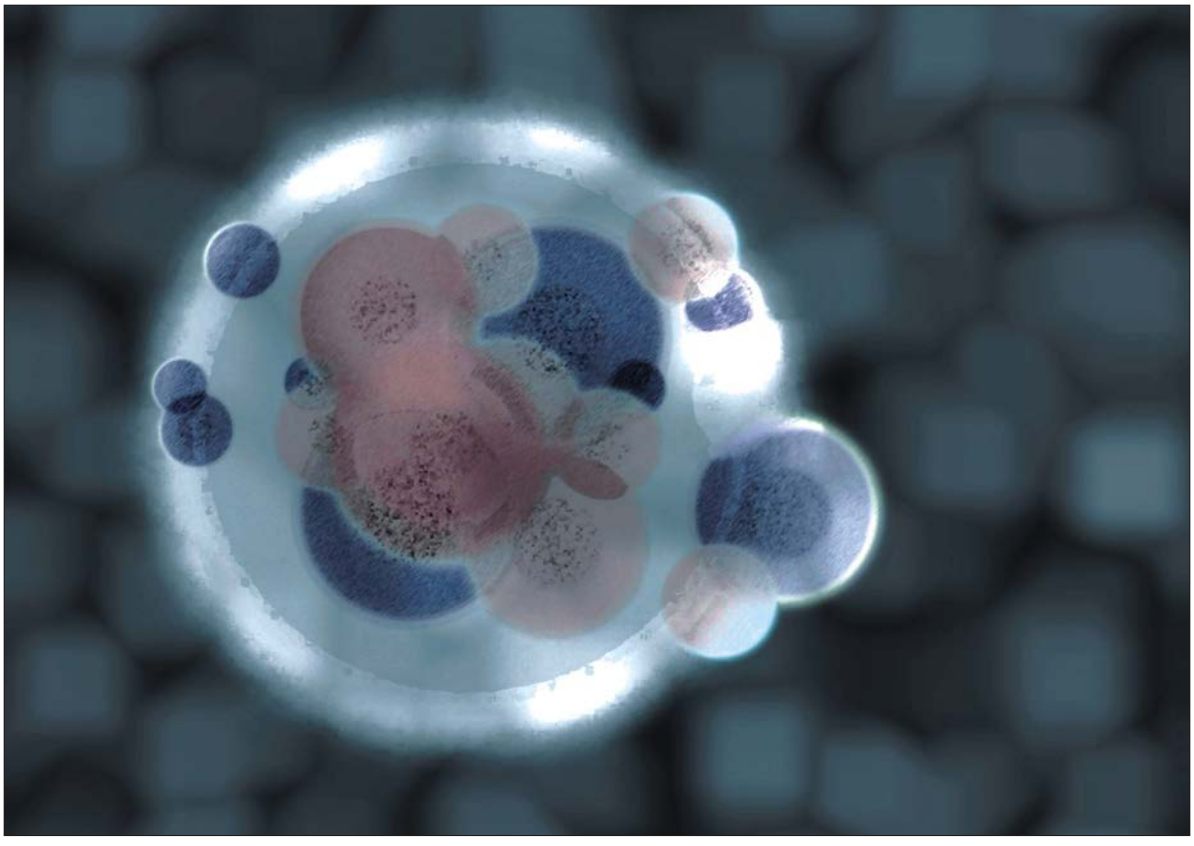

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Research Article
Toxicity of Aqueous Stem-Bark Extract of *Albizia chevalieri* on the Liver and Kidney of Juvenile African Catfish (*Clarias gariepinus*)

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Abstract

Background and Objective: Stem-bark of *Albizia chevalieri* has been used to kill fish by local fishermen over the years in some parts of Nigeria. This study investigated the effect of aqueous stem-bark extract of *Albizia chevalieri* on the liver and kidney of juvenile African catfish, *Clarias gariepinus*. **Materials and Methods:** *Clarias gariepinus* was purchased and acclimatized for 2 weeks in laboratory aquaria. An acute toxicity test was conducted with eighty fish divided into eight groups in aquaria containing ten fish each. The groups were respectively exposed to 800, 400, 200, 120, 80, 60, 40 and 0 mg L^{-1} of the aqueous extract of *A. chevalieri* to obtain the 96 hrs LC_{50} of 77.915 mg L^{-1}. In the sub-acute studies, a total of 48 acclimatized fish were divided into four groups containing 12 fish in each aquarium. Group 1 served as the normal control, while groups 2-4 were respectively exposed to three sub-lethal concentrations of 19.48, 9.74 and 7.79 mg L^{-1} of the extract, corresponding to the 1/4th, 1/8th and 1/10th of the 96 h $LC_{50,}$ for 16 days. **Results:** The result from the serum collected every 4 days showed significant ($p<0.05$) increases in the activities of alanine aminotransferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase (ALP), with significant ($p<0.05$) increases in the concentrations of direct bilirubin, total protein, creatinine, urea, sodium and potassium. From the acute toxicity test result, there was no survival in aquaria containing 200-800 mg L^{-1} of aqueous extract of *A. chevalieri*. **Conclusion:** The results showed that the death of fish on exposure to aqueous stem-bark extract of *A. chevalieri* could be a result of liver and kidney damages.

Key words: *Albizia chevalieri*, *Clarias gariepinus*, acute toxicity, liver function parameters, kidney function parameters

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Fishing is one of the sources of income in some parts of Ebonyi State, Nigeria, as well as some other parts of the country. Locally, piscicidal plants (plants that kill fishes) are usually used in fishing in streams and ponds due to their availability and low or no cost effect. Some of these plants have medicinal properties in humans and some other animals, yet, they are very toxic and lethal to fishes. *Albizia chevalieri* is one such plants. Literature reviews on the mechanism of toxicity of the plant on fishes are scanty. Hence, there is a need to ascertain the mode of toxicity of *A. chevalieri* and/or the organs affected in fish (*Clarias gariepinus*), especially the liver and kidney.

Albizia chevalieri (locally called 'urom' or 'ugbanta' in Ebonyi State) is one of the plants used in fish farming in Ebonyi State. It belongs to the plant family Fabaceae that grows up to a height of 12 m. Sometimes it is seen under harsher conditions of the dry savannah as a shrub in Senegal, Niger and Nigeria. It has leaves with 8-12 pairs of pinnate and 20-40 pairs of leaflets, twigs pubescent with white lenticels, an open and rounded or umbrella-shaped canopy and pale-greyish bark each[1]. A previous study on *Albizia* species indicated the presence of phenolic compounds from *Albizia amara* with significant antioxidant activity[2]. The leaf extract of *A. chevalieri* is used either as a cold-water decoction or dried, ground and sieved, then mixed with pap, for the management of diabetes mellitus by traditional medical practitioners in some parts of the Niger Republic and Sokoto, Nigeria. Hypoglycaemic effects of the leaves[3] and roots[4] have also been reported.

Previous study showed that *A. chevalieri* aqueous stem bark extract contains different phytochemicals such as flavonoids, alkaloids, phenols, steroids, saponins, tannins and cardiac glycosides[5]. Also, the study revealed that the aqueous stem bark extract of *A. chevalieri* induced oxidative stress in fish, which could be the possible cause of the death of the fish, resulting from the different phytochemical constituents of the plants[5].

Clarias gariepinus, also known as African catfish, belongs to the air-breathing catfishes of the family *Clariidae*. Catfish are currently produced worldwide in various production systems ranging from very low yielding extensive to high yielding intensive systems. It is widely cultured due to its tolerance to extreme conditions of the environment, high production and good feed conversion rate[6]. Hence, they are mostly used for fish farming in Nigeria and are normally used as samples in aquatic toxicity studies, due to their easy management when compared to some other fish species[7].

The liver is the largest solid organ, the largest gland and one of the most vital organs that functions as a centre for the metabolism of nutrients and excretion of waste metabolites[8]. Liver parenchyma serves as a storage organ for several products like glycogen, fat and fat-soluble vitamins. It is also involved in the production of a substance called bile that is excreted to the intestinal tract. Bile aids in the removal of toxic substances and serves as a filter that separates harmful substances from the bloodstream and excretes them[9]. The central role played by the liver in the clearance and transformation of chemicals exposes it to toxic injury[9]. The death of an animal could occur in a few minutes as a result of the total loss of liver function. The kidneys are responsible for maintaining homeostasis. This involves the management of fluid levels, electrolyte balance, excretion and other factors that keep the internal environment of the body consistent and in a good position. Renal failure can occur as a result of intrinsic or extrinsic causes. Extrinsic causes include cardiovascular disease, obesity, diabetes, sepsis and lung and liver failure. Intrinsic causes include glomerular nephritis, polycystic kidney disease, renal fibrosis, tubular cell death and stones[10]. Hence, serious kidney damage would result in the accumulation of fluids and other harmful substances which can result in death. Both liver and kidney are indispensable organs in animals and their loss of functions is detrimental.

Therefore, since the liver and kidney are responsible for detoxification and excretion of waste products respectively, poisonous substances can affect their functions and ultimately lead to the death of the animal. Hence, the study is aimed at investigating the toxicity of aqueous stem-bark extract of *A. chevalieri* on the liver and kidney of juvenile African catfish, *Clarias gariepinus*.

Furthermore, this study carried out gas chromatography and mass spectrometric (GC-MS) analyses of both the ethanol and n-hexane extracts of *A. chevalieri* stem bark.

MATERIALS AND METHODS

Study area: This study was carried out at the animal house and the laboratory of the Biochemistry Department of Ebonyi State University, Abakaliki, Nigeria.

Materials: Freshly cut stem-bark and leaves of *Albizia chevalieri* were collected from Nkaliki Enyibuchiri in the Ikwo Local Government Area of Ebonyi State, Nigeria. The plant was identified and authenticated by Prof. S.S. Onyekwelu of the Applied Biology Department, Ebonyi State University, Abakaliki, Nigeria. One hundred and fifty (150) juvenile *Clarias gariepinus* were purchased from Chi-boy Farms, Abakaliki, Ebonyi State.

Equipment: Spectrophotometer (Spectro 21D PEC Medicals USA), rotary evaporator, oven, weighing balance, measuring cylinder, glasswares (pyrex), centrifuge (binatone), refrigerator, sample containers.

Chemicals/reagents: All the chemicals and reagents used in this research were of the purest analytical grade commercially available. The assay kits were products of Randox Laboratories Limited, BT29 4QY, United Kingdom.

Methods

Sample preparation: The stem-bark of *A. chevalieri* was dried under laboratory conditions for two weeks. Afterwards, it was ground to powder using a mechanical grinder and stored in a sealed container before use. About 200 g of the ground stem-bark of *A. chevalieri* was soaked in 1000 mL of distilled water and allowed to stand for 48 hrs in an air-tight container. The mixture was filtered using a muslin cloth and squeezed thoroughly to let all the filtrate out, while the residue was discarded. The water in the filtrate was evaporated using a rotary evaporator at 60°C for some days to get the dry aqueous extract of the *A. chevalieri* stem-bark[5].

Acute toxicity test: Acute toxicity test to determine the 24, 48, 72 and 96 hrs LC_{50} values of aqueous stem-bark extract of *A. chevalieri* on *C. gariepinus* was conducted in a semi-static system in the laboratory. The water with the extract of *A. chevalieri* concentrations was changed after every 24 hrs by adding fresh water and extract of *A. chevalieri* to counterbalance their decreasing concentrations. Exactly 80 fish weighing between 240.00±20.00 and 180.00±60.00 g with lengths 30.80-25.50 cm were selected and divided into eight groups containing 10 fish in each aquarium, for acute toxicity testing. The groups were, respectively exposed to 800, 400, 200, 120, 80, 60, 40 and 0 mg L^{-1} of the aqueous stem bark extract of *A. chevalieri*. The experiment was conducted in aquaria containing 40 L of aerated tap water. Afterwards, the percentages of survival and mortality were calculated[5].

Sub-acute toxicity tests: The sub-acute toxicity tests were carried out with 77.915 mg L^{-1}, being the 96 hrs LC_{50} value of *A. chevalieri* on *C. gariepinus*. Three groups of acclimatized fish containing twelve fish in each aquarium were exposed to 19.48, 9.74 and 7.79 mg L^{-1} of *A. chevalieri*, corresponding to the 1/4th LC_{50}, 1/8th LC_{50} and 1/10th LC_{50}, for 16 days. Another aquarium of 12 fish without the plant extract was simultaneously maintained to serve as the normal control[5].

Blood collection: At the end of every 4 days, three fish were taken from each aquarium and their blood was collected from the head and caudal fin region. The blood was centrifuged at 3000 ppm for 20 min and the serum collected was used for biochemical assays.

Biochemical assays: Standard methods were used for all the biochemical analyses using Randox kits. They include serum activities of alanine aminotransferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase (ALP), serum concentrations of direct bilirubin, total protein, urea, creatinine, sodium and potassium.

Statistical analysis: Statistical analysis was performed using one-way Analysis of Variance (ANOVA) followed by Duncan's multiple range test procedures of SAS software version 9.1. All the results obtained were expressed as Mean±Standard Deviation (SD) of three replicates of each sample and the differences between means were regarded as significant at $p<0.05$.

RESULTS

Acute toxicity observation: The result of the acute toxicity effect of aqueous stem bark extract of *Albizia chevalieri* on juvenile *Clarias gariepinus* is presented in Table 1. There were 10 fish in each group before the introduction of *A. chevalieri* extract. No death (100% survival/0% mortality) was recorded in the aquaria exposed to the concentrations of 0-60 mg L^{-1} of *A. chevalieri* extract after 24, 48, 72 and 96 hrs. While a total of 2, 4, 7 and 8 fish died, respectively after 24, 48, 72 and 96 hrs (20% survival/80% mortality) in the aquarium exposed to 80 mg L^{-1}, a total of 6, 7, 8 and 9 fish died, respectively after 24, 48, 72 and 96 hrs (10% survival/90% mortality) in the aquarium containing 120 mg L^{-1} of the plant extract. Whereas, all the fish died (0% survival/100% mortality) in the aquaria containing 200-800 mg L^{-1} after 24 hrs. The mean lethal concentrations (LC_{50}) of *A. chevalieri* expressed in mg L^{-1} for 24, 48, 72 and 96 hrs are 107.289, 96.244, 83.807 and 77.915 mg L^{-1}, respectively.

The mean lethal concentrations (LC_{50}) expressed in mg L^{-1} at various exposure times (95% confidence intervals) are shown below:

- 24 hrs LC_{50} = 107.289 (91.160-132.461)
- 48 hrs LC_{50} = 96.244 (81.470-117.524)
- 72 hrs LC_{50} = 83.807 (70.697-99.802)
- 96 hrs LC_{50} = 77.915 (66.584-90.492)

Table 1: Acute toxicity test of *Clarias gariepinus* juveniles exposed to aqueous bark extract of *Albizia chevalieri*

A. chevalieri concentration (mg L^{-1})	Number of fish exposed	Number of deaths				Mortality (%)			
		24 hrs	48 hrs	72 hrs	96 hrs	24 hrs	48 hrs	72 hrs	96 hrs
00	10	10	10	10	10	00	00	00	00
40	10	10	10	10	10	00	00	00	00
60	10	10	10	10	10	00	00	00	00
80	10	02	04	07	08	20	40	70	80
120	10	06	07	08	09	60	70	80	90
200	10	10	10	10	10	100	100	100	100
400	10	10	10	10	10	100	100	100	100
800	10	10	10	10	10	100	100	100	100

Table 2: Effects of sub-lethal concentrations of aqueous stem-bark extract of *Albizia chevalieri* on some serum liver function parameters of *Clarias gariepinus*

Days/groups	AST (U L^{-1})	ALT (U L^{-1})	ALP (U L^{-1})	Direct bilirubin (µmol L^{-1})	Total protein (g L^{-1})
4th day					
Control	44.56±0.69^{a}	10.78±0.25^{a}	13.12±0.24^{a}	19.84±0.38^{a}	37.85±0.41^{a}
1/10th LC_{50}	45.44±0.50^{a}	11.33±0.17^{a}	16.08±0.09^{b}	28.37±0.38^{b}	37.85±0.41^{a}
1/8th LC_{50}	51.56±0.69^{b}	12.78±0.58^{b}	17.78±0.16^{c}	38.46±0.38^{c}	51.54±0.31^{b}
1/4th LC_{50}	58.89±0.69^{c}	15.56±0.42^{c}	20.32±0.16^{d}	43.05±0.49^{d}	55.45±0.20^{c}
8th day					
Control	45.11±0.84^{a}	11.01±0.35^{a}	13.04±0.37^{a}	19.88±0.25^{a}	37.96±0.55^{a}
1/10th LC_{50}	60.89±0.84^{b}	23.83±0.33^{b}	24.56±0.00^{b}	37.72±0.38^{b}	55.39±0.55^{b}
1/8th LC_{50}	68.44±1.35^{c}	32.61±0.67^{c}	25.50±0.41^{c}	42.15±0.38^{c}	60.35±0.55^{c}
1/4th LC_{50}	74.78±0.84^{d}	38.17±0.33^{d}	29.06±0.37^{d}	48.30±0.38^{d}	69.78±0.55^{d}
12th day					
Control	44.89±0.84^{a}	10.89±0.44^{a}	13.20±0.47^{a}	20.12±0.25^{a}	37.26±0.46^{a}
1/10th LC_{50}	80.78±0.51^{b}	38.78±0.25^{b}	27.65±0.29^{b}	46.49±0.43^{b}	58.58±0.46^{b}
1/8th LC_{50}	89.22±0.39^{c}	41.89±0.25^{c}	29.53±0.29^{c}	49.12±0.38^{c}	64.84±0.70^{c}
1/4th LC_{50}	102.00±0.33^{d}	46.00±0.17^{d}	36.70±0.19^{d}	57.89±0.38^{d}	78.72±0.91^{d}
16th day					
Control	45.21±0.85^{a}	10.10±0.29^{a}	13.24±0.39^{a}	20.11±0.25^{a}	38.33±0.36^{a}
1/10th LC_{50}	94.22±0.84^{b}	60.83±0.50^{b}	34.85±0.50^{b}	61.99±0.49^{b}	75.22±0.55^{b}
1/8th LC_{50}	99.44±1.02^{c}	62.89±0.19^{c}	37.01±0.32^{c}	67.90±0.49^{c}	80.06±0.55^{c}
1/4th LC_{50}	111.56±1.26^{d}	69.33±0.50^{d}	37.01±0.32^{d}	79.38±0.38^{d}	89.37±0.55^{d}

Data are presented as Mean±Standard deviation of 3 fish in each group, values with different alphabet superscripts differ significantly ($p<0.05$) between durations within the concentration, AST: Aspartate aminotransferase, ALT: Alanine aminotransferase and ALP: Alkaline phosphatase

Sub-lethal toxicity/biochemical parameters

Liver function parameters: The result of the effects of sub-lethal concentrations of *A. chevalieri* on some serum liver function parameters of *Clarias gariepinus* is shown in Table 2. The result showed significantly ($p<0.05$) increases in the activities of aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphatase (ALP), with concomitant significant ($p<0.05$) increases in the concentrations of direct bilirubin and total protein from the 4th, 8th, 12th and 16th day in a dose and time-dependent manner when compared with that of the normal control. Thus, the activity of AST increased from 45.44±0.50-51.56±0.69 and 58.89±0.69 U L^{-1} on the 4th day and finally, from 94.22±0.84-99.44±1.02 and 111.56±1.26 U L^{-1} on the 16th day, in the groups exposed to 1/10th, 1/8th and 1/4th LC_{50} of *A. chevalieri*, respectively, when compared to the control group with 44.56±0.69 and 45.21±0.85 U L^{-1}, respectively. Similarly, the activity of ALT increased from 11.33±0.17-12.78±0.58 and 15.56±0.42 U L^{-1} on the 4th day and from 60.83±0.50- 62.89±0.19 and 69.33±0.50 U L^{-1} on the 16th day, in the groups exposed to 1/10th, 1/8th and 1/4th LC_{50} of *A. chevalieri*, respectively, when compared to the control group with the peak value of 11.01±0.35 U L^{-1} observed on the 8th day. Also, ALP activity increased on the 4th day from 16.08±0.09-17.78±0.16 and 20.32±0.16 U L^{-1} and on the 16th day from 34.85±0.50-37.01±0.32 and 37.01±0.32 U L^{-1}, in the groups exposed to 1/10th, 1/8th and 1/4th LC_{50} of *A. chevalieri*, respectively, when compared to the control group with the peak value of 13.24±0.39 U L^{-1} on the 16th day. The level of serum direct bilirubin increased from 28.37±0.38-38.46±0.38 and 43.05±0.49 µmol L^{-1} on the 4th day and from 61.99±0.49-67.90±0.49 and 79.38±0.38 µmol L^{-1} on the 16th day, in the groups exposed, respectively to 1/10th, 1/8th and 1/4th LC_{50} of *A. chevalieri*, when compared to the control group with the peak value of 20.11±0.25 µmol L^{-1} on the 16th day. Likewise, serum 75.22±0.55-80.06±0.55 and 89.37±0.55 g L^{-1} on the 16th day, in the groups exposed to 1/10th, 1/8th and 1/4th

Table 3: Effects of sub-lethal concentrations of aqueous stem-bark extract of *Albizia chevalieri* on some serum kidney function parameters of *Clarias gariepinus*

Days/groups	Creatinine (mg dL^{-1})	Urea (mg dL^{-1})	Sodium (Na^+) (mEq L^{-1})	Potassium (K+) (mEq L^{-1})
4th day				
Control	5.55±0.31[a]	7.49±0.08[a]	14.30±0.35[a]	3.80±0.01[a]
1/10th LC_{50}	8.32±0.20[b]	9.96±0.08[b]	19.07±0.20[b]	4.88±0.01[b]
1/8th LC_{50}	9.27±0.12[c]	10.46±0.08[c]	20.93±0.35[c]	5.55±0.00[c]
1/4th LC_{50}	11.84±0.12[d]	11.90±0.08[d]	23.49±0.53[d]	6.87±0.01[d]
8th day				
Control	5.48±0.27[a]	7.55±0.17[a]	14.32±0.17[a]	3.79±0.01[a]
1/10th LC_{50}	10.51±0.09[b]	13.85±0.11[b]	21.69±0.40[b]	6.30±0.01[b]
1/8th LC_{50}	11.82±0.09[c]	14.80±0.06[c]	24.01±0.37[c]	6.96±0.01[c]
1/4th LC_{50}	13.90±0.00[d]	15.57±0.13[d]	26.10±0.70[d]	8.86±0.01[d]
12th Day				
Control	5.53±0.38[a]	7.78±0.00[a]	14.26±0.41[a]	3.82±0.06[a]
1/10th LC_{50}	13.70±0.08[b]	16.26±0.17[b]	24.25±0.62[b]	7.98±0.02[b]
8th LC_{50}	14.55±0.08[c]	17.71±0.13[c]	25.61±0.41[c]	8.59±0.06[c]
1/4th LC_{50}	15.48±0.15[d]	19.65±0.09[d]	28.73±0.62[d]	10.86±0.02[d]
16th Day				
Control	5.50±0.24[a]	7.51±0.20[a]	14.31±0.23[a]	3.86±0.01[a]
1/10th LC_{50}	14.58±0.05[b]	21.85±0.13[b]	26.31±0.23[b]	10.98±0.01[b]
1/8th LC_{50}	15.34±0.10[c]	22.74±0.20[c]	28.85±0.46[c]	11.71±0.01[c]
1/4th LC_{50}	17.62±0.05[d]	23.40±0.15[d]	31.85±0.23[d]	13.60±0.02[d]

Data are presented as Mean±Standard deviation of 3 fish in each group, values with different alphabet superscripts differ significantly ($p<0.05$) between durations within concentration

LC_{50} of *A. chevalieri*, respectively, compared to the control group with 37.85±0.41 and 38.33±0.36 g L^{-1}, respectively. Therefore, the highest effects were observed in the group exposed to 1/4th LC_{50} of *A. chevalieri*, while the lowest effects were observed in those exposed to 1/10th LC_{50} per day of sample collection.

Kidney function parameters: Table 3 shows the result of the effects of sub-lethal concentrations of *A. chevalieri* on some serum kidney function parameters of *Clarias gariepinus*. The result showed significant ($p<0.05$) increases in the concentrations of urea, creatinine, sodium and potassium in a dose and time-dependent manner in serial order of days of sample collections when compared with that of the normal control. Hence, serum creatinine level increased from 8.32±0.20-9.27±0.12 and 11.84±0.12 mg dL^{-1} on the 4th day and from 14.58±0.05-15.34±0.10 and 17.62±0.05 mg dL^{-1} on the 16th day, in the groups exposed to 1/10th, 1/8th and 1/4th LC_{50} of *A. chevalieri*, respectively, when compared to the control group with peak value of 5.55±0.31 µmol L^{-1} on the 16th day. Serum urea concentration increased from 9.96±0.08-10.46±0.08 and 11.90±0.08 mg dL^{-1} on the 4th day and from 21.85±0.13-22.74±0.20 and 23.40±0.15 mg dL^{-1} on the 16th day, in the groups exposed to 1/10th, 1/8th and 1/4th LC_{50} of *A. chevalieri*, respectively, compared to the control group with peak value of 7.78±0.00 mg dL^{-1} on the 12th day. Also, the serum sodium (Na^+) level increased from 19.07±0.20-20.93±0.35 and 23.49±0.53 mEq L^{-1} on the 4th day and from 26.31±0.23-28.85±0.46 and 31.85±0.23 mEq L^{-1} on the 16th day, in the groups exposed to 1/10th, 1/8th and 1/4th LC_{50} of *A. chevalieri*, respectively, when compared to the control group with peak value of 14.32±0.17 mEq L^{-1} on the 8th day. Lastly, serum potassium (K^+) concentration increased from 4.88±0.01-5.55±0.00 and 6.87±0.01 mEq L^{-1} on the 4th day and from 10.98±0.01-11.71±0.01 and 13.60±0.02 mEq L^{-1} on the 16th day, in the groups exposed to 1/10th, 1/8th and 1/4th LC_{50} of *A. chevalieri*, respectively, compared to the control group with peak value of 3.86±0.01 mEq L^{-1} on the 16th day. Therefore, the lowest effects were observed in the group exposed to 1/10th LC_{50} of *A. chevalieri*, while the highest effects were observed in those exposed to 1/4th LC_{50} on the 4th and 16th days, respectively.

DISCUSSION

Erratic swimming, quick and sudden movement, slow movement, bottom settlement and dorsal floating before death were observed amongst the fish in the aquaria containing high concentrations (120-800 mg L^{-1}) of aqueous stem-bark extract of *A. chevalieri*. Less erratic and quick movements were displayed by the fish in the aquaria with lower concentrations of aqueous stem-bark extract of *A. chevalieri*, but showed weakness after 96 hrs. The normal movement was displayed by the control group (0.00 mg L^{-1}) after 96 hrs. Table 1 shows the results of aqueous stem-bark extract of *A. chevalieri*. The deaths and weaknesses observed in the fish groups with high and low concentrations of

A. chevalieri, respectively showed that the plant extract is toxic to the fish. The 24 hrs LC_{50} of the aqueous stem bark extract of *A. chevalieri* (107.29) obtained from this study is in line with the work of Singh *et al.*[11] in which the 24 hrs LC_{50} of acetone stem-bark extract of *Thevetia peruviana* on the fish, *Catla catla*, in laboratory condition was 99.43 mg L^{-1}. Therefore, from the results of the acute toxicity test obtained in this study, it could be suggested that the stem-bark of *Albizia chevalieri*, a piscicidal plant, is toxic to fish at high concentrations.

The result showed that serum ALT, AST and ALP activities, with direct bilirubin and total protein levels in *Clarias gariepinus* exposed to an aqueous stem-bark extract of *A. chevalieri* significantly ($p<0.05$) increased compared to the normal control in time and concentration-dependent manner from the 1/10th LC_{50} to 1/4th LC_{50} of the fourth day to sixteenth day (Table 2). The most significant effects were observed in the 1/4th LC_{50} of the 16th day, whereas the least effects were observed in the 1/10th LC_{50} of the 4th day. The increase in the activities of ALT (31-84%), AST (2-60%), ALP (18-69%), with the increased levels of bilirubin (30-75%) and total protein (18-58%) within 16 days observed in current study is an indication of liver damage. Current study is in agreement with the work of Saidu *et al.*[4], who reported that there was an increase in the activities of ALT, AST, ALP and in the levels of bilirubin and total protein in the serum of albino rats exposed to the aqueous root extract of *Albizia chevalieri*. A similar report was given by Tiwari and Singh[12]. Also, increases in liver function parameters in *C. gariepinus* exposed to glyphosate[7], aqueous stem-bark extract of *Bridelia ferruginea* and butachlor[13] have been reported.

Serum ALT and AST are the most widely used clinical biomarker of liver damage[14,15]. The ALT level is found to be higher in the liver compared to other organs and is responsible for the transamination of alanine. When a hepatocellular injury occurs, ALT will leak into the extracellular space and enter the blood[14]. However, lower ALT activities are also found in heart tissue and skeletal muscles. This enzyme is used to detect hepatocellular necrosis[16]. The AST also helps in detecting hepatocellular necrosis but is considered a less specific biomarker enzyme for hepatocellular injury[14]. Besides the liver, it is also found in other organs like the heart, muscle, brain and kidney. Injury to any of these tissues can cause an elevated blood level of AST[17]. Increase in alkaline phosphatase and/or bilirubin with little or no increase in ALT is primarily a biomarker of hepato biliary effects and cholestasis[9,18]. In humans, increased ALP levels have been associated with drug-induced cholestasis[19].

An increase in tissue or serum bilirubin concentration results in jaundice and it occurs in toxic or infectious diseases of the liver e.g. hepatitis or bile obstruction[20]. Elevated bilirubin is an indication of liver cell impairment. Bilirubin measurement is also a useful index for determining the excretory function of the liver and assessment of haemolytic anaemia. In the liver, bilirubin is conjugated with glucuronic acid in a reaction catalysed by bilirubin-UDP-glucuronyl transferase which renders it soluble and subsequently excreted into the bile[4]. Increased plasma total protein concentration observed in the current work at high doses may be due to dehydration and/or increased plasma immunoglobulin concentration due to infection[4].

The result of the effect of aqueous stem-bark extract of *A. chevalieri* in the serum of *C. gariepinus* showed a gradual increase in the levels of creatinine (from 33-69%), urea (from 45-77%), sodium (from 25-55%) and potassium (from 22-72%) when compared to the control, in a dose and time-dependent manner within 16 days. The increase in the kidney function parameters observed in this study could be a result of kidney damage in the fish caused by stem-bark extract of *Albizia chevalieri*. Our result is in line with the study of Winkaler *et al.*[21], who reported a damaged gill and kidney tissue in *Prochilodus lineatus* (fish) exposed to an aqueous extract of neem leaves. Saidu *et al.*[4] reported that serum urea levels of the rats treated with aqueous root extract of *A. chevalieri* were significantly increased while serum creatinine decreased and the electrolytes were not affected by the extract. The differences in the effects of *Albizia chevalieri* in the kidney function parameters of rats and fish could be a result of the differences in the anatomy, physiology and metabolic processes in mammals and Pisces.

Creatinine is the major catabolic product of the muscle and it is excreted in the kidneys. Creatinine levels are used as an indicator of renal failure[22]. Serum creatinine is an important indicator of renal health because it is an easily-measured by-product of muscle metabolism. Creatinine itself is an important biomolecule because it is a major by-product of energy usage in muscle, via a biological system involving creatine phosphate and adenosine triphosphate[23]. The increased level of urea observed is an indication of azotemia. Urea is a major nitrogenous end product of protein and amino acid catabolism, produced by the liver and distributed throughout the intracellular and extracellular fluid. In the kidneys, urea is filtered out of the blood by glomeruli and is partially being reabsorbed with water[24,25]. The most frequently determined clinical indices for estimating renal function depends upon the concentration of urea in the serum. It is useful in the differential diagnosis of acute renal failure and prerenal condition where blood urea nitrogen-creatinine ratio is increased[26].

The electrolytes, Na^+ and K^+, levels also showed a significant ($p<0.05$) increase with increased concentration of the aqueous stem bark extract of *A. chevalieri* with days of exposure. This increase in the levels of sodium and potassium could be attributed to renal impairment. Sodium and potassium are essential mineral macronutrients and are the main extracellular and intracellular ions respectively, for all types of cells. They are important in maintaining fluid and electrolyte balance in the bodies of humans and animals[27]. Therefore, an impairment of the kidney interferes with the excretion of Na^+ and K^+ leading to their accumulation in the serum, resulting in hyponatremia and hyperkalemia, respectively.

CONCLUSION

This study reveals that a high concentration of aqueous stem-bark extract of *Albizia chevalieri* is toxic to fish (*Clarias gariepinus*). The toxicity could be attributed to alteration of liver and kidney functions leading to the damages of these essential organs in fish, as a result of the various bioactive compounds in the plant, thereby leading to the death of fish.

SIGNIFICANT STATEMENT

This study discovered that the toxicity and death of fish exposed to the stem-bark of *Albizia chevalieri* are partly due to liver and kidney damages caused by the plant. This research will help researchers to uncover some of the bioactive compounds in this plant that are responsible for its toxicity to fish and use them in other toxicological studies, which many researchers have not explored. Also, such active compounds could be of pharmacological importance, since the plant has been used to treat some ailments locally.

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