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Research Article
Toxicological Implication of Sub-chronic Administration of Aqueous Leaves Extract of *Cochlospermum planchonii* in Albino Rats

Fisayo Abraham Bamisaye, Abdulhakeem Olarewaju Sulyman, Emmanuel Oladipo Ajani and Victoria Aduke Alabi

Kwara State University, Malete, P.M.B. 1530, Ilorin, Nigeria

Abstract

Background and Objective: *Cochlospermum planchonii* is used for various purposes such as food additive, dye, rope making and medicine for treatment of various ailments in Nigeria. The plant is consumed indiscriminately by users. This study aims to evaluate toxicological effects of aqueous leaves extract of *Cochlospermum planchonii* (*C. planchonii*) in albino rats. **Materials and Methods:** The qualitative phytochemical screening of aqueous leaves extract of *C. planchonii* was carried out to ascertain the bioactives present in the extract. Albino rats weighing between 200 and 224 g were grouped into four (A-D) consisting of ten rats each. Group A (control) orally received 1 mL of distilled water while groups B-D received 50, 100 and 200 mg kg^{-1} b.wt., of the extract, respectively for 28 days. At the end of the experiment, these experimental rats were sacrificed and their blood, liver and kidney were collected for toxicological studies. In addition, histopathological studies of liver and kidney of the rats were also carried out. Statistical analysis was done by one-way analysis of variance (ANOVA) and Duncan multiple range test. **Results:** The aqueous leaves extract of *C. planchonii* contain alkaloids, cardenolides, cardiac glycosides, flavonoids, phenolics, saponnins, terpenoids and tannins while antraquinones and steroids were not detected. The extract supports growth in a dose dependent manner with best growth shown in rats treated with the highest concentration (200 mg kg^{-1} b.wt.). In addition, the extract reduced the electrolyte concentrations while it increased bilirubin and blood creatinine concentrations in rats. Furthermore, histopathological studies of the liver and kidney of extract-treated rats revealed dissociation of hepatic cords in the hepatic parenchyma of the liver while few foci of nephrosis (coagulative tubular necrosis) were seen in their kidney at the end of the experiment. **Conclusion:** Aqueous leaves extract of *C. planchonii* may not be safe when consumed for long period of time, especially at high concentrations.

Key words: *Cochlospermum planchonii*, phytochemicals, growth support, toxicology

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Corresponding Author: Fisayo Abraham Bamisaye, Phytomedicine and Toxicology Research Laboratory, Department of Biochemistry, School of Basic Medical Sciences, College of Pure and Applied Sciences, Kwara State University, Malete, P.M.B. 1530, Ilorin, Nigeria Tel: +2348066352359

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

INTRODUCTION

Medicinal plants contain both organic and inorganic constituents. Many of them are found to be rich in one or more individual phytochemicals, thereby providing a possible link to therapeutic action of medicines[1]. Medicinal herbs are nowadays put to use by many people that are seeking remedies and health approaches. This may be due to the fact that they have countless numbers of benefits to the society, especially in line of medicine and pharmacology. For a variety of reasons, more individuals nowadays prefer to take personal control over their health with the use of medicinal herbs[2]. This is commonly done for a wide variety of illnesses such as common cold, scratches, malaria fever, stomach disorders, typhoid fever, wounds but to mention just a few, which are readily treated at home. One such plant that is widely claimed in traditional medicine of Nigeria to be used in the management of several ailments[3] without recourse to its safety or toxicity risk is *C. planchonii*.

Cochlospermum planchonii is a shrub with woody subterranean root stock. It is a common weed reproducing naturally from seeds and rhizomes[4]. It flowers towards the end of rainy season in Nigeria. *C. planchonii* is a small family comprising 15 species in 2 genera[5]. The plant is shown in Fig. 1 below.

Traditional medicine practitioners in Northern Sierra Leone use decoction of *C. planchonii* root stock for the treatment of gonorrhoea. Mali people include the root stock preparations prescribed against diuretic and jaundice. Dried powdered root stock mixed with shea butter is applied on burn wounds to promote healing. Root stocks are also rubbed on the body against snake-bites. A decoction of the root stock or leaves is used as a bath or drunk against malaria. The powdered root stock, diluted in water and mixed with lemon juice, is drunk as a tonic[6]. Powdered dried leaves are taken for the treatment of palpitations. Its rhizomes and leaves are used to treat many diseases: Malaria, hepatitis, diabetes, infertility, tryponosomiasis and sexual transmissible infections in some African countries[6].

Due to this sudden strong rise in the use of medicinal plants like *C. planchonii*, their thorough scientific investigations are imperative in order to provide information on their safety and toxicity risk. Hence, overall aim of this study was to identify the risk associated with consumption of *C. planchonii* leaves in the kidney and liver of albino rats.

Fig. 1: *Cochlospermum planchonii* plant showing leaves, flower and fruits[5]

MATERIALS AND METHODS

Materials

Collection and preparation of plant material: Leaves of *C. planchonii* were collected at Kwara State University, Malete, Nigeria, in October, 2014. The plant was identified, authenticated and voucher specimen (No.UIH001/923) was deposited in the herbarium of University of Ilorin Botany Department, Ilorin, Nigeria.

Experimental animals: Adult male albino rats weighing between 200 and 224 g used for this study were procured from the breeding colony, Kwara State University, Malete, Nigeria. They were maintained at 37°C on a 12 h light/dark cycle with free access to food and water for 1 week before commencement of the study.

Chemicals, reagents and assay kits: Creatinine, urea, bilirubin, alkaline phosphatase, alanine amino transferase, aspartate amino transferase, gamma glutamyl transferase and albumin kits were all obtained from Randox laboratories Ltd, Antrim UK. Similarly, electrolyte (potassium, sodium and chloride) kits were purchased from Teco Laboratories Ltd, UK. All other chemicals and reagents were of analytical grade obtained from British drug house; Poole, UK.

Methods

Extract preparation: Leaves of the plant were harvested, rinsed with distilled water and then dried at room temperature until a constant weight was obtained. The dried leaves were pulverized using an electric blender (Philip comfort blender, mode HR1727, Holland). Two hundred and fifty grams of it

were macerated with distilled water for 24 h with intermittent shaking. It was then filtered using muslin cloth, dried with oven at 40°C to give a yield of 20.5% extract and then stored in air-tight container at 4°C.

Qualitative phytochemical screening: Crude leaves aqueous extract of *C. planchonii* was subjected to various qualitative phytochemical screening of glycoside, anthraquinones, cardiac glycoside, saponins, steroid, triterpenes, flavonoids, tannins and alkaloids; using standard methods[7].

Experimental design: Experimental rats were grouped into four (A-D) consisting of ten rats each. Group A (control) orally received 1 mL of distilled water for 28 days. Groups B, C and D were treated like the control except that they received 50, 100 and 200 mg kg^{-1} b.wt., of leaves aqueous extract of *C. planchonii*, respectively. The animals were handled humanly in accordance with guidelines of National Institute of Health on the care and use of laboratory animals[8].

After 28 days, these rats were sacrificed and their blood, liver and kidney, were collected and used for toxicological and histopathological tests.

Collection of blood sample: Blood was withdrawn from the rats by cardiac puncture after they have been anaesthetized with diethyl ether. Blood collected were centrifuge for 10 min at 3000 rpm using Uniscope Laboratory Centrifuge. The serum (clear supernatant) obtained was separated and kept frozen for estimate of renal, liver function tests and marker enzymes' activities.

Determination of enzymes activities in the serum: ALT, AST, ALP and GGT activities were monitored using their respective kits.

Determination of albumin, total bilirubin, creatinine, urea and electrolytes concentration in the serum: Their concentrations were monitored using respective kits.

Determination of protein concentration: This was carried out using the Biuret method as described by Plummer[9].

Preparation of tissue sections: A fraction of selected organs (liver and kidney) was removed and fixed in 10% buffered normal formalin (BNF) solution for 72 h at room temperature[10].

Examination of slides: The stained tissue sections were examined using 4 objective lens of the microscope for focusing and later viewed with the 10 objective lens of the microscope for a higher magnification[10].

Data analysis: Data were expressed as mean±SD of ten determinations except otherwise stated. The statistical tools used were one-way analysis of variance (ANOVA) and Duncan multiple range test[11]. Differences were considered statistically significant at $p<0.05$.

RESULTS

Phytochemical status: Phytochemical screening of aqueous leaves extract of *C. planchonii* indicates the presence of cardenolides, phenolic, cardiac glycoside, saponins, terpenoids, flavonoids, tannins and alkaloids while antraquinones and steroids were not detected (Table 1).

Growth effects of aqueous leaves extrac*t* of *C. planchonii* on experimental rats: All extract-treated animals (groups B, C and D) had significant growth compared with the control group (A) at the end of experimental analysis. Group D had highest weight gain compare with other extract-treated groups (Table 2).

Liver function indices in experimental rats: Serum albumin concentrations in extract-treated rats were not significantly

Table 1: Phytochemical status of aqueous leaves extract of *C. planchonii*

Phytochemicals	Status
Alkaloids	+
Antraquinones	-
Cardenolides	+
Cardiac glycoside	+
Flavonoids	+
Phenolic	+
Saponins	+
Steroids	-
Terpenoids	+
Tannins	+

+: Present, -: Not detected

Table 2: Growth effects of aqueous leaves extract of *C. planchonii* on the experimental rats

	Body weight (g)		
Groups	Initial	Final	Average weight gained
A	170.00±12.67[a]	204.40±19.17[c]	34.40
B	162.60±12.67[a]	182.40±20.40[c]	39.80
C	154.20±10.84[a]	196.80±8.07[c]	42.60
D	152.80±13.24[a]	212.00±19.57[c]	59.20

Results are Mean±SD, n = 10. Means with the same letter across the column are not significantly different ($p>0.05$)

Table 3: Liver function indices in experimental rats fed with aqueous leaves extract of *C. planchonii*

	Biomarkers					
Groups	Albumin (g L^{-1})	T.BIL (mmol L^{-1})	ALT (U L^{-1})	AST (U L^{-1})	ALP (U L^{-1})	GGT (U L^{-1})
A	29.93±3.49[a]	299.52±23.43[a]	611.40±50.11[a]	226.00±10.76[a]	67.68±10.99[a]	193.04±98.18[a]
B	30.26±3.13[a]	284.53±6.220[a]	587.60±33.57[a]	213.00±22.89[a]	67.16±10.55[a]	193.77±96.59[a]
C	28.06±2.26[a]	290.45±13.85[a]	564.40±12.47[a]	213.50±22.68[a]	137.00±42.01[b]	192.99±97.11[a]
D	29.41±1.86[a]	378.70±32.19[b]	575.75±16.47[a]	217.50±20.05[a]	67.00±12.88[a]	191.14±88.44[a]

Results are Mean±SD, n = 10. Means with the same letter across the columns are not significantly different (p>0.05)

Table 4: Renal function indices in experimental rats fed with aqueous leaves extract of *C. planchonii*

	Biomarkers				
Groups	Creatinine (mg dL^{-1})	Urea (mg dL^{-1})	Sodium (mEq L^{-1})	Chloride (mEq L^{-1})	Potassium (mEq L^{-1})
A	7.44±2.14[a,b]	60.66±26.95[a]	146.49±1.52[b]	68.40±7.26[b]	25.12±0.38[ab]
B	3.23±1.45[a]	72.40±15.14[a]	145.82±202[b]	88.62±6.01[c]	16.83±0.62[ab]
C	3.18±1.11[a]	72.52±14.70[a]	140.79±3.40[b]	50.41±4.84[a,b]	27.68±6.11[b]
D	15.60±2.60[b]	76.49±10.66[a]	124.85±7.78[a]	48.33±2.79[a]	13.83±1.12[a]

Results are Mean±SD, n = 10. Means with the same letter across the columns are not significantly different (p>0.05)

Table 5: Protein concentrations in the serum of experimental rats fed with aqueous leaves extract *of C. planchonii*

Groups	Protein concentrations
A	42.27±0.47[a]
B	42.25±0.74[a]
C	42.06±0.67[a]
D	42.00±1.46[a]

Results are Mean±SD, n = 10. Means with the same letter across the columns are not significantly different (p>0.05)

difference compared with control at the end of the study. Similarly, there was no significant difference in the activities of ALT, AST and GGT except that of ALP in group C, which was significantly higher, compared with the control. A significant increase in concentration of total bilirubin in group D was observed compared with control at the end of the study (Table 3).

Renal function indices in experimental rats: There was a significant increase ($p<0.05$) in creatinine level and a significant decrease ($p>0.05$) in sodium, chloride and potassium ions levels of group D compared with the control. However, there was no significant difference in the concentrations of urea of extract-treated rats compared with the control. The electrolytes' concentrations of extract-treated rats were concentration dependent with group D having the lowest (Table 4).

Protein concentrations in experimental rats: Protein concentrations in the serum of treatment rats (groups B-D) showed no significant difference when compared with control group A after the experimental period (Table 5).

Histopathology study of rat liver: At the end of 28 days of treatment, the extract-treated animals showed significant difference in their liver histopathology compared with the control (Group A):

- **Group B:** This group showed closely-packed hepatic plates and moderate random single-cell hepatocellular necrosis
- **Group C:** Showed closely packed hepatic plates and moderate dissociation of hepatic cords in the hepatic parenchyma
- **Group D:** The liver of group D animals marked widespread dissociation of hepatic cords and individualization of hepatocytes (Fig. 2)

Histopathology study of rat kidney: Similarly, histopathological results of kidney of extract-treated animals showed significant difference compared with control group (Group A):

- **Group B:** The kidney of group B showed few foci of nephrosis (coagulative tubular necrosis)
- **Group C:** The kidney showed moderate sloughing off of epithelium of tubules in the renal medulla making the tubules appearing cystic
- **Group D:** The histopathologies of the kidney in this group were exactly the same with those of group C (Fig. 3)

DISCUSSION

Growth supporting nutrients are likely to be present in the extract apart from phytochemicals. This is evidenced in

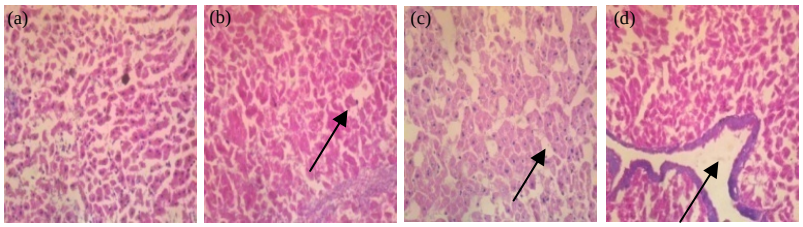

Fig. 2(a-d): Photomicrographs of liver 400X

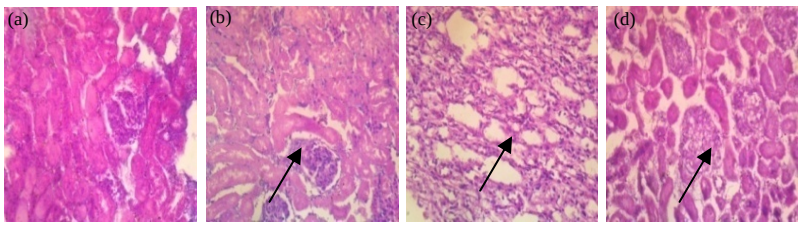

Fig. 3(a-d): Photomicrographs of kidney 400X

animals' growth performs which is dose dependent. It is further evidenced in the weight gained by the animals treated with the extract.

Liver is the largest organ of human body weighing approximately 1500 g and performs more than 500 vital metabolic functions[12]. Smooth endoplasmic reticulum of the liver is the principal 'metabolic clearing house' for both endogenous chemicals like cholesterol, steroid hormones, fatty acids and proteins and exogenous substances like drugs and alcohol. The central role played by liver in the clearance and transformation of chemicals exposes it to toxic injury[13]. Hepatotoxicity may result not only from direct toxicity of the primary compound but also from a reactive metabolite or from an immunologically-mediate response affecting hepatocytes, biliary epithelial cells and/or liver vasculature[14,15]. The blood main protein is albumin and is made by the liver[16]. The non-significant difference in the concentrations of albumin at the end of the study in extract-treated animals may suggest that aqueous extract of *C. planchonii* has no effect on their albumin contents. Bilirubin is a chemical normally present in the blood in small amounts and used by the liver to produce bile. Bilirubin is excreted from the liver in the bile. When the liver cells are damaged, they may not be able to excrete bilirubin in normal way, causing a build-up of bilirubin in the blood and extracellular (outside the cells) fluid[14]. The observed increase in bilirubin in the blood of extract-treated animals at a higher dose, with no increase in hepatic enzyme activities may suggest cholestasis[17]. This may suggest slight hepatotoxicity of the extract at high doses. It may also impair production of bile of rats treated with the highest concentration of extract in this study.

Kidneys perform the function of removal of waste products of metabolism and also serve homeostatic functions[18]. Renal function indices are usually required to assess the normal functioning of different parts of the nephrons[19].

Creatinine, a breakdown product of creatine phosphate in muscle, usually produced at a fairly constant rate by the body depending on muscle mass[20] is removed from the blood chiefly by the kidneys[21]. A sharp rise in blood creatinine level observed in rats treated with the highest concentration of the aqueous leaves extract of *C. planchonii* (group D) may have indicated damage to functioning nephrons of the kidneys[22,23] at this dose.

Urea is a major nitrogenous end product of protein and amino acid catabolism, produced by liver and distributed throughout intracellular and extracellular fluid. Kidney urea is filtered out of blood by glomerulli and is partially being reabsorbed with water[19,24]. An alternate estimation of renal function can be made when interpreting the blood (plasma) concentration of creatinine along with that of urea. Blood urea nitrogen (BUN)-to-creatinine ratio (the ratio of blood urea nitrogen to creatinine) can indicate other problems besides those intrinsic to the kidney; for example, a urea level raised out of proportion to the creatinine may indicate a prerenal problem such as volume depletion[25]. However, the observed values of urea in the aqueous extract-treated animals implied that the extract has no effect on the glomerullli of their kidney. Electrolyte disorders are usually multifactorial in nature. Various pathophysiological factors, such as nutritional status, gastrointestinal absorption capacity, coexistent acid-base abnormalities, pharmacological agents, other comorbid

diseases (mainly renal disease) or acute illness, alone or in combination, play a key role[26]. Electrolytes are salts that conduct electricity and are found in the body fluid, tissue and blood. Electrolytes are important because they are needed in the cells (especially nerves, heart, muscles) to maintain voltages across their cell membranes and to carry electrical impulses (nerve impulses, muscle contractions) across themselves and to other cells[27]. The kidneys regulate fluid absorption, excretion and maintain a narrow range of electrolyte fluctuation. Normally, sodium and potassium are filtered and excreted in the urine and faeces according to the body's needs[27]. Too much or too little sodium or potassium may be caused by poor diet, dehydration, medication and disease, resulting in an imbalance[28]. Sodium can act with other electrolytes especially potassium in the intracellular space to regulate the osmotic pressure and maintain proper water balance within the body. It is required for glucose absorption and transportation of other nutrients across cell membranes. In addition, normal functioning of the nervous system depends on sodium. Potassium also plays an important role in the regulation of acid base balance in the cell, water retention and is essential for protein biosynthesis by ribosomes[1]. In this study, these important functions may be impaired in extract-treated rats especially at higher concentrations of the extract. Furthermore, the observed decrease in electrolytes in this study may lead to hypokalemia arrhythmias, hyponatremia and hypochloremia[26,29-31] conditions due to a decrease in K^+, Na^+ and Cl^-, respectively in the fluids of extract-treated rats.

Estimation of total proteins in the body is helpful in differentiating between a normal and damaged liver function as the majority of plasma proteins such as albumins and globulins are produced in the liver. Total protein is often reduced slightly but the albumin to globulin ratio shows a sharp decline during hepatocellular injury[16]. This observation of insignificant change in protein concentration and albumin concentration in extract-treated animals may suggest no hepatocellular injury in these animals.

The histopathological study of liver and kidney of rats treated with aqueous leaves extract of *C. planchonii* revealed that the extract can induce alteration in the organs architecture and it should be taken with care since it may not be completely safe.

CONCLUSION

This study revealed that the aqueous leaves extract of *C. planchonii* contains cardenolides, phenolic, cardiac glycoside, saponins, terpenoids, flavonoids, tannins, alkaloids which may be responsible for its medicinal and or toxicological properties. Antraquinones and steroids were not detected. Its effect on growth of animals is concentration dependent. Furthermore, the observed effects of aqueous leaves extract of *C. planchonii* in the blood, liver and kidney of experimental albino rats suggest that it may not be completely safe for consumption, especially at high doses.

SIGNIFICANCE STATEMENTS

The effect of *C. planchonii* on growth of animals is concentration dependent. Furthermore, this study confirms that despite the medicinal value of this plant, it may not be completely safe for consumption, especially at high doses.

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