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Research Article

Toxic Effects of Aqueous Extract of Two Medicinal Plants, *Mansonia altissima* and *Sarcocephalus nervosus* on Biochemical Indices of *Clarias gariepinus* (Burchell 1822)

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ABSTRACT

The acute and sub-lethal biochemical responses of *Clarias gariepinus* to aqueous extracts of *Sarcocephalus nervosus* (AESN) and *Mansonia altissima* (AEMA) were assessed through static renewal laboratory bioassays. The fish were exposed to sub-lethal doses (1/10th, 1/50th, 1/100th of 96h LC_{50}) of AESN and AEMA for 21 days. The LC_{50} values for AESN were 30.71, 23.00, 15.81, and 13.36 g/L at 24, 48, 72, and 96 hours, respectively. No mortality occurred at 100 g/L of AEMA over 96 hours. ANOVA revealed significant differences (p<0.05) in mortality and biochemical responses; tissue levels of protein, cholesterol, triglycerides, and glucose, which ranged from 5.28 to 41.80 mg/dL, 18.46 to 89.35 mg/dL, 18.46 to 117.03 mg/dL, and 27.90 to 63.01 mg/dL, respectively. The study concluded that AESN and AEMA induce stress and significantly affect liver function, energy demand, and lipid metabolism in *C. gariepinus*.

Keywords: Acute toxicity, Aqueous extracts, Biochemical, Blood, Fish, Medicinal plants, Sub-lethal
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INTRODUCTION

The economic value of plants cannot be overemphasized as all the body components serve useful purposes such as fuel, energy, income generation (including foreign exchange), medicine, cosmetics, pesticides and horticulture (Fafioye, 2001). Many plants have medicinal properties which have been used as base chemicals in the pharmaceutical industries (Tyler, 1986; Singh *et. al*., 2014). In developing and non-industrialized countries, plant infusions are commonly used to treat a wide range of diseases (Teixeira *et al.*, 2003). In Congo, the bark-decoction of *Lamnea welwitschii*, with active ingredients of saponins and tannins is taken by women for menstrual pains and sterility to treat dysentery, diarrhea, urethral discharge and haemorrhoid (Burkill, 1985). Also in Nigeria, processed bark of *Khaya gradifoliolia* is used to treat typhoid fever and the seeds of *Picralina* sp. which contain crystalline alkaloids that are used in place of quinine for the treatment of malaria fever (Chukwu & Okeowo, 2006). Virtually all the drugs synthesized today in pharmaceutical laboratories were first obtained from plants (Balandrin et al., 1985).

The processed bark of *Sarcocephalus nervosus* commonly known as Opepe ira in Nigeria is used to treat typhoid fever. *Mansonia altissima* (African black walnut, also called Ofun by Yorubas in Nigeria) is a popular West African timber tree; it has been used in Ivory Coast as an arrow poison and in the treatment of leprosy as well as an aphrodisiac in man (Ogbamgba & Wekhel, 2005).

In Nigeria, trees /plants used for medicinal purposes, furniture and energy are transported via water courses of Lagos Lagoon from Niger Delta to Okobaba sawmill at Ebute Metta, Lagos. According to Omitoyin *et al.* (1999), transportation of woods/trees is done by tying the logs together in batches and on arrival at the mill they are left in the water or an area of the water called the log pond until needed. The floating logs exude substances that are capable of creating pollution problems. These substances contain poisonous alkaloids that are presumed to protect the

plants against various insects, bacteria and epiphytes. The substances are directly or indirectly toxic to both pelagic and benthic organisms at high concentrations when they enter the water body (Olaifa et al., 1987).

Some of the secondary metabolites in the crude or refined form of medicinal plants may be toxic to lower beings or man, or indeed both (Satoh et al., *2001*). The present study investigates the acute toxicity and sublethal effects of AESN and AEMA on biochemical parameters (tissue protein, triglyceride, glucose and cholesterol) of *Clarias gariepinus*.

Clarias gariepinus (Family Claridae), *Clarias sp.* mostly inhabit fresh water and are widely distributed in Africa and Asia Minor. *Clarias gariepinus* is a common Nigeria catfish that is available all year round in pool and water logged marshy areas. The fish can survive for a long time in laboratory aquaria.

MATERIALS AND METHODS

Test Organisms

Clarias gariepinus (catfish) fingerlings with a total length of 7 - 10cm were obtained from Agboola fish farm at Ayobo- Ipaja in Lagos, Nigeria and transported in an oxygenated polythene bag to the Aquatic Toxicology and Eco-physiology Laboratory, Department of Marine Sciences, University of Lagos. They were acclimated for 14 days in a rectangular glass tank (70 x 95 x 70 cm). The tank was filled to quarter capacities with dechlorinated tap water. This tap water had been allowed to stand for 24 hours for dechlorination before the introduction of the fish and aerated using a Ferrari aquarium air pump (CTB-608). During the period of acclimatization, the animals were fed on Coppens® feed. The dechlorinated tap water in the holding tank was changed every other day to prevent pollution by fish exude and food remnants.

Test Compounds

The test compounds are the aqueous bark extracts of *Mansonia altissima* and *Sarcocephalus nervosus*. The bark of the *M. altissima* and *S. nervosus* were obtained at Okobaba saw-mill, Ebute-Metta, Lagos, Nigeria. They were sun-dried for 7 days and milled into powder form using a grinding machine. Extraction of the aqueous form from the *S. nervosus* and *M. altissima* was done separately.

Physico-Chemical Parameters of the Test Media

Physico-chemical parameters such as dissolved oxygen, pH and temperature of the test media were measured during the experimental period for the various bioassays. The pH and temperature were measured using the Hanna instrument (HI 991301). The Dissolved Oxygen (DO) was determined using a Jenway D O meter (Model, 9071).

Aqueous Extracts Preparation

100g of each powdered bark extract were separately soaked in 1 litre of dechlorinated water for 2 days (48hrs) to ferment. After the 2 days of soaking, the solutions were filtered using a muslin cloth to separate the aqueous extract from the residue. The aqueous solution was then kept in a plastic container at room temperature ready to be used. The prepared aqueous extracts were used for acute and sub-lethal toxicity tests.

Acute Toxicity Bioassay

A static bioassay procedure was adopted for all the toxicity tests. Depending on the test concentrations, a given volume of dechlorinated tap water was measured into a bioassay glass tank (22 x 15x 18 cm) and a predetermined volume of aqueous extract (of *S. nervosus* and *M. altissima*) was added into the water to make it up to 2000ml (total volume of test media) to achieve the desired test concentration. Ten active fishes were introduced into the test medium containing either aqueous *S. nervosus* (AESN) or *M. altissima* (AEMA). Each treatment was replicated thrice, given a total of 30 fish per treatment, including untreated media (control). The concentrations of test compound tested were as follows: AESN: 10, 22, 24, 26, 28 and 0 g/L (control) and AEMA: 80, 85, 90, 95, 100 and 0 g/L (control).

Assessment of Quantal Response (Mortality)

Mortality assessment was carried out every 24 hours over a 96 hours experimental period. Fish were assumed to be dead when there was no body or operculum movement, even when prodded with a probe. Dead animals were removed and recorded immediately observed.

Sub-Lethal (Biochemical Bioassay) Analysis

Three replicates per tank of different concentrations of both the test media (seven tanks include control) were taken to the laboratory randomly on 4, 7, 14 and 21 days for biochemical analysis of the exposed organisms. In this series of experiments, *C. gariepinus* was exposed to sub-lethal concentrations

(1/10th, 1/50th and 1/100th of 96hLC_{50}) of AESN and AEMA as follow: AESN: 1.23, 0.25, 0.12 and 0 g/L (control) and AEMA: 10.00, 2.00, 1.00 and 0 g/L (control).

A total of 40 test animals were exposed per sub-lethal concentration including control. A drastic change in concentration of the test media via evaporation and reduction in dissolved oxygen levels was avoided by changing the test media to the same concentration every 48 hours. The test animals were weighed to determine their individual weight.

Aliquot fish samples (0.5g of tissue) of the test organisms in different concentrations of different test media were collected and then homogenized using a homogenizer. Appropriate kits and standard methods as described by Barham and Trinder (1972) were employed in the determination of total protein, cholesterol, glucose and triglyceride in the tissue of *C. gariepinus* exposed to sub-lethal concentration of the test media for 21 days.

Statistical Analysis

The quantal response (dose-mortality response) of the 96h toxicity tests were analysed after Finney (1971). The indices of toxicity measurement derived from the analysis were; .LC_{50} (the concentration that kills 50% of the population), and TF (Toxicity factor for relative potency measurements of the test chemical based on their 96hLC_{50}).

$$\mathrm{T.F} = \frac{\text{LC50 of test compound at 24hrs}}{\text{LC50 of test compound at other hours } (24, 48, 72 \text{ and } 96\text{hrs})}$$

One-way analysis of Variance (ANOVA) and student Newman Keul's (SNK) tests were used to test for significant differences (5% level) in the mean mortality response of *C. gariepinus* to different concentrations of AESN at 24, 48, 72 and 96 hrs of exposure. All analysis was carried out using SPSS 10.0 for Windows. This analysis was not run for AEMA, this is because no mortality was recorded- during the acute test even at 100 g/L of the aqueous concentration.

RESULTS

Physico-Chemical Characteristics of the Test Media

The mean values obtained for the physico-chemical parameters of the test media throughout the period of the experiment were pH (7.42), Total Dissolved Solid (4.38mg/L), Dissolved Oxygen (7.40 mg/L), Salinity (0.01 ‰) and Temperature (25.5^0C).

Acute Toxicity of AESN against *Clarias gariepinus*

The result of acute toxicity test based on quantal response measurements of AESN against *Clarias gariepinus* fingerlings at 24, 48, 72 and 96 hours of exposure is shown in Table I. The concentration of aqueous extract that caused 50% mortality of *C. gariepinus* (LC_{50}) at 24, 48, 72 and 96 hrs of exposure were 30.71, 23.00, 15.81, and 13.36 g/L respectively.

The median lethal concentration of aqueous extract against *C. gariepinus* decreased as the duration of exposure increased (Table 1). Analysis of variance (ANOVA) computed for the acute test showed that there was a significant difference ($p < 0.05$) in the quantal response of test organisms to different concentrations of AESN at 24, 48, 72 and 96 hrs of exposure. Further analysis using the Student Newmans Keul (SNK) test at 5% significant level revealed that the mortality response at 10, 22, 24, 26 and 28 g/L were significantly different ($p < 0.05$) from the control (0 g/L) at 48, 72 and 96 hrs of exposure to AESN (Table 2).

Biochemical Effects of AESN on *C. gariepinus*

The result of the biochemical changes in tissue cholesterol, protein, glucose and triglyceride of *C. gariepinus* exposed to sub-lethal concentrations of AESN for 21 days are presented in Table 3. The cholesterol content in the tissue of the test organism exposed to AESN ranged from 18.46 to 88.33mg/dl. The lowest value of cholesterol, 18.46 mg/dl was recorded on day 14 in organisms exposed to 0.25 g/L while the highest value, 88.33 mg/dl was recorded on day 4 in organisms exposed to 0.25 g/L (Table 3). Analysis of variance (ANOVA) revealed that there was a significant difference ($P<0.05$) in the mean tissue cholesterol of *C. gariepinus* exposed to different concentrations of AESN at day 7 and 21, while there was no significant difference ($P>0.05$) in the mean tissue cholesterol of *C. gariepinus* exposed to AESN at day 4 and 14 (Table 3). Further analysis using DMRT showed that there was no significant difference ($P>0.05$) in the mean tissue cholesterol of *C. gariepinus* exposed to 0.00 g/L and 0.25 g/L of AESN at day 14 (Table 3).

The mean glucose level in the tissue of *C. gariepinus* exposed to AESN ranged from 27.90 to 48.59 mg/dl, with the lowest value of 27.90 mg/dl which was recorded at day 21 in organisms exposed to

Table 1: Relative acute toxicity of AESN against *Clarias gariepinus*

Exposure Time	LC50 (95%CL) g/l	Slope ± S.E	D.F	Prohibit line Equation	T.F
24h	30.71 (35.25 - 29.89)	5.05±1.48	3	Y= -2.54 + 5.07x	1.00
48h	23.00 (28.33 - 19.33)	2.96±0.74	3	Y= -1.00+ 2.94x	1.34
72h	15.81 (18.69 - 11.48)	3.05±0.68	3	Y= -1.34+3.05x	1.94
96h	13.36 (15.79 - 10.01)	3.66±0.70	3	Y= -0.88+3.66x	2.30

L.C. = Lethal concentration, D.F. = Degree of freedom, T.F=Toxicity factor

Table 2: Percentage mortality of *C. gariepinus* exposed to different concentrations of AESN

Concentration (g/L)	Number of Organisms	Percentage mortality/time			
		24h	48h	72h	96h
Control	30	0.00^{A}	0.00^{A}	0.00^{A}	0.00^{A}
10	30	3.33^{A}	16.67^{B}	30.00^{B}	40.00^{B}
22	30	10.00^{B}	36.67^{B}	56.67^{C}	76.67^{C}
24	30	20.00^{C}	50.00^{D}	66.67^{D}	80.00^{C}
26	30	36.67^{D}	60.00^{C}	76.67^{C}	83.33^{C}
28	30	60.00^{C}	66.67^{C}	86.67^{E}	93.33^{CD}

Mean values on the same column with different subscript letters are significantly different in the SNK test (p<0.05).

Table 3: Mean biochemical response in the tissue of *C. gariepinus* exposed to sub-lethal concentration of AESN for 21 days.

Concentration (g/L)	Biochemical response (mg/dl) during treatment (Days)			
	4	7	14	21
		Cholesterol		
Control	$50.45±1.32^{A}$	$23.28±0.60^{AB}$	$23.95±4.24^{A}$	$29.06±0.39^{A}$
0.12	$50.97±0.59^{A}$	$28.46±0.40^{B}$	$25.78±3.49^{A}$	$56.18±5.36^{C}$
0.25	$88.33±17.62^{C}$	$21.89±2.97^{A}$	$18.46±2.25^{A}$	$61.87±3.77^{C}$
1.23	$57.85±3.61^{A}$	$45.57±1.30^{C}$	$20.90±1.53^{A}$	$42.70±3.31^{B}$
		Protein		
Control	$31.40±6.34^{A}$	$17.08±0.44^{C}$	$17.33±2.40^{A}$	$24.35±1.13^{A}$
0.12	$27.93±3.94^{A}$	$7.87±0.81^{A}$	$39.15±2.25^{B}$	$22.66±0.51^{A}$
0.25	$28.60±2.80^{A}$	$5.28±0.69^{A}$	$16.71±0.46^{A}$	$23.28±0.72^{A}$
1.23	$24.00±1.22^{A}$	$11.43±1.53^{B}$	$16.79±0.97^{A}$	$22.83±0.19^{A}$
		Glucose		
Control	$43.78±0.07^{A}$	$34.84±1.47^{A}$	$38.39±2.07^{A}$	$27.90±1.31^{A}$
0.12	$43.05±1.56^{A}$	$37.0±2.00^{A}$	$36.17±1.59^{A}$	$28.05±2.30^{A}$
0.25	$47.97±3.64^{B}$	$33.33±2.69^{A}$	$37.62±1.14^{A}$	$28.89±4.31^{A}$
1.23	$46.71±2.84^{B}$	$48.59±1.00^{B}$	$35.36±0.71^{A}$	$29.20±0.35^{A}$
		Triglyceride		
Control	$50.00±2.39^{A}$	$23.28±0.60^{C}$	$23.95±4.24^{A}$	$29.06±0.39^{A}$
0.12	$50.02±1.57^{C}$	$28.46±0.40^{A}$	$25.78±2.49^{A}$	$56.18±5.36^{A}$
0.25	$52.48±1.28^{C}$	$21.90±2.97^{B}$	$18.46±2.25^{A}$	$61.87±3.77^{A}$
1.23	$51.17±1.97^{A}$	$45.57±1.30^{AB}$	$20.90±1.53^{A}$	$42.70±3.31^{A}$

Means (±S.E, standard error) with the same superscript letter in a column are not significantly different in the DMRT test (p<0.05).

0.00 g/L (control) while the highest value, 48.59 mg/dl was recorded at day 7 in organisms exposed to 1.23 g/L (Table 3). Analysis of variance (ANOVA) showed that there was a significant difference (p<0.05) in the mean tissue glucose of *C. gariepinus* exposed to different concentrations of AESN at day 7. However, there was no significant difference (p<0.05) in the mean tissue glucose of *C. gariepinus* exposed to different concentrations of AESN at days 4, 14 and 21(Table 3).

The mean triglyceride content in the tissue of the test organism exposed to AESN ranged from 18.46 to 61.87 mg/dl (Table 3). The lowest value of 18.46 mg/dl was recorded on day 14 in organisms exposed to 0.25 g/L and the highest value 61.87mg/dl was observed on day 2l in organisms exposed to 0.25 g/L. (Table 3). Analysis of variance (ANOVA) showed that there was a significant difference (p<0.05) in the mean tissue triglyceride of C. *gariepinus* exposed to different Concentrations of AESN at days 4 and 7. However, there was no significant difference (20.05) in the mean tissue triglyceride of *C. gariepinus* exposed to different concentrations of AESN at days 14 and 21. Post hoc analysis using DMRT showed that there was no significant difference (p>0.05) in the mean tissue triglyceride of *C. gariepinus* exposed to 0.00, 0.12, 0.25 and 1.23 g/L at days 14 and 21 (Table 3).

The mean protein content in the tissues of the test organism exposed to AESN ranged from 528 to 39.15 mg/dl (Table 3). The lowest value of protein, 5.28 mg/dl was recorded on the 7th day in organisms exposed to 0.25 g/L and the highest value, 39.15 mg/dl was recorded on 14th day in organisms exposed to 0.12 g/L (Table 3). Analysis of variance (ANOVA) showed that there was no significant difference (p>0.05) in the mean tissue protein content of *C. gariepinus* exposed to different concentrations of AESN at day 21. Further analysis using the Duncan multiple range test (DMRT) showed that there was a significant difference (P<0.05) in the mean tissue protein of *C. gariepinus* exposed to 0.00, 0.25 and 1.23 g/L concentration of AESN at day14 (Table 3)

Biochemical Effects of AEMA on *C. gariepinus*

The result of the biochemical changes in tissue cholesterol, protein, glucose and triglyceride of *C. gariepinus* exposed to sub-lethal concentrations of AEMA for 21 days are presented in Table 4. The cholesterol content in the tissue of the test organism exposed to AEMA ranged from 18.96 to 89.35 mg/dl. The lowest value of cholesterol, 18.92 mg/dl was recorded on day 14 in organisms exposed to 2.00 g/L while the highest value, 89.35 mg/dl was recorded on day 7 in organisms exposed to 2.00g/L (Table 4). Analysis of variance (ANOVA) revealed that there was a significant difference (p<0.05) in the mean tissue cholesterol of *C. gariepinus* exposed to different concentrations of AESN at day 7 and 21, while there was no significant difference (P>0.05) in the mean tissue cholesterol of *C. gariepinus* exposed to different concentration of AEMA at day 7 and 21 (Table 4). Further analysis using DMRT showed that there was no significant difference (P>0.05) in the mean tissue cholesterol of *C. gariepinus* exposed to all concentrations at day 14 (Table 4).

The mean glucose level in the tissue of *C. gariepinus* exposed to AEMA ranged from 27.90 to 63.01 mg/dl, with the lowest value of 27.90 mg/dl which was recorded at day 21 in organisms exposed to 1.00 and 0.00 g/L (control) while the highest value, 63.01 mg/dl was recorded at day 4 in organisms exposed to 1.00 g/L (Table 4). Analysis of variance (ANOVA) showed that there was a significant difference (p<0.05) in the mean tissue glucose of *C. gariepinus* exposed to different concentrations of AEMA at day 4. Further analysis using DMRT showed that there was no significant difference (P>0.05) in the mean tissue glucose of *C. gariepinus* exposed to 0.00, 1.00, 2.00 and 10 g/L of AEMA at days 14 and 21 (Table 4).

The mean triglyceride content in the tissue of the test organism exposed to AEMA ranged from 20.38 to 117.78 mg/dl (Table 4). The lowest value of 20.38 mg/dl was recorded on day 7 in organisms exposed to 1.00 g/L and the highest value of 117.78 mg/dl was observed at day 2l in organisms exposed to 0.00 g/L (control) (Table 4). Analysis of variance (ANOVA) showed that there was a significant difference (p<0.05) in the mean tissue triglyceride of *C. gariepinus* exposed to different Concentrations of AEMA at days 4 and 7. Post hoc analysis using DMRT showed that there was no significant difference (p>0.05) between the mean tissue triglyceride of *C. gariepinus* exposed to 0.00, 1.00, 2.00 and 10.00 g/L at days 14 and 21 (Table 4).

The mean protein value of the test organisms exposed to sub-lethal concentration of AEMA ranged from 6.64 mg/dl to 41.80 mg/dl. The lowest value of 6.64 mg/dl was recorded on day 7 in organisms exposed to 100 g/L and the highest value of 41.80 mg/dl was observed on day 4 in organisms exposed to 200 g/L (Table 4). Analysis of variance (ANOVA) showed that there was a significant difference (p<0.05) in the mean tissue protein of *C. gariepinus* exposed to different concentrations of AEMA on day 7 (Table 4).

Table 4: Mean Biochemical Response in the Tissue of *C. gariepinus* exposed to sub-lethal concentration of AEMA for 21 days.

Concentration (g/L)	Biochemical response (mg/dl) during treatment (Days)			
	4	7	14	21
		Cholesterol		
Control	50.45±1.32^{A}	23.28±0.60^{A}	23.95±4.24^{A}	29.06±0.39^{A}
1.00	50.87±0.69^{A}	8160±1.96^{C}	23.95±1.98^{A}	60.83±2.52^{B}
2.00	73.67±14.48^{A}	89.35±10.53^{C}	18.92±1.70^{A}	36.40±4.81^{A}
10.00	50.58±1.47^{A}	62.49±0.72^{B}	20.29±0.67^{A}	29.51±5.47^{A}
		Protein		
Control	31.40±6.34^{A}	17.08±0.44^{C}	17.33±2.40^{A}	24.35±1.13^{A}
1.00	24.93±1.93^{A}	6.64±0.21^{A}	19.34±1.85^{A}	27.90±0.20^{AB}
2.00	41.80±0.61^{B}	11.55±2.34^{B}	16.42±0.41^{A}	31.10±3.52^{AB}
10.00	31.53±4.84^{AB}	13.51±1.06^{BC}	17.90±0.39^{A}	33.10±3.52^{B}
		Glucose		
Control	43.78±0.07^{A}	34.84±1.47^{A}	38.39±2.07^{A}	27.90±1.31^{A}
1.00	63.01±3.25^{A}	43.63±4.38^{B}	35.65±0.20^{A}	27.90±0.20^{A}
2.00	44.38±0.29^{A}	42.31±0.56^{AB}	39.36±0.61^{A}	31.19±2.99^{A}
10.00	42.78±1.75^{C}	37.67±0.58^{AB}	37.68±2.12^{A}	33.10±3.52^{A}
		Triglyceride		
Control	81.17±3.95^{A}	43.3±5.05^{A}	70.80±2.70^{A}	111.78±19.08^{A}
1.00	77.5±4.64^{A}	20.38±2.49^{A}	84.37±18.48^{A}	114.49±19.91^{A}
2.00	81.45±9.29^{A}	49.78±0.51^{B}	80.04±7.87^{A}	117.03±14.77^{A}
10.00	125.52±10.23^{B}	60.85±2.10^{C}	73.95±4.26^{A}	104.35±5.44^{A}

Means (±S.E, standard error) with the same superscript letter in a column are not significantly different in the DMRT test (p<0.05).

However, there was no significant difference (p>0.05) in the mean tissue protein of *C. gariepinus* exposed to AEMA at days 4, 14 and 21 (Table 4). Post hoc analysis using DMRT showed that there was a significant difference (p<0.05) in the mean tissue protein of *C. gariepinus* exposed to 0.00 and 1.00 g/L of AEMA at day 7 (Table 4).

DISCUSSION

The toxicity effect of aqueous extract of *Sarcocephalus nervosus* (AESN) on *C. gariepinus* for static non-renewal bioassay increased with time of exposure. This can be related to Dahunsi *et al*. (2011), who observed that the longer the period of exposure of an organism to a given toxicant, the lower the quantity of toxicant needed to reach its tolerance levels.

In this study, the acute toxicity level based on the $96hLC_{50}$ value of aqueous extract of *Sarcocephalus nervosus* (AESN) was found to be 13.36g/L when tested against C. *gariepinus*. Analysis of variance (ANOVA) showed that there was a significant difference (P<0.05) in the quantal response of *C. gariepinus* to different concentrations of AESN at 24, 48, 72, and 96 h of exposure, which was in tandem with the findings of Ajima *et al*. (2010), on the Effects of Fish Bean (*Tephrosia vogelii*) leave extract exposed to freshwater Cichlid fish – *Tilapia zilli*. On the basis of the relative sensitivity of test organisms to AESN and AEMA, *C. gariepinus* was highly susceptible to AESN (96hLC50 = 13.36g/L). Based on the derived $96hlC_{50}$ the computed toxicity factor recorded that AESN at 96 hours was 2.30 times higher than 24 hours.

The exposure of *C. gariepinus* to AESN and AEMA at sub-lethal concentrations showed variation in the level of protein, cholesterol, glucose and triglycerides content in the *C. gariepinus* tissue. This can be related to Dahunsi *et al.* (2011) and Thiagarajan *et al.* (2019), who reported that the composition of blood and other tissue of fish may be altered by a wide variety of environmental factors such as disease, stress and gill damage due to mechanical abrasion.

It was also observed that the tissue glucose level of *C. gariepinus* indicated that the glucose level in individual organisms exposed to both AESN and AEMA decreases on further exposure from day 4 to 14. This was in agreement with Lawal *et al.* (2013) as reported in *Poecilia reticulata* exposed to sub-lethal concentration of surfactant and corroborated by Ajani

et al. (2007). The observed decrease in glucose levels in *Clarias gariepinus* after exposure to environmental stressors like AESN and AEMA likely indicates the depletion of the fish's energy reserves and impaired ability to restore them. This is consistent with findings from studies on similar pollutants, where a reduction in glucose levels was associated with stress and energy expenditure needed for repairing damaged cells and maintaining homeostasis under adverse conditions (Owolabi & Abdulkareem, 2021).

In the present study, the level of tissue triglyceride in *C. gariepinus* exposed to AESN and AEMA decreased as the period of exposure increased from days 4 to 21 irrespective of the concentration to which they were exposed. This was also in agreement with the report of Lawal *et al.* (2013) on the toxicity of two household liquid soaps in *Poecilia reticulata*. This was also corroborated by Samuel and Ojikutu (2020).

The cholesterol value in the tissue of *C. gariepinus* exposed to AESN and AEMA decreased as the time of exposure increased from days 4 to 21. The reduction could be due to the high energy demanded to be able to cope with the toxicant (Banaee *et al.*, 2019). The reduction in the level of cholesterol could also be due to tissue damage in the internal organs (Heydarnejad, 2013). Similarly, the progressive reduction observed in the cholesterol value could also indicate the deficiency of hepatic metabolites compounds as well as abnormal physiological function of the metabolism (Omitoyin, 2007)

The tissue protein of the exposed test organisms recorded in this study showed that individuals exposed to AESN and AEMA showed an increase in protein content on day 14 of 0.12 g/L and an increase in protein content on day 4 of 0.25 g/L concentration respectively. According to Tiwari and Singh (2006), an increase in protein level may lead to increased osmotic pressure and osmolarity of the plasma in the cellular compartment. This implied that the exposed test organisms at this period of exposure and in this concentration experienced a higher toxicity effect than others. The tissue protein values of the test organisms decreased in content on further exposure to the varying concentrations when exposed to AESN and AEMA for 4 days (0.25 g/L) and 4 days (2 g/L) respectively.

The observed increase and decrease could be a result of the sub-lethal concentration to which the test organism was exposed. This was in agreement with the description of Neelima *et al.* (2017) on the quantity of protein degradation or its rate of synthesis. Also, increase in the protein level of the organism under the influence of xenobiotics has been reported to be a result of protein in the body tissue of *C. gariepinus* exposed to different concentrations of AESN and AEMA may be a result of the low production of protein due to the high protein utilization in glucose formation to overcome stress induced by the toxicant (Lawal *et al.*, 2013). However, analysis of variance (ANOVA) showed a significant difference ($p<0.05$) for day 14 and no significant difference for day 21 for AESN and a significant difference for day 4 and no significant difference for day 21 for AEMA.

The cholesterol content in the tissues of *C. gariepinus* decreased with further exposure to 0.25 g/L of AESN from day 4 to 17 and 2 g/L of AEMA from day 7 to 14, aligning with the findings of Samuel and Ojikutu (2020). Based on the present study's data, it can be concluded that medicinal plants have both lethal and sub-lethal effects on *C. gariepinus*.

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