Influence of Alkaloid from *Cnidoscolous aconitifolius* (Miller) Johnston Leaves on Kidney and Liver Functional indices of Male Albino Rats

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Abstract: The possible toxic effect of crude alkaloid from *Cnidoscolous aconitifolius* in the kidney and liver of male albino rats was investigated. The twenty four male albino rats involved were grouped into four groups of six each. Group A (the control) received 0.5ml of distilled water once daily. Groups B, C, and D were first administered orally with 0.5ml each and also with 250, 500 and 1000 mg/kg body weight of the alkaloid respectively once daily. For the kidney, administration of alkaloid at all doses significantly (P≤0.05) reduced the level of creatinine, sodium ions and calcium ions in the serum of the animal. In contrast, all the doses of the alkaloid significantly (P≤0.05) increased the potassium and phosphate ion content of the serum. The alkaloid at 1000 mg/ kg body weight did not significantly (P>0.05) alter the level of serum urea content. The serum chloride ion of the rats administered with the 500 mg alkaloid per body weight was significantly (P≤0.05) higher than at other doses. For the liver, the alkaloid at all doses significantly (P≤0.05) increased the serum albumin while it is only the rats administered with 250 and 500 mg/kg that had significant increase (P≤0.05) in total and conjugated bilirubin level. The serum globulin level significantly (P≤0.05) increased at the 1000 mg/kg body weight. This study indicated that the crude alkaloid from *C. aconitifolius* leaves altered the basic function of the organs.
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1. Introduction

C*nidoscolous aconitifolius* (Miller) Johnston (Fam: Euphorbiaceae) known as tree spinach (English), *efo iyana ipaja*, or *efo Jerusalem* (Yoruba) is commonly found growing in western Nigeria. The leaves are commonly eaten as vegetables (Kuti and Torres, 1996)**.** The shoot and leaves of *C. aconitifolius* are used as laxative, diuretic, circulation and lactation stimulants.

The kidneys have three basic mechanisms of filtration, reabsorption, and secretion separating the various components of the blood. These three processes occur in the nephron, which is the most basic functional unit of the kidney. The kidney **e**xcretes unwanted substances by purifying the blood through the process of ultra-filtration and re-absorption (Ramalingam, 1997). The functional capacity of the kidney can be measured by the dye excretion tests, clearance test, concentration and dilution tests and method for examination of blood concentrations of excretory and electrolyte constituents. Furthermore, renal function tests are required either to demonstrate the presence or absence of active lesion in the kidney, or to assess the normal functioning capacity of different parts of the functioning unit, nephron (Panda, 1989). Inorganic electrolytes occur in large quantities in both extracellular and intracellular fluids. Due to their ability to dissociate readily into their constituent ions or radicals, they comprise the single most important factor in the transfer and movement of water and electrolytes between three divisions of the extracellular and intracellular compartment (Zilva *et al.*, 1991).

The various functions of the mammalian liver are carried out by the liver cells or hepatocytes. The liver which is full of blood vessels can store up to 1500 cm^3 of blood and they are capable of regulating the level of blood in general circulation. The liver converts many toxic and biologically active foreign substances into harmless or inactive forms in a process known as detoxication. The concentration of albumin, globulin and bilirubin in the serum can indicate the state of the liver and can be used to ascertain types of liver damage (Yakubu *et al.*, 2003a).

The phytochemical constituents of most leafy vegetables have been found to contain alkaloid and they are often consumed fresh or steamed thus this was reported to be biotoxic to mammals (Bianco *et al.*, 2006; Yakubu *et al.*, 2008). There is the need to provide scientific information on the alkaloid toxicological activity on mammalian organs. This study was therefore designed to evaluate the toxicological potentials inherent in the alkaloid from *C. aconitifolius* leaves on male albino rats as an indicator.

2. Materials and Methods

The plant samples were collected from a single population within the premises of the main campus of University of Ilorin, Ilorin, Kwara State, Nigeria, and were authenticated at the Forestry Research Institute of Nigeria (FRIN), Ibadan, Nigeria. A voucher specimen (FHI 107768) was deposited at the FRIN herbarium.

Male albino rats (*Rattus novergicus*) of Wistar strain weighing 163.58±3.52 g were obtained from the Animal Holding Unit of the Department of Biochemistry, University of Ilorin, Ilorin, Nigeria.

The assay kits for the determination of serum albumin, globulin, bilirubin, urea, uric acid and creatinine are products of Randox Laboratory, Ltd, Co-Atrim, UK. Assay kits for the determination of serum Na^+, K^+, Ca^{2+} and PO_4^{2-} are products of Agappe Diagnostics Ltd, Kerala, India. The assay kit for the determination of serum Cl^- is a product of Dialab production Ltd, Austria. All other reagents used were of analytical grade and were prepared in volumetric flask using glass wares and distilled water.

Crude alkaloid was extracted following the method reported by Manske (1965). Briefly, fresh leaves of *C. aconitifolius* were oven dried at 40^{o}C for 48 h until a constant weight was obtained. The dried leaves were thereafter pulverized in a blender (Mikachi Blender with Mill, Model MK-1830, China) and the resulting powder weighing 600 g was soaked in 1.2 L of hexane for 72 h. The mixture was then filtered using a Whatman No. 1 filter paper. The filtrate (hexane-extract) containing fats, oils, terpenes, waxes was discarded. The residue was then soaked in 1.5 L of methanol and allowed to stand for one week, thereafter, the mixture was filtered again. The filtrate was evaporated using a rotatory evaporator (R110, Gallenkamp, UK, England); the recovered methanol was poured into the residue again and allowed to stand for two hours. This was repeated for one more time to extract most of the alkaloids from the residue. The three filtrates were combined together and further concentrated with water bath until most of the methanol was evaporated from the extract. The resulting watery methanoly alkaloid slime was kept for the next step. The residue (post methanol) was treated with 200 ml 1M HCl to extract the remaining alkaloids present, the mixture was also filtered. The residue was discarded, while the resulting filtrate was added to the watery methanoly slime, more dilute HCl was added to the solution. This acidic solution was then basified by adding 150 ml 5M NaOH with continuous stirring until the precipitate becomes cloudy. Equal volume of chloroform was then added to the solution, shaken and placed in a separating funnel and allowed to separate into two layers, plenty of 1M NaCl was added to facilitate this separation by breaking the emulsion formed. This was done three times to extract most of the alkaloids from the aqueous (upper) layer into the organic (lower) layer. The organic (lower) layer containing alkaloids was carefully collected into a conical flask. The three portions were combined together and later backwashed by adding a solution of NaCl and dilute NaOH, placed in a separating funnel and separate the layers as above. The aqueous (upper) layer was discarded. The collected organic layer was then evaporated using water bath to get rid of the chloroform. The resulting brownish-black slurry (crude alkaloids) was then reconstituted with distilled water to give the required doses used for this study.

A total of twenty four (24) male albino rats of proven maturity were housed in clean aluminium cages contained in well-ventilated standard housing conditions (temperature: 28-31^{o}C; photoperiod: 12; humidity: 50-55%). The animals were allowed free access to rat pellets (Bendel Feeds and Flour Mills Ltd., Ewu, Nigeria) and tap water. They were acclimatized for two weeks before the commencement of the experiment. The rats were completely randomized into four groups of six each. Group A: Control, received 0.5 ml of distilled water once daily. Groups B, C and D: received 250, 500 and 1000 mg/kg body weight of the alkaloid respectively once daily. Extract administration was done for 60 days between (10:00-10:45 a.m) using plastic syringes attached to metal oropharyngeal cannula.

The procedure described by Yakubu *et al.* 2008 was employed. Briefly, under ether anaesthesia, the veins after being slightly displaced (to prevent blood contamination by interstitial fluid) were cut with a sterile scapel blade and 5 ml of the blood was collected into clean and dry centrifuge tubes. The blood was then left for 10 mins to clot at room temperature. The tubes were thereafter centrifuged at 224 x g for 15 mins using Uniscope Laboratory Centrifuge (Model SM800B, Surgifriend Medicals, Essex, England). The sera were later aspirated with Pasteur pipettes into clean, dry, sample bottles and then used within 12 h of preparation for the various biochemical assays.

The method used for the determination of urea in the serum was diacetylmonixine using thiosemicarbazide as described by Veniamin and Vakirtzi (1970). Serum uric acid was determined according to the method described by Tietz (1995). Serum creatinine was determined using the method described by Bartels and Bohmer *et al.* (1972). Flame photometry method described by Tietz (1955) was used to determine the level of Na^+, K^+ in the serum. The O-cresolphthalein direct method described by Tietz (1995)

was used for the determination of Ca^{2+} in the serum. Cl^- was determined using the mercuric nitrate method described by Tietz (1995). The method described by Fiske and Subbarow (1925) was used for the determination of PO_4^{2-} concentration in the serum.

Serum albumin was determined by the methods described by Doumas *et al.* (1971). The determination of serum globulin level was done using the method described by Tietz (1995) by subtracting the concentration of serum albumin from the total serum protein content. The concentration of globulin was expressed in g/L. Serum bilirubin was determined using the method described by Sherlock (1951).

Results were expressed as the mean of six replicates ± SD. Statistical analysis used was ANOVA and Duncan Multiple Range Test using SPSS version 16.0. Differences were considered statistically significant at P≤0.05.

3. Results

The extract from *C. aconitifolius* leaves was positive to Mayer's and Wagner's reagents by showing a creamy precipitate and a reddish-brown colour respectively. The amount quantitatively analyzed was 15.42 g i.e. percentage weight of 0.50±0.01.

Administration of alkaloid at all doses significantly (P≤0.05) reduced the level of uric acid, creatinine, sodium ion and calcium ions in the serum of the animals (Table 1). In contrast, all the doses of the alkaloid significantly (P≤0.05) increased the potassium and phosphate ion content of the serum. The alkaloid at 1000 mg/ kg body weight did not significantly (P>0.05) alter the level of serum urea content. The serum chloride ion of the rats administered with the 500 mg alkaloid per body weight was significantly (P≤0.05) higher than at other doses.

Administration of alkaloid from *C. aconitifolius* leaves to male rats at all doses investigated significantly (P≤0.05) increased the serum albumin (Table 2). Furthermore, the alkaloid increased the serum total and conjugated bilirubin levels only at 250 and 500 mg/kg body weight whereas the 1000 mg/kg body weight did not significantly (P>0.05) altered the level of the bilirubin. In contrast, the alkaloid at 250 and 500 mg/kg body weight produced values of globulin that compared well with the control value whereas the 1000 mg/kg body weight increased it significantly.

Table 1: Effect of administration of alkaloid from *C. aconitifolius* leaves on some kidney function parameters of male rats

	Alkaloid (mg/kg body weight)			
Parameters	**Control**	**250**	**500**	**1000**
Serum urea (mmol/L)	14.50 ± 2.74^a	15.25 ± 0.82^a	24.50 ± 3.83^b	14.30 ± 3.01^a
Serum uric acid (µmol/L)	1.35 ± 0.16^a	0.87 ± 0.03^b	1.28 ± 0.08^a	0.81 ± 0.20^b
Serum creatinine (mmol/L)	290.45 ± 3.01^a	185.83 ± 12.46^b	30.33 ± 0.87^c	50.26 ± 3.69^d
Serum Na^+ (mmol/L)	$2.30\times10^{-2}\pm10.95^a$	$2.10\times10^{-2}\pm5.48^b$	$2.10\times10^{-2}\pm10.95^b$	$1.98\times10^{-2}\pm2.70^c$
Serum K^+ (mmol/L)	4.25 ± 0.27^a	4.75 ± 0.27^b	5.00 ± 0.55^b	4.75 ± 0.27^b
Serum Ca^{2+} (mmol/L)	8.10 ± 0.00^a	2.35 ± 0.55^b	3.53 ± 0.08^c	0.63 ± 0.08^d
Serum Cl^- (mmol/L)	$3.30\times10^{-2}\pm16.43^b$	$5.0\times10^{-2}\pm54.77^d$	$2.93\times10^{-2}\pm2.74^a$	$4.28\times10^{-2}\pm2.74^c$
Serum PO_4^{2-} (mmol/L)	1.50 ± 0.11^a	1.90 ± 0.33^b	2.65 ± 0.38^c	2.86 ± 0.08^c

Values are mean of six replicates ± SD. Means with the same letter(s) across the row are not significantly different at (P≤0.05) by DMRT.

Table 2: Effect of administration of alkaloid from *C. aconitifolius* leaves on some liver function parameters of male rats

	Alkaloid (mg/kg body weight)			
Parameters	**Control**	**250**	**500**	**1000**
Serum albumin (g/L)	11.02±1.34[a]	14.04±1.14[bc]	14.51±0.67[b]	13.14±0.38[c]
Serum globulin (g/L)	26.44±1.47[ab]	24.43±0.85[a]	24.87±1.70[bc]	27.59±1.36[c]
Serum total bilirubin (µmol/L)	16.41±2.10[a]	25.04±3.84[b]	41.72±13.57[c]	16.96±1.46[a]
Serum conjugated Bilirubin (µmol/L)	84.54±1.79[a]	111.85±8.55[b]	139.23±1.45[c]	44.20±1.51[d]

Values are mean of six replicates ± SD. Means with the same letter(s) across the row are not significantly different at ($p \leq 0.05$) by DMRT

Discussions

Urea is the major nitrogen-containing metabolic product of protein catabolism. The increased level of serum urea at 250 and 500 mg/kg body weight may be an indication that the alkaloid impaired the clearance functioning of the kidney. The decrease in serum uric acid concentration as observed in this study may suggest impairment of the renal function and may be an indication of glomerular dysfunction of the nephron as corroborated by Chawla (1999). Creatinine clearance is measured as an indication of glomerular filteration rate. Decreased creatinine content of the serum at all doses may suggest high creatinine clearance and may be an indication of compromise of the renal function. The significant decrease in serum sodium ion concentration by the alkaloid at all doses may be adduced to attempt at compromising the functional capacity of the nephron probably resulting from excessive loss of Na^+ pool body fluids. It may also be due to either decrease production of aldosterone and other mineralcorticoids which will in turn decrease the tubular reabsorption of Na^+ or increased production of either antidiuretic hormone or increased tubular sensitivity to the hormone (Tietz *et al.*, 1994).

Potassium ions play an important role in the way in which nerve impulses are propagated along the nerve cells and transmitted to receptor cells. The sodium pump maintains the intracellular K^+ concentration of 140 mM as against the extracellular K^+ concentration of 5mM (Horton *et al.*, 1993). The increase in serum K^+ at all doses suggested a possible adverse effect on the pump that maintains the constancy of its extracellular concentration. This increase is a more dangerous trend because of its effect on the heart, but it rarely occurs unless renal function is depressed. It may result in the paralysis of the atria and ventricular arrhythmias may develop. The muscle fibres may eventually become unexcitable, and the heart stops in diastole (Ganong, 2001).

Calcium ion is important in many biological processes such as muscle contraction, serves as an intracellular second messenger for hormones. It is also important in nerve cells for effective transfer of nerve impulses and also for blood clotting (Guyton and Hall, 2000). It is also known to activate a number of enzymes. The decreased level of serum calcium ion concentration by the alkaloid at all doses may be due to decreased mobilization from the bones and this may adversely affect several other calcium dependent activities within the system. It could also be due to a direct or indirect effect on hormones like calcitonin and parathyroid, which are needed to maintain calcium homeostasis.

The reduction in serum chloride ion concentration at 250 and 1000 mg/kg dose may be due to renal tubular dysfunction as corroborated by Mayne (1998). The significant increase in serum phosphate ion concentration at all doses may suggest glomerular dysfunction of the nephron. This agreed with findings by Chawla (1999).

Albumin is the major protein present within the blood and represents a reliable test to assess the degree of liver damage in animals. The increase in serum albumin by the alkaloid at all doses may be attributed to increased rate of hepatic synthesis of albumin without proportionate in the rate of its catabolism. Consequently, the amino acid pool may no longer be maintained within normal limits (Yakubu *et al.*, 2003b).

Serum globulins are a heterogeneous complex mixture of protein molecule whose role is to regulate osmotic pressure-homeostasis (Ganong, 2001). The

reduction in serum globulin concentration at 250 and 500 mg/kg body weight, may suggest a reduced rate of transportation of nutrients, defence, coagulation processes, buffering capacity of the blood and homeostasis as corroborated by Ganong (2001). Bilirubin is an important metabolic product of blood with biological and diagnostic values (Moudgil and Narang, 1989). The increase in 250 and 500 mg/kg body weight of the alkaloid could result when any portion of the biliary tree becomes partially or totally blocked by components of the alkaloid. This may affect the ability of the liver in transforming bilirubin to the bile pigment-bilirubin glucuronide (Naganna, 1989). Such increased bilirubin content is an indication of impairment of the liver's functional capacity as extensive liver damage lead to increase in serum total and conjugated bilirubin concentration (Moudgil and Narang, 1989).

The available evidence in this study indicated alterations in functional indices of the kidney and liver by alkaloid which might be responsible for some toxic and deleterious effects on the basic function of the organs.

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