

Hepatoprotective Effect of Aqueous Leave Extract of *Cyphostemma glaucophilla* on Carbon Tetrachloride Induced Hepatotoxicity in Albino Rats

¹Ojogbane Elejo, ²Daikwo Moses Alilu and ³Nwodo Okwesili Fred Chiletugo

¹Department of Biochemistry, Kogi State University, Anyigba, Nigeria

²Department of Biochemistry, University of Nigeria Nsukka, Enugu State, Nigeria

Corresponding Author: Nwodo Okwesili Fred Chiletugo, Department of Biochemistry, University of Nigeria Nsukka, Enugu State, Nigeria

ABSTRACT

Several literatures exist on the efficacy of *Cyphostemma glaucophilla* in the treatment of diverse ailment ranging from malnutrition disorders to systemic diseases such as hypertension and kidney problems. Effect of aqueous leave extract of *Cyphostemma glaucophilla* was investigated on Carbon tetrachloride induced hepatotoxicity by evaluating its biochemical activities on Aspartate Amino Transferase (AST), Alanine Amino Transferase (ALT), Alkaline Phosphatase (ALP), Bilirubin and creatinine. The negative control group A were served daily doses of saline (0.85% NaCl; 5 mL kg⁻¹) positive control group B received (0.85% NaCl; 5 mL kg⁻¹ and a single concentration 5 mL kg⁻¹ of CCl₄) while groups C, D, E and F were given 5.0, 10.0, 15.0, 20.0 mg kg⁻¹ body weight of extract using stomach tubes for 7 days, respectively. CCl₄ (5 mL kg⁻¹) single concentration was administered to groups B, C, D, E and F on the 8th day however, extract administration continued for groups C, D, E and F on day 9 and 10. The activities of the enzyme markers in the positive control group were observed to increase significantly ($p < 0.05$) when compared with the negative control and the test groups C, D, E and F. The test groups administered varying doses of extract after exposure to CCl₄ decreased significantly ($p < 0.05$) and in a dose dependent manner the activities of ALT, AST and ALP. There was also a significant ($p < 0.05$) dose dependent decrease in the level of unconjugated, conjugated bilirubin and creatinine. It is observed, that *Cyphostemma glaucophilla* among its numerous pharmacological properties may serve as a cheap alternative in maintaining liver and kidney disorders.

Key words: *Cyphostemma glaucophilla*, carbon tetrachloride, liver enzyme marker, kidney parameters, hepatoprotective

INTRODUCTION

Many plant species have served as sources of medicine for people all over the world. *Cyphostemma glaucophilla* is a flowering plant which belongs to the family of vitaceae. These species are caudiform and use to belong to the genus *Cissus*. It is a perennial herb usually seen by streams and rivers and can be found in such places as Togo, Nigeria, Sudan, Democratic Republic of Congo and Angola. The leaf extract is used in ethno medicine for the treatment of diverse ailments ranging from malnutrition disorders to systemic disease such as hypertension and kidney problems in Kogi and Kwara states of Nigeria (Omale *et al.*, 2006).

Malnutrition is a serious and debilitating condition that threatens health. It is a nutritional disorder common in young children and the elderly, the incidence rate varies from country to country depending on the biological and socioeconomic status of a population (Wardlaw and Kessel, 2002).

Analysis of the aqueous leave extract of *Cyphostemma glaucophilla* showed the presence of phytochemical as vitamin C, E, flavonoids, proteins, carbohydrate, polyphenols, o and c glycoside (Omale *et al.*, 2006).

Investigation of various species from the genus *Cyphostemma* has been described. The leave of *C. rheifolia* contains quinolizidine, alkaloid, flavonoid, terpenoids and allenic ketone (Al-Duais *et al.*, 2009). The stem wood of *C. Pallida* showed presence of stilben, triterpenoids and steroids (Beltrame *et al.*, 2002).

Diverse literatures exist on the efficacy of *Cyphostemma glaucophilla* in the management of ailments. Ojogbane *et al.* (2007) had reported its potential in cardiovascular health. The aqueous leave extract induced increases in protein synthesis has also been investigated and validated (Ojogbane and Nwodo, 2010). However, James *et al.* (2009) also investigated and confirmed plant potential as dietary supplement for the management of nutritional related disease such as kwashiorkor and also reported that the plant could be used in the management of cardiovascular disease.

Various substances are known to cause liver and kidney damage and one of them is carbon tetrachloride which is a well known hepato and nephrotoxin. Within the body, carbon tetrachloride breaks down to highly toxic CCl_3 and CCl_2O free radicals by Cytochrome p450 enzyme and causes damage to hepatocytes (Ali *et al.*, 2011).

In this study, the effect of aqueous extract of *C. glaucophilla* was evaluated on CCl_4 induced hepatotoxicity in albino rats.

MATERIALS AND METHODS

Plant material: *Cyphostemma glaucophilla* leaves were collected from Idah, along Ibaji road in Kogi State of Nigeria in the month of March, 2011.

Animals: The experimental animals used in this study were Wister albino rats of either sex, between 7 and 9 weeks old with average weight of 100-150 g. They were obtained from the Animal House of the Department of Biochemistry Kogi State University Anyigba.

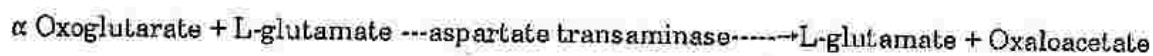
Chemical/reagent: Carbon tetrachloride used in this study was a product of British Drug House (BDH), England. Reagents used for all the assays were commercial kits obtained from Randox, USA.

Extraction procedure: Four hundred grams quantity of pulverized leaves was macerated in five volumes (w/v) of water for 18 h and then filtered. The Whatman No. 4 filtrate was evaporated in a water bath to get the dried residue.

Experimental design: Thirty Wister rats of either sex were housed in separate cages, acclimatized for seven days and then divided into six groups of five rats each. All administrations were through the oral route. They were all fed with their normal diet and access water throughout the period of experiment. Group A was the negative control which was given (0.85% NaCl; 5 mL kg⁻¹). Group

B represented the positive control and received normal saline and a single dose of CCl_4 (5 mL kg^{-1}). Group C, D, E and F were given daily doses of 5.0, 10.0, 15.0 and 20.0 mg kg^{-1} , respectively. CCl_4 (5 mL kg^{-1}) was administered to the test groups on the 8th day. However extract administration continued on day 9th and 10th: Twenty four hours after the last administration, animals were anaesthetized by ether and blood samples were collected via cardiac puncture were spun at 10,000 rpm for 5 min to separate the plasma from the particulate substances.

Assay of aspartate amino transferase activity (Randox commercial enzyme kit): This method is based on the principle that aspartate amino transferase activity was measured by monitoring the concentration of oxaloacetate formed with 2,4-dinitrophenylhydrazine.

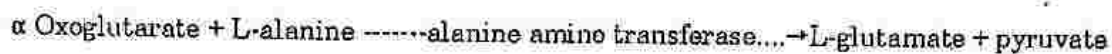


The AST substrate phosphate buffer of 0.5 mL each was pipetted into the sample Blank (B) and sample Test (T) test tubes, respectively. The serum sample of 0.1 mL was added to the sample test (T) only and mixed immediately; then incubated in a water bath for exactly 30 min at 37°C .

A volume of 0.5 mL of 2,4-dinitrophenylhydrazine was added to both sample Blank (B) and sample t-test is used to test tubes immediately after incubation. Also, 0.1 mL of the sample was added to the Sample Blank (B) only. The medium was mixed and allowed to stand for exactly 20 min at 25°C . Finally, 5.0 mL of Sodium Hydroxide solution (NaOH) was added to both the Sample Blank (B) and sample t-test is tested tubes and mixed thoroughly.

Absorbance of the sample (A-sample) was read at a wavelength of 550 nm against the sample blank after 5 min.

Assay of alanine aminotransferase activity (Randox commercial enzyme kit): This method, is based on the principle that alanine amino transferase is measured by monitoring the concentration of pyruvate hydrazone formed with 2,4-dinitrophenylhydrazine. Transaminase activity in plasma is stimulated by high concentration of aldehydes, sketones or oxoacids.



The ALT substrate phosphate buffer of 0.5 mL each was pipetted into two sets of test tubes labeled B (sample blank) and T (sample test), respectively. The serum (0.1 mL) sample was added to the sample test (T) only and mixed properly: then incubated for exactly 30 min in a water bath at a temperature of 37°C . A volume of 0.5 mL each of 2,4-dinitrophenylhydrazine was added to both test tubes labeled T (sample test) and B (sample blank) immediately after the incubation. Also, 0.1 mL of serum sample was added to the sample Blank (B) only. The entire medium was mixed thoroughly and allowed to stand for exactly 20 min at 25°C . After which 5.0 mL each of sodium hydroxide (NaOH) solution was added to the both test tubes and also mixed thoroughly. Absorbance of the Sample (A-sample) against the sample blank was read at a wavelength of 550 nm after 5 min.

Assay of alkaline phosphatase activity (QCA commercial enzyme kit): This method is based on the principle that serum alkaline phosphatase hydrolyses a colorless substrate of

phenolphthalein monophosphate giving rise to phosphoric acid and phenolphthalein which at alkaline pH values, turns into a pink colour that can be photometrically determined. Distilled water (1.0 mL) was pipetted into 2 sets of test tubes labeled SA (sample) and ST (standard), respectively. Then one drop each of chromogenic substrate was added to the distilled water in the two sets of test tubes. Their contents were mixed and incubated at 37°C for 20 min in a water bath. After which a standard solution of 0.1 mL was added to the Standard test Tube (ST) only; followed by the addition of the serum sample of 0.1 mL to the sample test tube (SA). The content was also mixed and incubated at 37°C for 20 min in a water bath. A color developer of 5.0 mL each was added to both sets of test tubes. Absorbance of the sample against the blank (water) was read at a wavelength of 550 nm.

Determination of bilirubin: Colorimetric method based on that described by Jendrassik and Grof (1938).

Statistical analysis: Results were expressed as Mean±SEM and tests of statistical significance were carried out using one way ANOVA. The statistical package used was Gen Stat Release 4.23DE, Lawes Agricultural Trust.

RESULTS

Effect of extract on aspartate amino transferase (AST): Result shown on Table 1 indicated that the activity of AST in groups C and D administered lower doses (5.0, 10.0 mg kg⁻¹) of extract before and after exposure to CCl₄ decreased (32.00±1.10, 30.50±0.08 IU L⁻¹) significantly (p<0.05) and in a dose dependent manner when compared with the positive control (40.00±0.01 IU L⁻¹). However, the difference between group C and D (1.50±1.02 IU L⁻¹) was not significant (p>0.05) when compared with the negative control. Although there was a significant (p<0.05) decrease in the activity of AST in groups E and F (35.50±0.10 IU L⁻¹) when compared to the positive control (40.00±0.01 IU L⁻¹), the effect is less when compared with groups C and D there was also a significant (p<0.05) difference between groups E, F and the negative control.

Effect of extract on alanine amino transferase: There was significant (p<0.05) difference between the positive and negative control on Table 1. Significant dose dependent decrease in the activity of ALT was observed in groups C and D when compared with the positive control. The difference in activity of ALT in groups E and F was not significant (p>0.05). However, there was

Table 1: Effect of Extract on plasma enzyme markers

Parameters (IU L ⁻¹)	Groups/treatment					
	A negative control	B positive control	C 5.0 mg kg ⁻¹ extract	D 10.0 mg kg ⁻¹ extract	E 15.0 mg kg ⁻¹ extract	F 20.0 mg kg ⁻¹ extract
Aspartate amino transferase (AST)	30.00±0.10 ^a	40.00±0.01 ^b	32.00±1.10 ^a	30.50±0.08 ^a	35.50±0.10 ^a	35.50±0.01 ^a
Alanine amino transferase (ALT)	19.00±0.02 ^a	30.10±0.10 ^b	23.50±0.09 ^a	20.00±1.10 ^a	25.60±1.10 ^a	26.00±0.15 ^a
Alkaline phosphatase	52.00±0.06 ^a	80.30±1.10 ^b	60.15±0.08 ^a	52.50±0.07 ^a	64.50±0.09 ^a	65.00±0.07 ^a

Means with different superscript in a row are statistically significant (p<0.05)

Table 2: Effect of Extract on some kidney parameters

Parameters mg dL ⁻¹	Treatment groups					
	A negative control	B positive control	C 5.0 mg kg ⁻¹ extract	D 10.0 mg kg ⁻¹ extract	E 15.0 mg kg ⁻¹ extract	F 20.0 mg kg ⁻¹ extract
Creatinine	0.40±0.01 ^a	0.50±0.001 ^b	0.40±0.05 ^a	0.38±0.01 ^a	0.42±0.02	0.42±0.02
Total bilirubin	0.18±0.05 ^a	0.38±0.01 ^b	0.20±0.10 ^a	0.18±0.01 ^a	0.23±0.01	0.23±0.01
Conjugated bilirubin	0.14±0.01 ^a	0.30±0.01 ^b	0.15±0.10 ^a	0.14±0.01 ^a	0.18±0.01 ^a	0.17±0.01

N = 5; Means with different superscript in a row are statistically significant ($p < 0.05$)

a significant ($p < 0.05$) decrease in ALT activity between E, F and the positive control but a significant ($p < 0.05$) Increase in ALT between groups E, F and the negative control.

Effect of extract on alkaline phosphatase: ALP of the test animals administered 5.0 mg kg⁻¹ and 10.0 mg kg⁻¹ extract were dose dependently and significantly ($p < 0.05$) decreased when compared with the positive control. This trend was also observed for groups E and F when compared with the positive control. However, the effect of group C and D is more on Table 1.

Effect of extract on creatinine: Table 2 shows a significant ($p < 0.05$) dose dependent decrease in the concentration of creatinine (0.40±0.05, 0.38±0.01 mg dL⁻¹) in group C and D when compared with the positive control (0.50±0.00 mg dL⁻¹). Also group E and F had a significant ($p < 0.05$) decrease (0.42±0.02 mg dL⁻¹) in creatinine level when compared with the positive control (0.50±0.00 mg dL⁻¹).

Effect of extract on total bilirubin: It is evident on Table 2 that significant ($p < 0.05$) decreases exist between the test groups C and D administered low doses (5.0, 10.0 mg kg⁻¹) of extract and the positive control. Significant difference was also observed between the negative and positive control.

Effect of extract on conjugated bilirubin: In Table 2, the same trend was observed with significant ($p < 0.05$) differences between the negative and positive control. Also the effect of extract at lower doses was more significant than at higher doses.

DISCUSSION

Result as shown on Table 1 indicated significant ($p < 0.05$) increases in the activities of the liver enzyme (AST, ALT, ALP) in the positive control when compared with the negative control. This is consistent with the finding of Ali *et al.* (2011) that CCl₄ is a potent toxicant that has the potential to cause hepatic toxicity when given in a single dose (5 mL kg⁻¹). Also Mounnissamy *et al.* (2008) had reported that elevated activities of (AST, ALT and ALP) are indicative of hepatotoxicity. Dhanasekaran and Ganapathy (2011) pointed out that AST and ALT may be elevated in other conditions such as the dysfunction of heart and skeletal muscle which also possess these marker enzyme.

Table 1 reveals that the test group C and D which were administered low doses (5.0, 10.0 mg kg⁻¹) of extract before and after exposure to CCl₄ elicited a more significant ($p < 0.05$) dose dependent decrease in the enzyme activities when compared with the positive control and had non significant ($p > 0.05$) differences when compared with the negative control. Even though there was significant ($p < 0.05$) decrease in enzyme activities in group E and F when compared with

the positive control, the effect is less compared to the effect of group C and D. Hence low doses between (1-10 mg kg⁻¹ extract) is recommended. It could be asserted that extract has a good potential in maintaining liver and kidney integrity. This result is consistent with the record of Omale *et al.* (2006) and Dhanasekaran and Ganapathy (2011).

Similar trend was observed on Table 2 with the positive control showing a significant ($p < 0.05$) increase in the level of creatinine when compared with the negative control. Adinarayana *et al.* (2011) had reported that any condition that impairs the function of the kidney will probably raise the creatinine level in the blood. However the most common source of long standing kidney disease in adults is high blood pressure and diabetes mellitus. Extract at lower doses in group C and D was able to significantly ($p < 0.05$) and in a dose dependent manner lower the creatinine level when compared to the positive control and a non dose dependent ($p > 0.05$) difference when compared with the negative control. This could be a possible mechanism by which extract is able to manage hypertension and other systemic disease. Concentrations of extract at higher doses, although significant ($p < 0.05$) when compared with the positive control is less effective than at lower doses. However, the effect of extract at higher dose is in contradiction with the view of Omale *et al.* (2006), further studies is required to authenticate the effect of high dosage.

The result of total bilirubin and conjugated bilirubin also indicated a significant ($p < 0.05$) increase in their levels in the positive control than in the negative control. Since bilirubin could leak out of the kidney due to damage thereby increasing its concentration in the blood, this is consistent with the results of Hemabarathy *et al.* (2009). Similarly the dose of extract at lower concentration was more effective in lowering the concentration of bilirubin in the test group when compared with the positive control.

CONCLUSION

These results have indicated potential effect of *Cyphostemma glaucophylla* extract as a hepatoprotective against carbon tetrachloride induced hepatic damage in rats. Among its numerous pharmacological properties, it may serve as a cheap alternative in maintaining liver and kidney disorders.

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