

Research Article

Effects of Stem Extract of *Adenia Cissampeloides* on Liver Function of *Clarias Batrachus* Fish

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Abstract

Screening for phytochemical composition of stem aqueous extract of *Adenia cissampeloides* and the effects on selected parameters of liver function in *Clarias batrachus* fish were carried out. Standard screening procedures were used. One hundred and sixty (160) fish of average weight were exposed to 0.0, 0.625, 1.25, 2.5 and 5.0 g/L of the extract for eight hours in triplicates. Blood samples were collected from the fish at one-hour interval and analysed for aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP) and unconjugated bilirubin (UB) and the results analysed using ANOVA. The phytochemical screening showed presence of rotenone, tannins, alkaloids, glycosides and saponin. Steroids were not detected. The result showed significant ($p < 0.05$) increases in AST activities and concentrations of UB. Increases in the activities of ALT and ALP were not significant ($p > 0.05$). Large effect size (ω^2) of 0.42 and 0.52 for UB and AST, respectively, were obtained. AST/ALT ratio of 1.5 indicated damages to liver cells and disruption of vital processes that elicited cytotoxic reactions in the fish. It is possible that the same effects may occur in man if such fish harvested using this fish poisoning plant is consumed.

Keywords: *Adenia cissampeloides*, cytotoxicity, liver function, phytochemical.

Introduction

Adenia cissampeloides also known as monkey rope or snake climber is a robust semi-woody climber of the family passifloraceae. The plant contains the toxin modeccin [1] and its stem is used as fish poison. However, fresh leaves of *Adenia cissampeloides* are used as vegetables in Ngbo, Ebonyi State; in Ivory Coast, the leaf extract is rubbed on the breast after child birth to promote lactation [2]. Leaves and roots of the plant are used in the treatment of itching and ringworm, haemorrhoids, sores and wounds [3].

Some active principles found in plants make fish dizzy or kill them, such as rotenones and saponins [4]. Some plants which liberate cyanide in water are also used as fish poison, as well as plants which contain high level of ichthyothereol, triterpene and other ichthyotoxins. Fish poisons affect a number of cellular processes in the fish. They inhibit $\text{Na}^+ \text{K}^+ \text{ATPase}$, interfere with oxidative capacity and glutathione metabolism of major organs and nerves; and cause other toxic effects [5, 6].

The ancient practice of poisoning fish was an important method of securing food and has continued to flourish in many cultures today. People consume fish produced by this method in almost every part of the world. In Ebonyi State, and possibly in many other parts of Nigeria, the stem extract of *Adenia cissampeloides* is used as fish poison and such fish are widely consumed in the state. To the best of our knowledge, no research has been carried out on the biochemical effect of using this plant as fish poison. Hence, the study aimed at carrying out phytochemical screening of *Adenia cissampeloides* stem extract and determining the effects of the stem extract on selected biomarkers of liver function such as Aspartate aminotransferase (AST), Alanine aminotransferase (ALT), Alkaline phosphatase (ALP) and Unconjugated bilirubin(UB).

Materials and Methods

Extraction

The extraction process was done by soaking 400 g of shredded fresh stem of the plant in 400 ml of distilled water for 24-hours. To

circumvent the effect of temperature on the active principle during drying, as was the case in the pilot study where both aqueous and ethanol extracts after evaporation could not kill the fish, fresh aqueous extract yield was estimated by soaking 400 g of the shredded fresh stem in 400 ml of distilled water and taking the weight (792 g). The chaff weighed 391.94 g after filtration with Whatman filter paper No 42 (125 mm). By subtracting the weight of water and chaff (783.94 g) from the weight of stem and water (792 g) which gave 8.06 g and dividing by 400 g of the stem, 1 g of the stem gave 0.02 g of the extract. This guided the amount/ quantity of the shredded fresh stem added to the aquaria directly without drying process. Figure 1.



Figure 1: Leaves and Stems of *Adenia cissampeloides*.

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Fish exposure and serum preparation:

A total of one hundred and sixty (160) *Clarias batrachus* fish of average weight of 122 g each were used in the study. *Clarias batrachus* is the major fish harvested in the area. Eight fish were exposed to 0.0 g/L, 0.625 g/L, 1.25 g/L, 2.5 g/L, 5.0 g/L, 10 g/L and 20 g/L of the stem extract in five aquaria (A, B, C, D, E) for LC₅₀ determination. Eight fish were exposed to 0.0 g/L, 0.625 g/L, 1.25 g/L, 2.5 g/L and 5.0 g/L in five different aquaria each in triplicate for determinations of effects on parameters. Blood was collected from one fish taken from each aquarium at one hour interval during the eight hour exposure for serum preparation.

Phytochemical Screening:

The chemical tests were carried out using the screening procedures described by Sofowara (1993) with little modifications to identify the constituents of the stem [7].

Test for tannins: Few drops of 0.1% of FeCl₃ were added to 3ml of the aqueous extract and observed for greenish precipitate formation.

Test for saponin: Exactly 10ml of the extract was boiled in a water bath, cooled and shaken vigorously for a stable persistent froth. The frothing was mixed with 3 drops of olive oil and shaken vigorously, then observed for the formation of emulsion.

Test for flavonoids (rotenone): Four drops of NaOH were added to 2ml of the aqueous extract. A brick-red colouration was an indication of a positive test for flavonoids.

Test for alkaloids: About 2 ml of the extract was mixed with 2 ml of HCl and few drops of Wagner's reagent added. Turbidity and dark precipitate indicated presence of alkaloids.

Test for cardiac glycosides: About 2 ml of the extract was added 5ml of H₂SO₄ and heated for 15 minutes and then cooled. About 1ml NaOH and 10ml Fehling's reagent were added and observed for brick-red precipitate.

Test for steroids: About 2 ml of acetic anhydride was added to 2ml of the extract with 2ml H₂SO₄ and observed for colour change from violet to blue or green indicating a positive test.

Assay / determination of the Parameters of Liver Function

Determination of aspartate aminotransferase (AST) in serum

Principle: α-oxoglutarate + L-aspartate GOT L-glutamate + Oxaloacetate.

AST was measured by monitoring the concentration of oxaloacetate hydrazone formed with 2, 4-dinitrophenylhydrazine. A red colour is produced on the addition of sodium hydroxide (0.4 Mol/L). Intensity of colour is related to enzyme activity [15, 16].

Procedure: AST kits manual (source: Randox Laboratories Ltd, UK). AST activity (u/L) was obtained from AST Standard graph of activity versus absorbance.

Determination of alanine aminotransferase (ALT) in serum

Principle: α-oxoglutarate + L-alanine ^{GPT} L-glutamate + pyruvate

Glutamic-pyruvic transaminase was measured by monitoring the concentration of pyruvate hydrazone formed with 2, 4-dinitrophenylhydrazine [8, 9].

Procedure: ALT kits manual (source: Randox Laboratories Ltd, UK). ALT activity (u/L) was obtained from ALT Standard graph of activity versus absorbance

Determination of alkaline phosphatase (ALP) in serum

Principle: P-nitrophenylphosphate + H₂O ^{ALP} phosphate + p-nitrophenol

Procedure: ALP kits manual (source: Randox Laboratories Ltd, UK). ALP activity (u/L) = 2760 × change in absorbance at 405 nm / min.

Determination of total and direct bilirubin in serum

Principle: Direct (conjugated) bilirubin reacts with diazotized sulphanic acid in alkaline medium to form a blue coloured complex. Total bilirubin is determined in the presence of caffeine, which releases albumin bound bilirubin by the reaction with diazotised sulphanic acid [10].

Procedure: Bilirubin kits manual (source: Randox Laboratories Ltd, UK). Total bilirubin concentration (mg/l) = 10.8 × Absorbance_{TB} (578 nm). Direct bilirubin concentration (mg/dl) = 14.4 × Absorbance_{DB}. Unconjugated bilirubin (UB) concentration (mg/dl) = Total bilirubin concentration – Direct bilirubin concentration.

Data Analysis

Analysis of variance (ANOVA): one factor completely randomized design was used. The causative relationship between the independent variable (extract) and dependent variable (activity/concentration of the biomarkers over time) was determined. This is because there is only single independent variable (extract of *Adenia cissampeloides*) with five levels or conditions (groups). The focus was upon different types of variance in the groups.

Hence, a null hypothesis was tested which stated that there was no difference among the five group means. The null hypothesis was rejected when the derived F-value exceeded the tabled critical value of F or otherwise accepted. When the null hypothesis was rejected the effect size, omega-squared (ω²), which is the measure of the magnitude of the effect of independent variable upon the dependent variable, was determined and multiple comparison test (Turkey's Honestly Significant Difference, HSD) was used to tell which of the means were significantly different from each other [11].

Results and Discussion

Results: The results obtained are presented in the table and figures below. Table 1.

The results show high content of tannin and flavonoids with three plus followed by alkaloids and glycosides with two plus and then saponin having just one plus. Table 2.

Figure 2. The activity increased significantly (p < 0.05) with time, Figure 3. The activity increased non-significantly (p > 0.05), Figure 4. The activity increased non-significantly (p > 0.05), Figure 5. The concentration increased significantly (p < 0.05) reaching peak at the fifth-hour before coming back to normal.

Discussion

The results of phytochemical screening showed the presence of flavonoids (rotenone), tannin and alkaloids, glycosides and saponin. Steroids were not detected [Table 1]. The results showed that *Adenia cissampeloides* contains principles that may be poisonous to fish.

Rotenone interferes with respiration by inhibiting the protective mechanisms which minimizes the toxic potential of oxygen

Table 1: Phytochemicals present in *Adenia cissampeloides*

Phytochemicals	Observations	Inferences
Tannin	Greenish precipitate	+++
Saponin	Stable form, no emulsion	+
Flavonoids (rotenone)	Brick-red colour	+++
Alkaloids	Turbid and dark precipitate	++
Glycosides	Brick-red precipitate	++
Steroids	No reaction	-

Table 2: Analysis of Variance on the Parameters.

Parameter	Source	some of squares	d f	mean squares	F-Cal	F-Tab	Ho	ω^2
AST	Treatment	8984.681 (SSB)	4	2246.170 (MSB)	11.64*	2.64	rejected	0.52
	Error	6753.250 (SSW)	35	192.950 (MSW)				
	Total	15737.931 (SST)	39					
ALT	Treatment	515.925	4	128.981	0.99	2.64	accepted	----
	Error	4544.053	35	129.830				
	Total	5059.978	39					
ALP	Treatment	747.506	4	186.877	0.06	2.64	accepted	----
	Error	102369.859	35	2924.853				
	Total	103117.365	39					
UB	Treatment	0.438	4	0.110	8.46*	2.64	rejected	0.43
	Error	0.471	35	0.013				
	Total	0.909	39					

*AST and UB were statistically significant ($P < 0.05$)

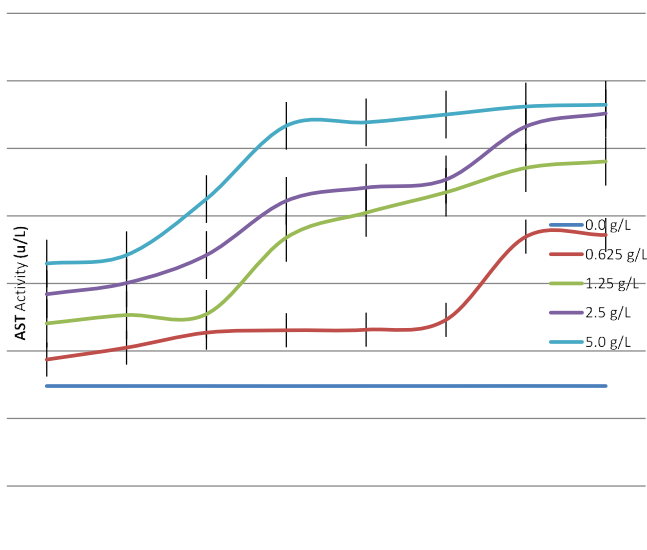


Figure 2: Effects of *Adenia cissampeloides* Stem Extract on AST Activity in *Clarias batrachus* Within Eight Hours of Exposure.

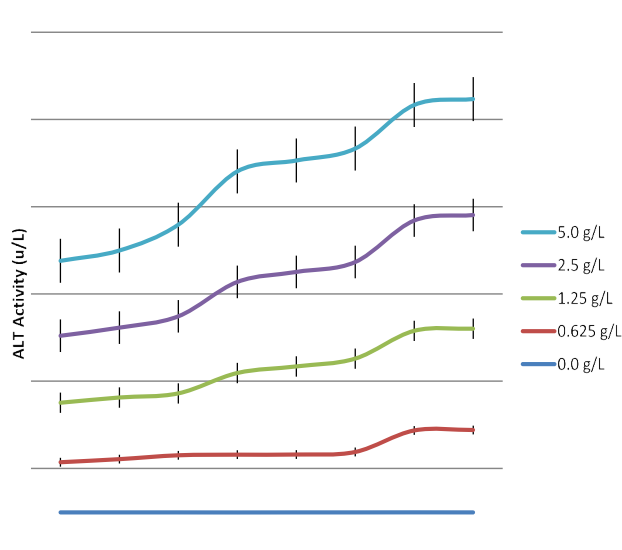


Figure 3: Effects of *Adenia cissampeloides* Stem Extract on ALT Activity in *Clarias batrachus* Within Eight Hours of Exposure.

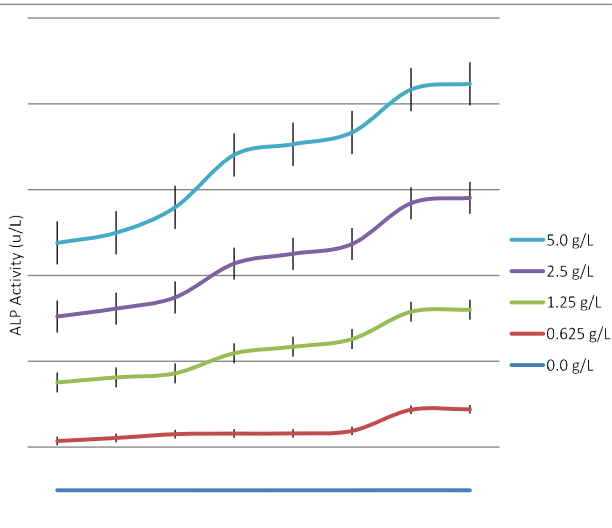


Figure 4: Effects of *Adenia cissampeloides* Stem Extract on ALP Activity in *Clarias batrachus* Within Eight Hours of Exposure.

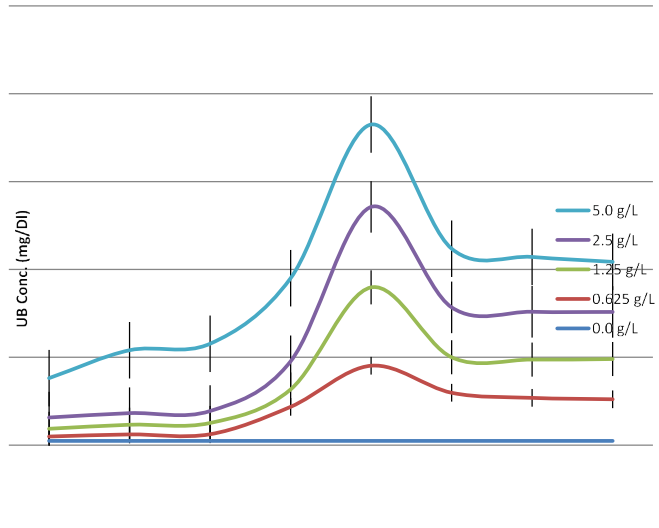


Figure 5: Effects of *Adenia cissampeloides* Stem Extract on concentration of Unconjugated Bilirubin (UB) in *Clarias batrachus* Within Eight Hours of Exposure.

intermediates; saponin has asphyxiation action while cardiac glycosides affect central nervous system and nerve mechanisms of heart [12]. Alkaloids have neurologic effects; they inhibit $\text{Na}^+\text{-K}^+$ ATPase, DNA polymerase, cytochrome P-450 system and cause damage to the liver cells. Tannins cause enzyme inhibition, substrate and metal ion deprivation and other slow acting effects [13].

The results showed significant increase in AST activity which was
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both time and concentration dependent [Figure 2]. Within the first one hour of exposure, the activity increased from 17.52u/L of the control to 46.25 u/L of the highest concentration of extract (5.0 g/L) and increased to 76.0U/L in eight hours. This could be attributed to the toxic effects of the extract on the liver of the exposed fish. A similar trend was obtained for ALT activity, though it was not significant ($p > 0.05$) [Figure 3]. However, AST/ALT ratio of 1.5 was obtained

and showed significant damage to the liver cells causing a release of membrane bound AST, reaction which is normally irreversible.

There were increases in the activities of ALP but was not significant ($p > 0.05$) [Figure 4]. Activities of ALP rose from 4.82 u/L of the control to 42.94 u/L in one hour and to 66.455u/L within eight hours at the highest exposure concentration of the extract (5.0 g/L). An elevated ALP is usually associated with liver or bone disease [9]; however, the increases were not statistically significant.

Furthermore, the concentration of unconjugated bilirubin increased with increase in extract concentration and time of exposure [Figure 5]. This suggests that haemoglobin was being destroyed or the liver was not actively treating the haemoglobin it received. This agrees with the result in elevated AST activity. Increase in unconjugated bilirubin concentration up to 0.473mg/dl coupled with AST/ALT ratio of 1.5 indicated liver damage [14].

Above all, the results revealed that the extract had large effect on AST and UB with ω^2 of 0.52 and 0.43 respectively [Table 2]. Effect size (ω^2) was not determined for ALT and ALP as they were not statistically significant ($p > 0.05$).

Conclusion

The study has shown that major phytochemicals of *Adenia cissampeloides* stem include tannins, alkaloids, flavonoids (rotenones), glycosides and saponin. The activities of AST increased significantly ($p < 0.05$) with increasing concentration of the extract and with increasing time of exposure. Increases in the activities of ALT and ALP were not significant ($p > 0.05$). The concentration of unconjugated bilirubin increased but started reducing after 5 hours.

Therefore, the phytochemicals in the extract are thought to be responsible for the observed adverse effects in the fish. Higher levels of unconjugated bilirubin (UB) indicated that too much haemoglobin was being destroyed or that the liver was not actively treating the haemoglobin it received; increased level of AST indicated damages to liver cells and tissues. The destruction of haemoglobin and disruption of conjugation of bilirubin in the liver amidst the presence of oxidants or other circulating xenobiotics in the blood might have elicited cytotoxic reactions and subsequently poisoning of the fish.

It is possible that the same effects may occur in man; therefore, proper heat treatment, thorough washing and cooking are required

in handling the fish before being consumed. Otherwise, fish killed with *Adenia cissampeloides* should not be eaten by humans to avoid extended poisoning effects.

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