40 ±0.5a	81.2± 2.0a	79.2± 3.3c	96.9 ±4.7 c	24.7 ±1.1 c	5.5± 0.3b	15.0± 0.1cd	1.8± 0.1b	3.5± 0.2b	4.6 ±0. 5b	2.2 ±0. 4b	243.9 ±2.4f
7.5g	346.4 ±3.5b	509.2 ±5.5b	84.2± 2.6c	41.2 ±1.8 c	7.5± 0.3b	3.7± 0.1a	14.0± 0.4bc	1.5± 0.1b	3.5± 0.3b	4.6 ±0. 3b	1.3 ±01 a	101.3 ±2.2b
8g	605.5 ±3.2c	92.6± 2.2a	60.9± 3.5b	21.1 ±1.8 a	9.13 ±0.4 b	8.9± 0.5c	13.7± 0.1b	1.6± 0.2b	5.7± 0.3c	1.9 ±0. 4a	0.9 ±0. 2a	179.8 ±1.63 c

Mean frequencies with the same superscript letter in a column are not significantly different in the DMRT (p = 0.05)

Total Protein (TP)

The mean TP in the blood of *C. gariepinus* exposed to varying concentrations of Ethanoic extract ranged from 11.31 ± 0.09 to 15.60 ± 0.41 (Table 4). The lowest mean 11.31 U/I was recorded at the concentration of 6grams and the highest 15.60 U/I was at 5.5 grams. The analysis of variance (ANOVA) of the TP levels in the blood for the Ethanoic extract showed a significant difference ($P < 0.05$). Furthermore, Post-hoc using Duncan Multiple Ranged Test (DMRT) revealed that there was a significant difference ($P<0.05$) in the TP levels in the blood of *C. gariepinus* exposed to varying Ethanoic extract concentrations (Table 4).

While the mean of TP levels in the blood of *C. gariepinus* exposed to varying concentrations of Aqueous extract ranged from 12.10 ± 0.29 to 15.30 ± 0.40 (Table 5). The lowest mean 12.10 U/I was recorded at the control and the highest 15.30 U/I was at 5.5 grams. The analysis of variance (ANOVA) of the TP levels in the blood for the aqueous extract showed a significant difference ($P < 0.05$). Furthermore, Post-hoc using Duncan Multiple Ranged Test (DMRT) revealed that there was a significant difference ($P<0.05$) in the TP levels in the blood of *C. gariepinus* exposed to varying aqueous extract concentrations (Table 5).

High Density Lipoprotein (HDL)

The mean HDL in the blood of *C. gariepinus* exposed to varying concentrations of Ethanoic extract ranged from 0.33 ± 0.19 to 3.69 ± 0.43 (Table 4). The lowest mean 0.33 U/I was recorded at the concentration of 8 grams and the highest 3.69 U/I was at 6 grams. The analysis of variance (ANOVA) of the HDL levels in the blood for the Ethanoic extract showed a significant difference ($P < 0.05$). Furthermore, Post-hoc using Duncan Multiple Ranged Test (DMRT) revealed that there was a significant difference ($P<0.05$) in the HDL levels in the blood of *C. gariepinus* exposed to varying Ethanoic extract concentrations (Table 4).

While the mean of HDL levels in the blood of *C. gariepinus* exposed to varying concentrations of Aqueous extract ranged from 0.50 ± 0.23 to 1.83 ± 0.06 (Table 5). The lowest mean 0.50 U/I was recorded at the concentration of 5 grams and the highest 1.83 U/I was at 6 grams. The analysis of variance (ANOVA) of the HDL levels in the blood for the aqueous extract showed a significant difference ($P < 0.05$). Furthermore, Post-hoc using Duncan Multiple Ranged Test (DMRT) revealed that there was a significant difference ($P<0.05$) in the HDL levels in the blood of *C. gariepinus* exposed to varying aqueous extract concentrations (Table 5).

Low Density Lipoprotein (LDL)

The mean LDL in the blood of *C. gariepinus* exposed to varying concentrations of Ethanoic extract ranged from 1.97 ± 0.19 to 4.99 ± 0.20 (Table 4). The lowest mean 1.97 U/I was recorded at the concentration of 6grams and the highest 4.99 U/I was at 7.5 grams. The analysis of variance (ANOVA) of the LDL levels in the blood for the

Ethanoic extract showed a significant difference ($P < 0.05$). Furthermore, Post-hoc using Duncan Multiple Ranged Test (DMRT) revealed that there was a significant difference ($P<0.05$) in the LDL levels in the blood of *C. gariepinus* exposed to varying Ethanoic extract concentrations (Table 4).

While the mean of LDL levels in the blood of *C. gariepinus* exposed to varying concentrations of Aqueous extract ranged from 2.28 ± 0.43 to 5.73 ± 0.27 (Table 5). The lowest mean 2.28 U/I was recorded at the concentration of 5 grams and the highest 5.73 U/I was at 8 grams. The analysis of variance (ANOVA) of the LDL levels in the blood for the aqueous extract showed a significant difference ($P < 0.05$). Furthermore, Post-hoc using Duncan Multiple Ranged Test (DMRT) revealed that there was a significant difference ($P<0.05$) in the LDL levels in the blood of *C. gariepinus* exposed to varying aqueous extract concentrations (Table 5).

Cholesterol (CHOL)

The mean Cholesterol in the blood of *C. gariepinus* exposed to varying concentrations of Ethanoic extract ranged from 2.09 ± 0.17 to 5.10 ± 0.30 (Table 4). The lowest mean 2.09 U/I was recorded at the concentration of 5 grams and the highest 5.10 U/I was at 7.5 grams. The analysis of variance (ANOVA) of the Cholesterol levels in the blood for the Ethanoic extract showed a significant difference ($P < 0.05$). Furthermore, Post-hoc using Duncan Multiple Ranged Test (DMRT) revealed that there was a significant difference ($P<0.05$) in the Cholesterol levels in the blood of *C. gariepinus* exposed to varying Ethanoic extract concentrations (Table 4).

While the mean of Cholesterol levels in the blood of *C. gariepinus* exposed to varying concentrations of Aqueous extract ranged from 1.99 ± 0.38 to 4.62 ± 0.31 (Table 5). The lowest mean 1.99 U/I was recorded at the concentration of 8grams and the highest 4.62 U/I was at 7.5 grams. The analysis of variance (ANOVA) of the Cholesterol levels in the blood for the aqueous extract showed a significant difference ($P < 0.05$). Furthermore, Post-hoc using Duncan Multiple Ranged Test (DMRT) revealed that there was a significant difference ($P<0.05$) in the Cholesterol levels in the blood of *C. gariepinus* exposed to varying aqueous extract concentrations (Table 5).

Triglyceride (TG)

The mean TG in the blood of *C. gariepinus* exposed to varying concentrations of Ethanoic extract ranged from 0.93 ± 0.22 to 1.87 ± 0.15 (Table 4). The lowest mean 0.93 U/I was recorded at the concentration of 6grams and the highest 1.87 U/I was at 5 grams. The analysis of variance (ANOVA) of the TG levels in the blood for the Ethanoic extract showed no significant difference ($P>0.05$). Furthermore, Post-hoc using Duncan Multiple Ranged Test (DMRT) revealed that there was a significant difference ($P>0.05$) in the TG levels in the blood of *C. gariepinus* exposed to varying Ethanoic extract concentrations (Table 4).

While the mean of TG levels in the blood of *C. gariepinus* exposed to varying concentrations of Aqueous extract ranged from 0.90 ± 0.22 to 2.17 ± 0.42 (Table 5). The lowest mean 0.90 U/l was recorded at the concentration of 5 grams and the highest 2.17 U/l was at 6 grams. The analysis of variance (ANOVA) of the TG levels in the blood for the aqueous extract showed a significant difference ($P < 0.05$). Furthermore, Post-hoc using Duncan Multiple Ranged Test (DMRT) revealed that there was a significant difference ($P<0.05$) in the TG levels in the blood of *C. gariepinus* exposed to varying aqueous extract concentrations except for 6grams which showed no significant difference ($P>0.05$)(Table 5).

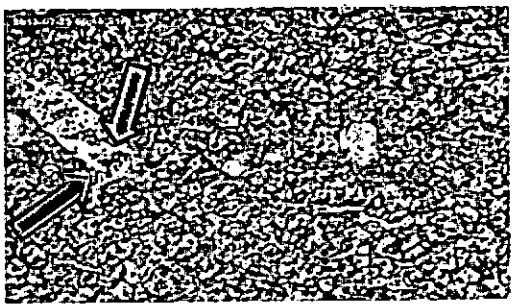
Alkaline Phosphatase (ALP)

The mean ALP in the blood of *C. gariepinus* exposed to varying concentrations of Ethanoic extract ranged from 13.63 ± 0.60 to 251.03 ± 3.51 (Table 4). The lowest mean 13.63 U/l was recorded at the concentration of 8 grams and the highest 251.03 U/l was at 7.5 grams. The analysis of variance (ANOVA) of the ALP levels in the blood for the Ethanoic extract showed a significant difference ($P < 0.05$). Furthermore, Post-hoc using Duncan Multiple Ranged Test (DMRT) revealed that there was a significant difference ($P<0.05$) in the ALP levels in the blood of *C. gariepinus* exposed to varying Ethanoic extract concentrations (Table 4).

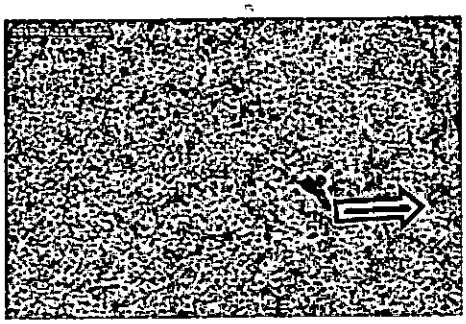
While the mean of ALP levels in the blood of *C. gariepinus* exposed to varying concentrations of Aqueous extract ranged from 55.60 ± 0.87 to 243.87 ± 2.40 (Table 5). The lowest mean 55.60 U/l was recorded at the concentration of 5.5 grams and the highest 243.87 U/l was at 6 grams (Fig. 5). The analysis of variance (ANOVA) of the ALP levels in the blood for the aqueous extract showed a significant difference ($P < 0.05$). Furthermore, Post-hoc using Duncan Multiple Ranged Test (DMRT) revealed that there was a significant difference ($P<0.05$) in the ALP levels in the blood of *C. gariepinus* exposed to varying aqueous extract concentrations (Table 5).

Histopathological Analysis

Histopathological Analysis photomicrographs (x400) of *C. gariepinus* Exposed to Ethanoic Extract of *Azadiractha indica* (Liver Slides)

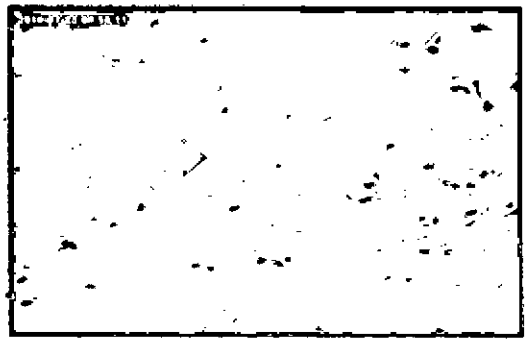


Liver A (Control)



Liver B (8 g of Ethanoic Extract)

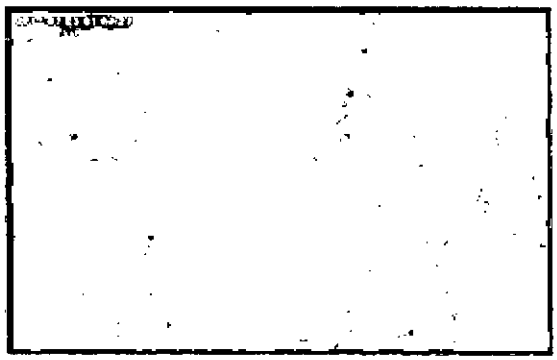
Control (A) shows hepatocytes (red arrow) surrounding a central vein (blue rrow).
B shows no pathologic changes.



Liver C (7.5 g of Ethanoic Extract)

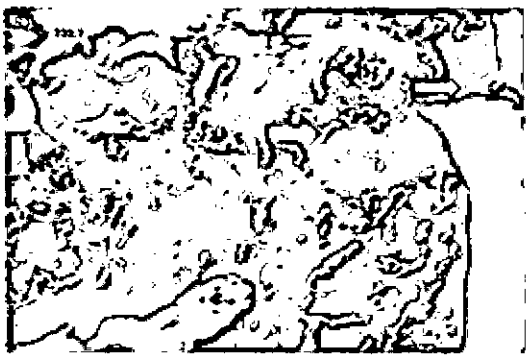
Liver D (5.5 g of Ethanoic Extract)

Hepatocytes showing no pathologic changes observed between Liver C and D



Liver E (5 g of Ethanoic Extract)
No pathologic changes seen in E

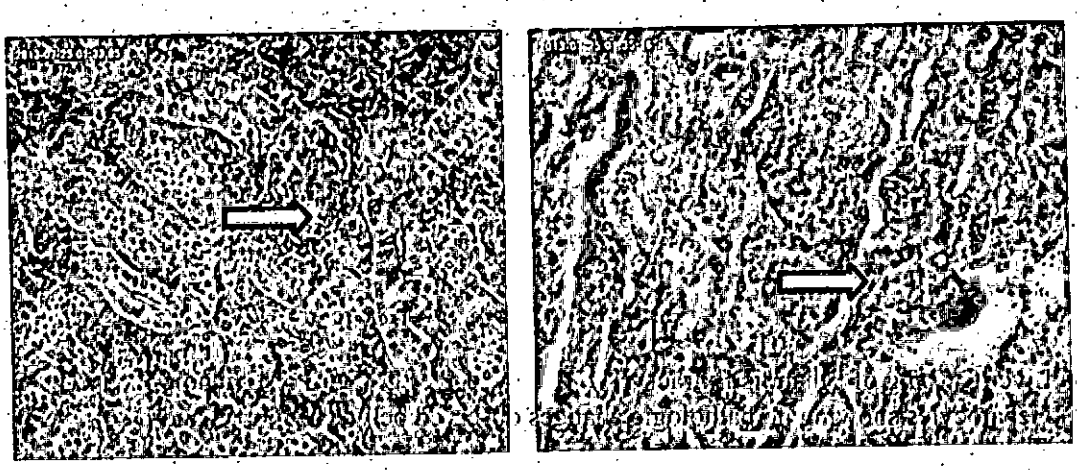
Histopathological Analysis photomicrographs (x400) of *C. gariepinus* Exposed to Ethanoic Extract of *Azadiractha indica* (Kidney Slides)



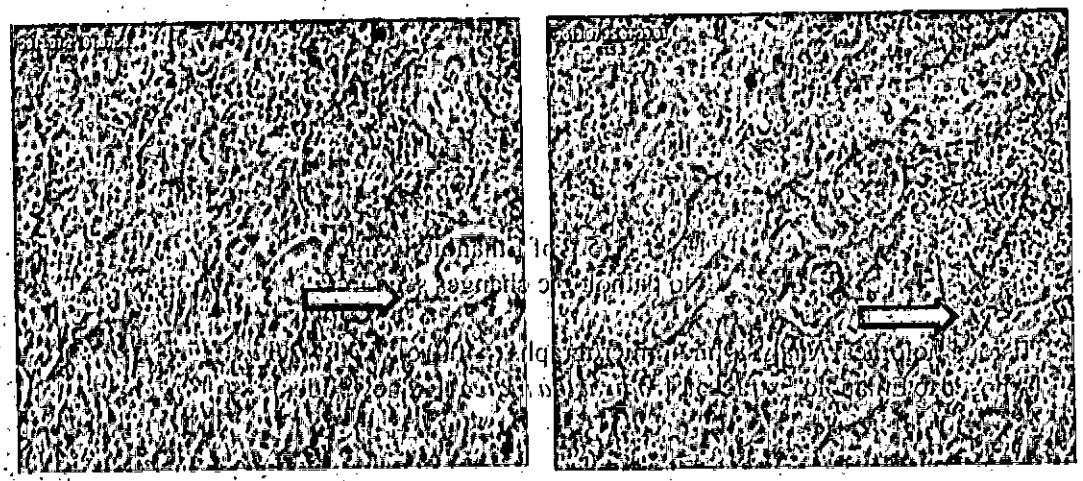
Kidney A (Control)

Kidney B (8 g of Ethanoic extract)

Blue arrow shows glomerulus. The red arrow shows tubules.
A is control. No pathologic changes seen in B

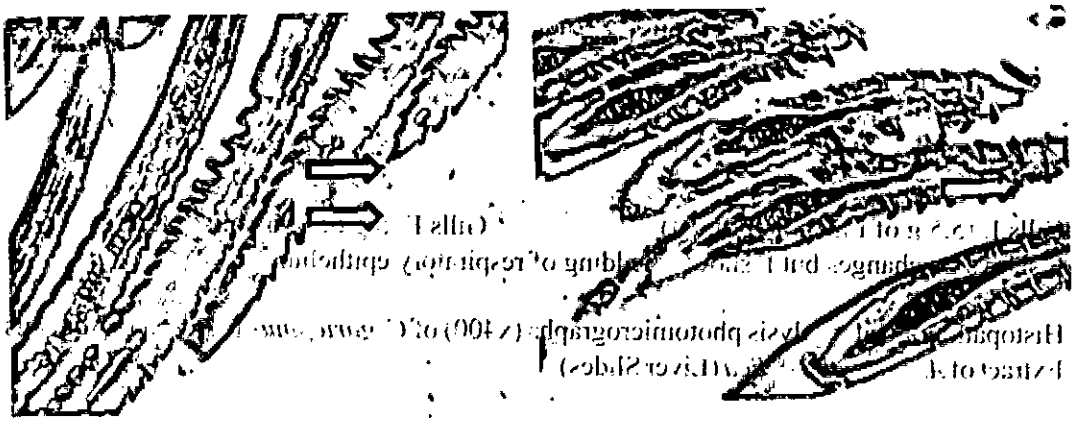


Kidney C (7.5 g of Ethanoic extract) Kidney D (6 g of Ethanoic extract)
Blue arrow shows the glomerulus. No pathology seen in C and D.



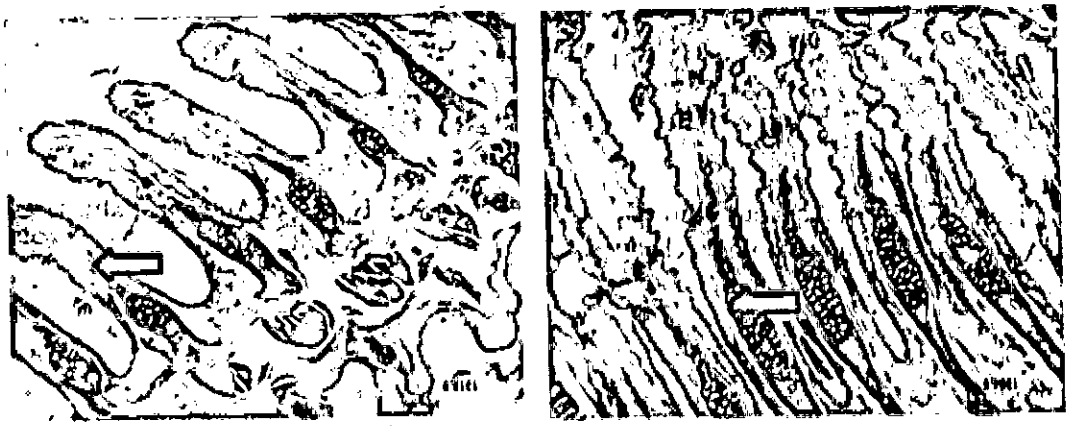
Kidney E (5.5 g of Ethanoic extract) Kidney F (5 g of Ethanoic extract)
No pathology changes observed in E and F.

Histopathological Analysis photomicrographs (x400) of *C. gariepinus* Exposed to Ethanoic Extract of *Azadiractha indica* (Gills Slides)



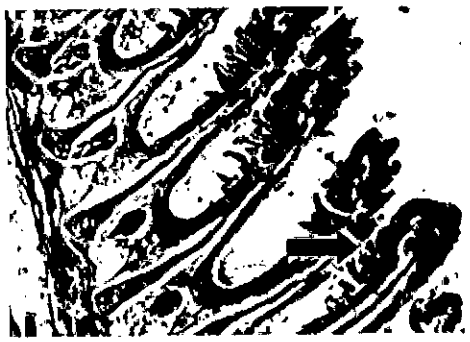
Gills A (Control) Gills B (8 g of Ethanoic Extract)

Blue arrows show respiratory epithelium. Red arrows show cartilage.



Gills C (7.5 g of Ethanoic Extract) Gills D (6 g of Ethanoic Extract)

No pathology seen in C and D

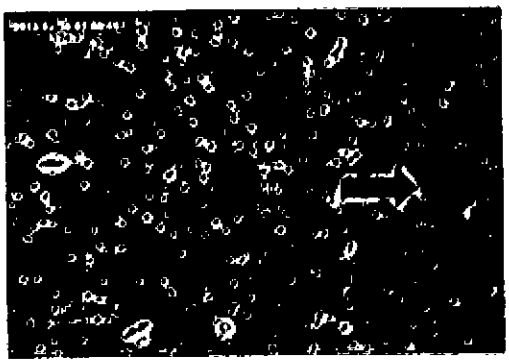


Gills E (5.5 g of Ethanoic Extract)

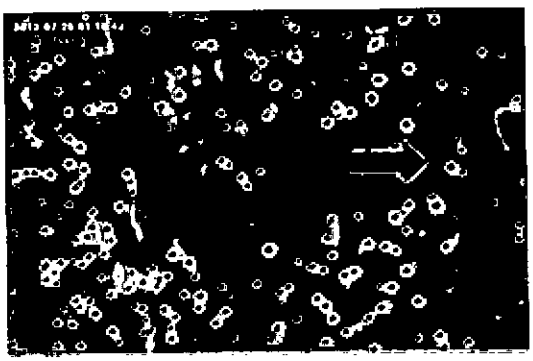
Gills F (5 g of Ethanoic Extract)

E shows no changes but F shows shedding of respiratory epithelium.

Histopathological Analysis photomicrographs (x400) of *C. gariepinus* Exposed to Aqueous Extract of *Azadiractha indica* (Liver Slides)

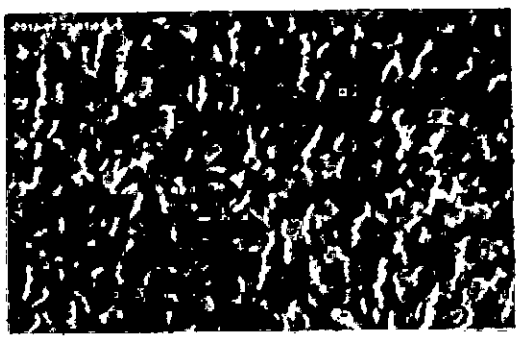


Liver A_1 (5.5 g of Aqueous Extract)

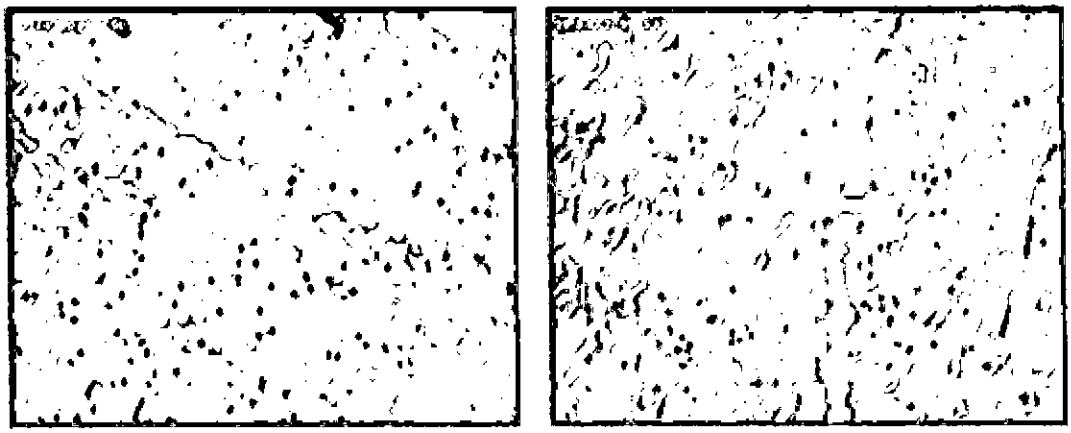


Liver B_1 (5 g of Aqueous Extract)

A_1 and B_1 show no pathologic changes.

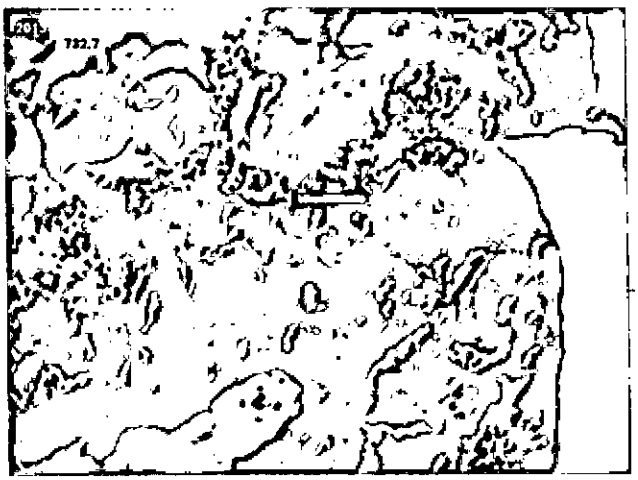


Liver C_1 (6 g of Aqueous Extract)

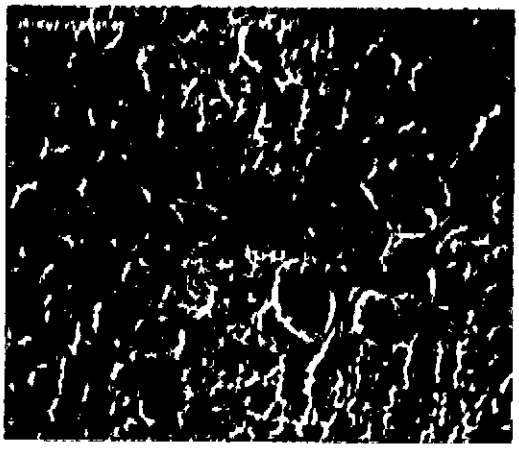


Liver D_1 (7.5 g of Aqueous Extract) Liver E_1 (8 g of Aqueous Extract)
C_1 shows vacuoles within the hepatocytes. E_1 shows areas of necrosis.

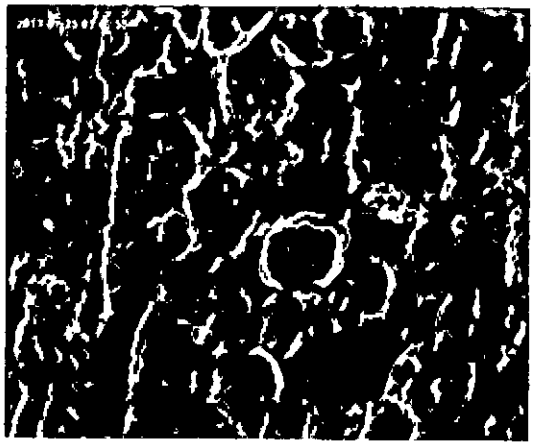
Histopathological Analysis photomicrographs (x400) of *C. gariepinus* Exposed to Aqueous Extract of *Azadiractha indica* (Kidney Slides)



Kidney A_1 (Control)
Blue arrow shows glomerulus. The red arrow shows tubules. A_1 is control.

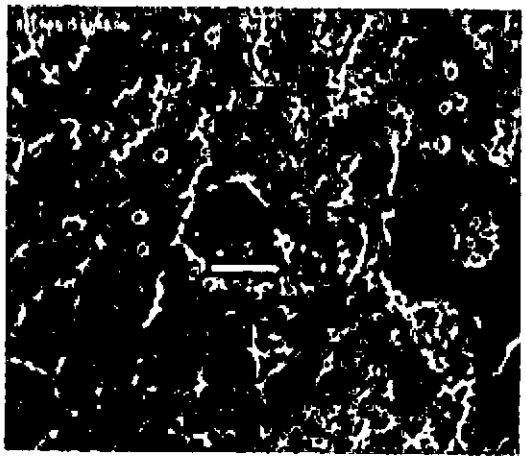


Kidney B_1 (7.5 g of Aqueous extract)

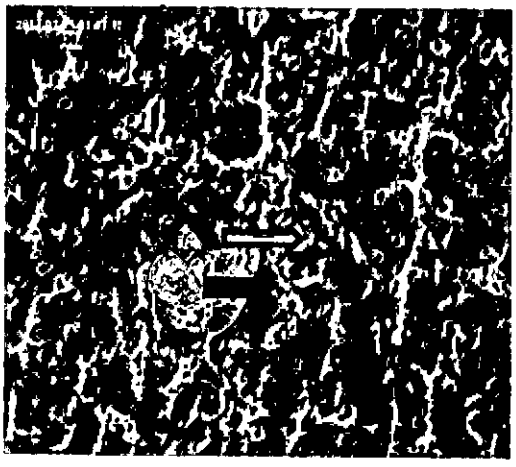


Kidney C_1 (5 g of Aqueous extract)

B_1 shows dense inflammatory infiltrate within the interstitium. No pathology seen in C_1.



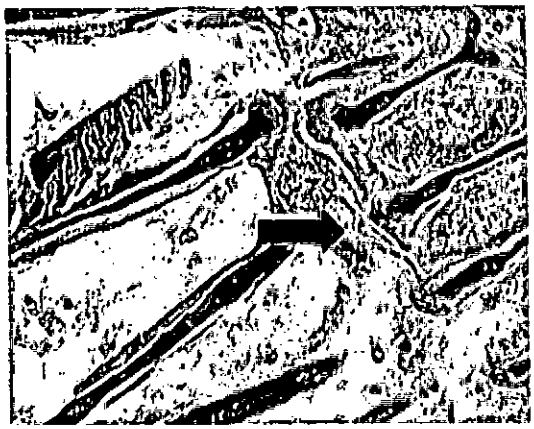
Kidney D_1 (6 g of Aqueous extract)

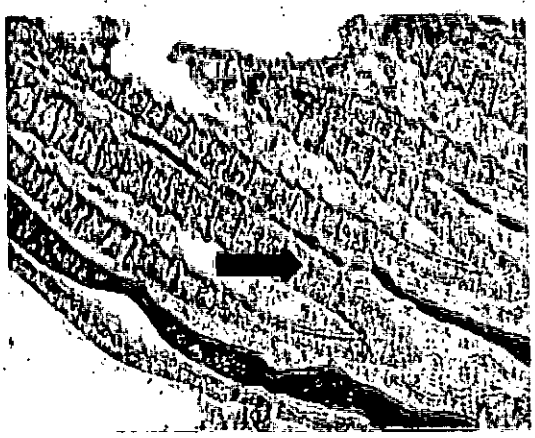


Kidney E_1 (8 g of Aqueous extract)

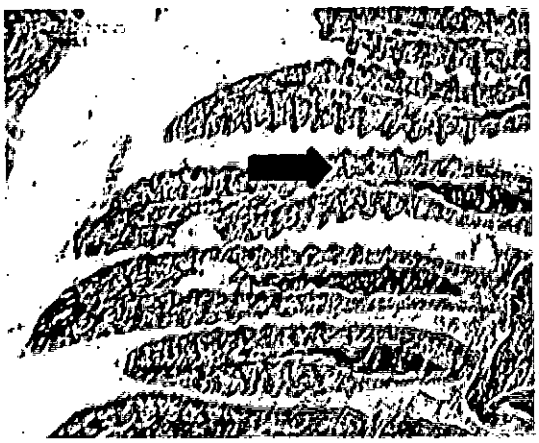
D_1 shows inflammatory infiltrate within the interstitium (red arrow). E_1 shows tubular necrosis (red arrow). The blue arrow indicates the glomerulus.

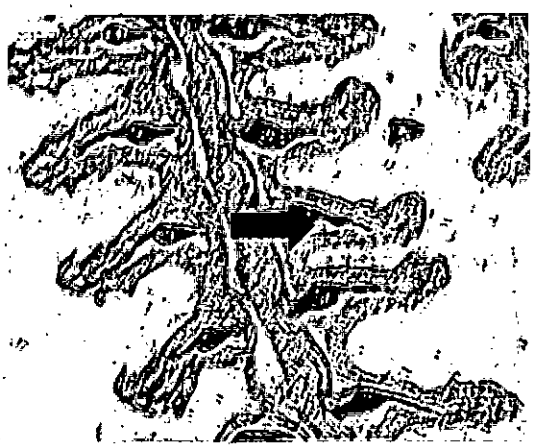
Histopathological Analysis photomicrographs (x400) of *C. gariepinus* Exposed to Aqueous Extract of *Azadiractha indica* (Gills Slides)





Gills A_1 (7.5 g of Aqueous extract) Gills B_1 (5.5 g of Aqueous extract)
A_1 shows no changes. B_1 shows destruction of respiratory epithelium.





Gills C_1 (5 g of Aqueous extract) Gills D_1 (6 g of Aqueous extract)
C_1 shows no changes. D_1 shows attenuation of the respiratory epithelium.

DISCUSSION

This present study showed that the ethanoic and aqueous extract of *Azadiractha indica* has some variable result outcome on mortality and histopathology. The results of the acute toxicity of ethanoic and aqueous extract on *C. gariepinus* showed that the concentration that affected 50% mortality (LC_{50}) at 96 hrs were 9.93 mL/L and 9.87 mL/L for ethanoic and aqueous extract. This shows that the ethanoic extract is more toxic

than the aqueous extract. This may be due to the extraction process, because, for the aqueous extraction, the grounded leaves was simply soaked in water and filtered and by so doing the alkaloids, though extracted, had been diluted with water, thereby reducing the potency of the alkaloids. But for the ethanoic extract the soxhlet extractor extracted only the alkaloids almost undiluted using ethanol as extraction medium (Ayoola *et al.*, 2011). Analysis of Variance (ANOVA) showed that there was significant difference ($P<0.05$) in the quantal response at 24, 48, 72, 96 hrs of exposure.

Physico-chemical parameters of the test media for the aqueous and ethanoics extracts are within the normal range for survival of aquatic organism most especially fish.This in accordance with the findings of Ayoola (2011).

Aspartate Aminotransaminase (AST), Alanine Aminotransaminase (ALT) and Alkaline Phosphatase (ALP) in the blood of *C. gariepinus* exposed to varying concentrations of Ethanoic and Aqueous extract showed a significant difference ($P < 0.05$). This is similar to the finding of Ayoola (2011). Enzymes such as phosphatase, dehydrogenase and transferase are often found in appreciable quantities in the serum, though they are not of extracellular fluid origin, hence serum enzyme measurement provides a valuable tool in clinical diagnosis because it provides information on the effect and nature of pathological damage to the tissue. This is similar to the finding of Ashafa *et al.* (2010).

The findings from the exposure activities of *C. gariepinus* to varying concentrations of Ethanoic and Aqueous extract *Azadiractha indica* was in accordance to the findings of Luskova *et al.* (2002) in *Cyprinus carpio* exposed to diazinon but contradicts that of Tiwari and Singh (2004) in *Channa punctatus* treated with sub lethal levels of alcoholic extracts of *Nerium indicum* of ALT and AST in the exposed fish corroborates .ALT and AST are non plasma specific enzymes that are localized in tissues cells of liver, heart, gills, kidneys, muscles and other organs and their presence in the blood (plasma) may give specific information about organ dysfunction (Wells, 1986; Gabriel and George, 2005). A decrease in the transaminases suggests that there was no tissue damage. This is similar to the finding of Ayalogu *et al.* (2001) and Luskova *et al.* (2002). The decline in ALP in exposed fish in this study may be due to the fall in the rate of synthesis of glycogen resulting from the low metabolic demands. This is in accordance with the finding of Shaffi (1979) and a decrease in metabolic transport. This is similar to the finding of Begum (2004) and Edquist *et al.* (1992). The decrease may also indicate that there was no kidney damage but a reduction in the hydrolytic action on a number of phosphor monoesters of organic origin such as glucose (Edquist *et al.* 1992). A reduction in the concentration of LDH in the plasma of the experimental fish infers a decrease in the glycolytic process due to lower metabolic rate. This is in accordance to the finding of Luskova *et al.* (2002), a shift towards anaerobic respiration (Tiwari and Singh, 2004), possibly due to a hypoxic internal environment.

From this study the optimal significant dose of 7.5 g of both ethanoic and aqueous extract is of most benefit to the fish due to the fact that it was able to reduce the leakage of the

liver enzyme into the serum thus helping to maintain the integrity of the hepatic cellular membrane.

Elevated levels of AST, ALP and ALT lower than 7.5 g for both ethanoic extract and aqueous extract observed could have occurred as a result of tissue damage or disrupted cell membranes that lead to the leakages of such enzymes from the tissue into the serum. This is in accordance to the finding of Morrone *et al.* (2009).

This alteration in the activity of AST, ALP and ALT at varying doses and extract could suggest inhibition, inactivation or activation of the enzyme molecule. (Akanji *et al.,* 2008). The similar elevated levels of ALP observed in these groups, could constitute a threat to the cells since the cells might be deprived of much needed energy as a result of indiscriminate hydrolysis of the phosphate ester. This is in accordance with the finding of Akanji *et al.* (2008). Albumin is one of several proteins made in the liver. These proteins are required to fight infections and to perform other functions. Lower than normal levels of albumin and total protein, may indicate liver damage or disease.

Elevated levels of serum protein such as Albumin and Globulin are good criteria for assessing the secretory function and capacity of the liver (Naganna *et al.,* 1989). The significant effect of the extract on Albumin in the serum at 8.0g dose for both extract could imply that the synthetic and secretory functions of the liver with respect to Albumin were not affected.

Bilirubin is a substance produced during the normal breakdown of red blood cells. Bilirubin passes through the liver and is excreted. Elevated levels of bilirubin (jaundice) may indicate liver damage or disease. This study shows that high concentration of ethanoic extract (8.0 g) and low dose of aqueous extract (5.0 g) induced some liver damages at the biliary sites. This is in accordance with the findings of Anofi *et al.* (2012).

In the present study, the two extract at various concentration seems to increase TG and HDL levels whereas it reduced the levels at 5.0 g of the aqueous extract, while the increase in HDL may be beneficial since the rate at which plasma cholesterol are carried to the liver and could also be increased. It could be hypothesize that both extract could regulate hepatic metabolism of lipids and could attenuate lipid abnormalities.

Increases in total cholesterol and LDL fraction are factors associated with the higher risk of atherosclerosis and coronary disease, while the increase in HDL is a protective factor.

The enhanced level of cholesterol (Hypercholesterolemia) may suggest the presence of a cardiovascular risk. Plasma cholesterol concentration elevation is therefore one of the important Chronic Renal Failure (CRFs) as its transportation within the lipoprotein is affected and is strongly associated with progression of atherosclerosis. This is similar to the work of Ogbonnia (2011). Hypercholesterolemia especially in the presence of increased free radical generation is atherogenic and maybe associated with increased

circulating immune cells. This is supported by studies carried out in 2003 by Panagiotakas *et al.,* they reported that these alterations were proven by an increase in the computed atherogenic index, a useful indicator of cardiovascular diseases.

The liver, kidney and gills from all the test concentration were carefully observed and compared with that of the control. The sub-lethal concentrations which the test organisms were subjected too are 5, 5.5,6, 7.5 and 8 g/L.

Liver shows hepatocytes and central vein at all concentration of the ethanoic extract of *Azadiractha indica* and no pathology changes at concentration of 5,5.5,6 but at 7.5 and 8 g/L there were vacuoles with the hepatocyte and areas of necrosis of the Aqueous extract of *Azadiractha indica.*

For the kidney, there was dense to mild inflammatory infiltration within the interstitial of the kidney exposed to 7.5 and 8 g/L of both Ethanoic and Aqueous extract of *Azadiractha indica* and no changes compared to control at 5,5.5,6 g/L.

The gills at 7.5 and 8 g/L of both ethanoic and aqueous extract of *Azadiractha indica* showed shedding of respiratory epithelium, destruction of respiratory epithelium and attenuation of the respiratory epithelium.

Generally cells died as a result of necrosis or apoptosis when they are challenged with toxins, noxious agent or injuries. This is similar with the findings of Eroschenko (2000). Toxic agents can cause all these changes observed in the liver of the test group, which means that the active constituent in the Neem leaves could cause damages to the liver, gills and kidney at high doses.

The results from the analysis carried out from this research work shows that sub-lethal concentration of *Azadiractha indica* doesn't lead to outright mortality but there were some side effects on the liver, kidney and gills of exposed organisms also in the blood of these organism which supports the findings of Olufayo and Fagbenro (2007), stating that sub-lethal concentrations of toxicants in the aquatic environment will not necessarily result in outright mortality of aquatic organisms but they have significant effects which can result in several physiological changes in the fish, but at a very low concentration, they (*Azadiractha indica*) can be used as an anti-stress, due to the result gotten, at lower concentration there was no mortality, no histopathological effects and the result from the bio-chemical analysis (AST,ALT,ALP,TP), were favourable to the organisms (*C. gariepinus*). Hence, *Azadiractha indica* could be employed as an Anti-stress agent but at a low concentration.

CONCLUSION

In conclusion, the observed changes during toxicity test of exposed fish showed that aqueous and ethanoic extracts of *Azadiractha indica* are both toxic to *C. gariepinus* at higher concentration of both extract.

The health hazard of *A. indica* plant leaf extract to aquatic organisms particularly in *C. gariepinus* has not been studied in detail. The findings of the present study showed that Neem leaf extracts (6g-8 g/5L) affects the histopathological and biochemical parameters of *C. gariepinus* even during a long-term exposure (28 days). These parameters could be effectively used as potential biomarkers of Neem leaf extracts toxicity to the freshwater fish in the field of environmental biomonitoring, therefore, both extracts can be exploited to either obtain fishes for human consumption and or eradicate unwanted fishes from water bodies. The observed LC_{50} value and altered parameters may help to establish the safer level of the aqueous extracts of *A. indica* to the aquatic environment and aquaculture farms.

Furthermore, studies on these parameters investigated showed that, there are possibilities of *Azadiractha indica.* (Neem leaf) serving as an anti- stress agent for the aqueous and ethanoic extract, this must be at a very low concentrations of 2-3 g/5L of water based on the findings of the analyzed results gotten which does not show a negative effects from the histopathological and biochemical parameters of *C. gariepinus* even during a long-term exposure (28 days).

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