

Phytochemical content of *Cnidoscolus aconitifolius* and toxicological effect of its aqueous leaf extract in Wistar rats

D. Akachukwu¹, P. N. Okafor¹, C. O. Ibegbulem²

ABSTRACT

Objective: Recent research on medicinal plants indicates that some plant extract are not only beneficial in the treatment of ailments, but could also be toxic. Aqueous extract of *Cnidoscolus aconitifolius* leaves is consumed for various reasons in traditional medicine. This study considers possible toxicological effects of aqueous leaf extract of *C. aconitifolius* using biochemical and histological indices of liver and kidney function as well as hematological indices in rats. **Materials and Methods:** In this study, phytochemical screening of *C. aconitifolius* leaves was carried out. Sub-acute toxicity tests were done using 20 male albino rats (4-6 weeks old; body weight, 132.20 \pm 48.10 g). They were grouped into four groups of five rats each and fed pelletized Grower's mash incorporated 100, 200, and 400 mg/kg body weight of the extract for 28 days. The control group received pelletized Grower's mash and water only. At the end of the feeding period, blood was collected through cardiac puncture for biochemical and hematological examinations. The liver and kidneys were also collected for histological studies and relative organ weights. **Results:** Phytochemical analysis of the plant leaves showed mean concentrations (%) of tannins, saponins, cyanogenic glycosides, alkaloids, phenols, flavonoids, and steroids at 0.14, 4.04, 0.003, 4.72, 0.19, 2.36, and 0.27, respectively. Incorporation of the aqueous extract into the diets of rats at 100, 200, and 400 mg/kg body weight for 28 days resulted in non-significant ($P > 0.05$) effects on the relative organ weights, total protein, globulin, aspartate aminotransferase, alanine aminotransferase and alkaline phosphatase activities, total and conjugated bilirubin, creatinine, platelet, red blood cell, white blood cell, neutrophils, lymphocytes, eosinophils, and basophils concentrations. However, blood monocyte concentrations were significantly decreased ($P < 0.05$) in rats administered 100 and 200 mg/kg body weight of extract. Histopathological studies showed that organs of the treated animals studied were not damaged. **Conclusion:** The results suggested that the aqueous leaf extract of *C. aconitifolius* had low toxicity at the concentrations investigated.

KEY WORDS: Aqueous leaf extract, *Cnidoscolus aconitifolius*, oral toxicity test, phytochemicals, rat

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INTRODUCTION

Traditional herbal medicines are naturally occurring plant derived substances, with minimal or no industrial processing, that have been used to treat illness within local or regional healing practices [1]. Plants are the oldest known sources of human and livestock healthcare and an important component of global biodiversity [2]. Medicinal plant can however be poisonous if wrong plant parts or wrong concentrations are used [3]. Nowadays, toxicity and safety of medicinal plants are the most discussed topics as herbal products have become popular worldwide.

Cnidoscolus aconitifolius is one of the plants used in traditional medical practice. There are two edible species of the plant; *Cnidoscolus chrymansa* and *C. aconitifolius*. Both appear similar morphologically, except in their leaf shape. The crop originated as a domesticated leafy green vegetable in the

Maya region of Guatemala, Belize, Southeast Mexico during pre-Cambrian period [4]. In ethnomedicine, decoctions of *C. aconitifolius* are administered as home-made prophylactic agent against a number of ailments such as acne, eye problems, kidney stones, and obesity. The antibacterial, anti-diabetic and ameliorative effects of various extracts of *C. aconitifolius* on anemia and osmotic fragility induced by protein energy malnutrition have been reported [5-8]. A wide variety of the folkloric use of this herb in ethnomedicine includes treatment for alcoholism, insomnia, gout, scorpion stings and as a cure for brain and vision improvement [9]. The currently observed rapid increase in the consumption of herbal remedies worldwide is stimulated by several factors, including the notion that all herbal products are safe and effective because they come from natural sources [10]. Despite the several claims to the efficacy of *C. aconitifolius* in the management of many diseases by herbalists, there is a dearth of information on the toxicological implications of the aqueous extract of the plant

leaves. This study analyzed the phytochemical contents of the oven-dried leaves of *C. aconitifolius*, conducted a rat feeding study using pelletized Grower's mash that were incorporated varying concentrations of its aqueous leaf extract for 28 days, and determined its effects on serological and hematological indices of the blood as well as histological features of the liver and kidneys.

MATERIALS AND METHODS

Procurement of Leaf Sample, Feeds and Rats

The fresh leaves of *C. aconitifolius* were collected at the living quarters of the National Root Crops Research Institute, Umudike, Nigeria. The leaves were authenticated by Mr. Ibe K. Ndukwe, a forester in the Herbarium Unit of the Department of Forestry and Environmental Management, Michael Okpara University of Agriculture, Umudike, Nigeria. The pelletized Grower's mash used (product of Vital Feed, Jos, Plateau State, Nigeria) was purchased at the Umuahia Market, Abia State, Nigeria. The 20 male albino rats (*Rattus norvegicus*) of the Wistar strain used in the study were purchased from the Animal Breeding Unit of the College of Veterinary Medicine, University of Nigeria, Nsukka, Nigeria.

All the chemicals used were of analytical-reagent grade.

Preparation of Extract

The leaves were washed in running tap water, oven-dried at 40°C and pulverized. The powder (200.0 g) was boiled for 20 min in 1000.0 ml of distilled water and allowed to cool for 2 h. The solution was filtered using Whatman No. 1 filter paper and concentrated at 45°C under reduced pressure using a rotary vacuum evaporator (Type: R-114A). The extract yielded 26.0 g dry matter which was kept at refrigeration temperature (4°C) until required for use.

Quantitative Analysis for Phytochemical Constituents

The total saponin content of the oven-dried leaves was determined using the double solvent extraction method [11]. Total tannin content was determined using ferricyanide method [12]. Total cyanide was determined by spectrophotometric method [13]. Total alkaloid was determined by alkaline precipitation gravimetric method [11]. Total phenol was determined by the colorimetric method [12]. Total flavonoid was determined by the gravimetric method [14] and total steroid was determined by the method described by Harbone [11].

Housing of Rats and Experimental Procedure

The United States National Institute of Health (NIH) guideline for animal use and care NIH [14] was followed.

The 20 male albino rats (*R. norvegicus*) of the Wistar strain used in the study were 4-6 weeks old and weighed 132.2 ± 48.1 g.

The rats were randomly distributed into four groups of five rats each and the groups labeled A, B, C, and D. They were housed in clean metabolic cages with provisions for feed and water troughs and acclimatized for 2 weeks.

Groups A, B, and C had pelletized Grower's mash incorporated with 100, 200, and 400 mg/kg body weight of the extract, respectively, while Group D served as the control and was administered the untreated feed. All the rats were placed *ad libitum* on tap water throughout the duration of the experiment and were sacrificed after 28 days.

Collection of Specimens

At the end of the experiment, the rats were euthanized in dichloromethane vapor and incisions made into their thoracic and abdominal cavities. Blood was collected by cardiac puncture using 10 ml hypodermic syringe and needle. Some blood (2.0 ml) was collected into ethylenediaminetetraacetic acid bottles and used for hematological studies while the remaining (6.0 ml) was allowed to clot and the sera collected by aspiration using Pasteur pipette for serological examinations. The organs (liver and kidneys) were excised from their abdominal cavities, washed in 10% formol saline, blotted dry, and weighed.

Determination of Biochemical Parameters

The concentrations of serum total protein, albumin, alkaline phosphatase (ALP), alanine aminotransferase (ALT), and aspartate aminotransferase (AST) activities, total and conjugated bilirubin, urea, and creatinine were determined using spectrophotometric method according to the method of Tietz [15], Cheesbrough [16] Recommendation German Society for Clinical Chemistry (DGKC) [17], Reitman and Frankel [18], Jendrassik and Grof [19], Weatherbun [20], Bartels and Böhmer [21]. Hematological parameters like red blood cells (RBC), hemoglobin (Hb), packed cell volume, white blood cell (WBC) count, neutrophils, monocytes, lymphocytes, eosinophils, basophils, and platelets were determined using the methods of Ochei and Kolhatkar [22].

Histopathological Studies

Histopathological studies were determined by the method described by Bancroft and Stevens [23]. Sections of the different organs from each group were collected in a sterile universal container containing 10 % neutral buffered formalin for 24 h. The tissues were dehydrated through graded series of ethanol (50%, 70%, 90% and 2 times of 100% ethanol) for complete dehydration, cleared in xylene to render the tissue transparent by removing ethanol from dehydrated sections and embedded in paraffin wax to provide a hard support for sectioning. The blocks were sectioned in the transverse plane at 7 μ m using LEICA microtome. Every third section was mounted on the glass slide and stained with hematoxylin and eosin. A drop of distrene tricresyl phosphate xylene mountant devoid of air bubbles was placed on the slide and cover slips were carefully placed over the slide. Photomicrographs of selected sections

were captured using Motic 2001 camera (Motican, UK) attached to a microscope at $\times 400$.

Statistical Analysis

Data were expressed as mean \pm standard deviation of five replicates. They were subjected to one way analysis of variance and means were separated with *post-hoc* multiple comparisons (option-least significant difference). $P \leq 0.05$ were considered to be significantly different.

RESULTS

The mean concentrations of tannins, saponins, cyanide, alkaloids, phenols, flavonoids, and steroids in the oven-dried leaves were 0.14%, 4.04%, 0.003%, 4.72%, 0.19%, 2.36%, and 0.27%, respectively [Table 1].

Administration of the extract at 100, 200 and 400 mg/kg body weight did not significantly ($P > 0.05$) alter the relative liver and kidney weights throughout the experimental period [Figure 1].

There were no significant ($P > 0.05$) effects on the serum total protein, globulin, and total and conjugated bilirubin contents of the animals throughout the experimental period [Figure 2]. There was a significant ($P < 0.05$) decrease in albumin concentration in animals administered 100 mg/kg body weight of extract [Figure 2].

Table 1: Concentrations of phytochemicals in *Cnidoscolus aconitifolius* leaves

| Phytochemical (%) | Concentration |
|-------------------|------------------|
| Saponins | 4.04 \pm 0.01 |
| Tannins | 0.14 \pm 0.00 |
| Cyanide | 0.003 \pm 0.01 |
| Alkaloids | 4.72 \pm 0.04 |
| Phenol | 0.19 \pm 0.00 |
| Flavonoids | 2.36 \pm 0.56 |
| Steroids | 0.27 \pm 0.28 |

Results are expressed as mean \pm SD of three replicates. SD: Standard deviation

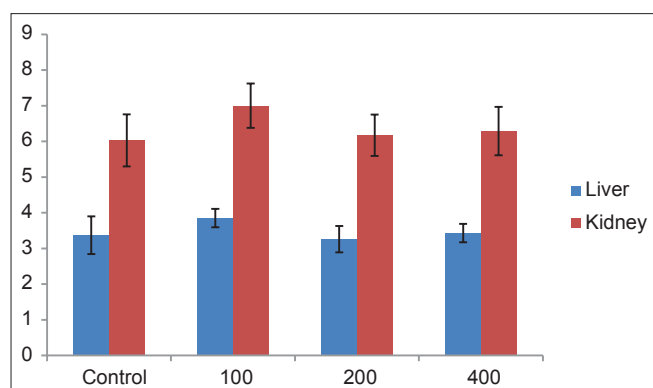


Figure 1: Effects of different concentrations of aqueous leaf extract of *Cnidoscolus aconitifolius* on relative organ weights of rats

The activities of ALP, AST and ALT were not significantly ($P > 0.05$) altered throughout the experiment [Figure 3].

The effects of administering 100, 200 and 400 mg/kg body weight of the extract on the kidney function indices of male rat is depicted in Figure 4. Serum creatinine contents of the animals were not significantly ($P > 0.05$) altered. Administration of 200 mg/kg body weight of extract significantly ($P < 0.05$) decreased the serum urea contents.

Administration of the different concentrations of the aqueous leaf extract did not significantly ($P > 0.05$) alter the concentrations of Hb, PCV, platelets, RBC, WBC, neutrophils, lymphocytes, eosinophils and basophils [Figure 5]. Administration at 100 and 200 mg/kg body weight significantly ($P < 0.05$) reduced the blood monocyte concentrations.

The photomicrographs of the liver and kidney of test and control animals were comparable [Figures 6-13].

DISCUSSION

Phytochemicals are known to perform many functions in plants and may exhibit different biochemical and pharmacological actions in animal species when ingested [24]. The presence of tannins in the plant could quicken the healing of wounds

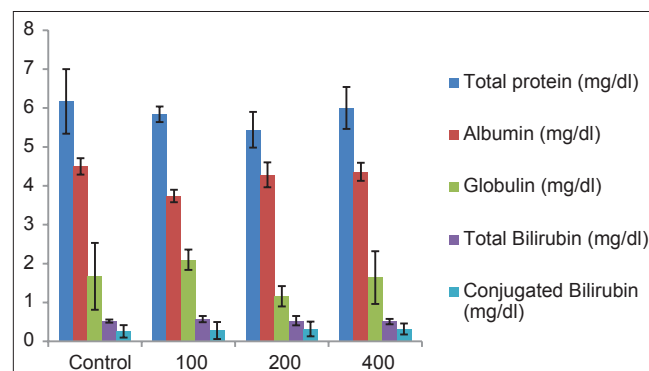


Figure 2: Effects of different concentrations of aqueous leaf extract of *Cnidoscolus aconitifolius* on some biochemical parameters of rats

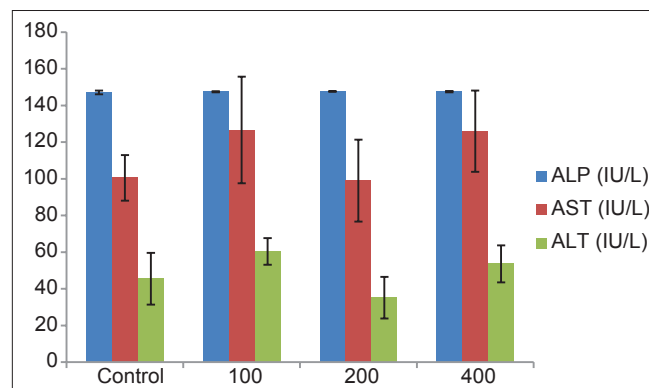


Figure 3: Effects of different concentrations of the aqueous leaf extract of *Cnidoscolus aconitifolius* on some liver function enzymes of rats

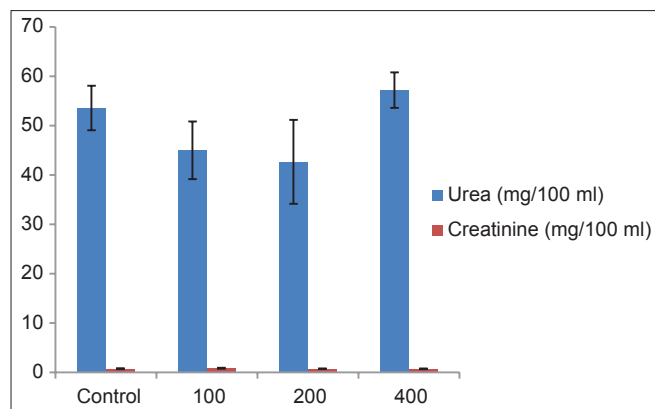


Figure 4: Effects of different concentrations of the aqueous leaf extract of *Cnidoscopus aconitifolius* on some kidney function indices of rats

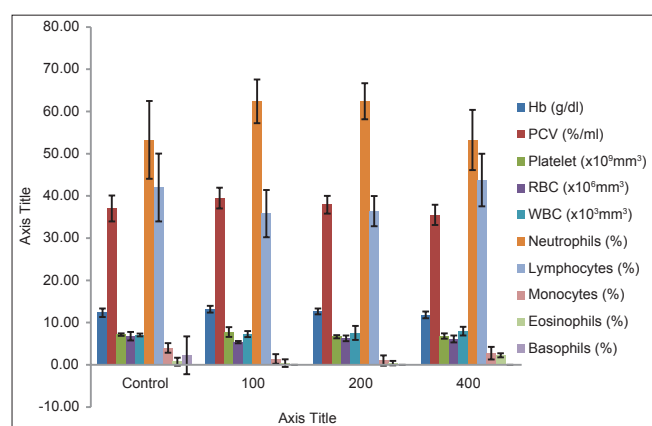


Figure 5: Effects of different concentrations of the aqueous leaf extract of *Cnidoscopus aconitifolius* on the hematological parameters of Wistar rats

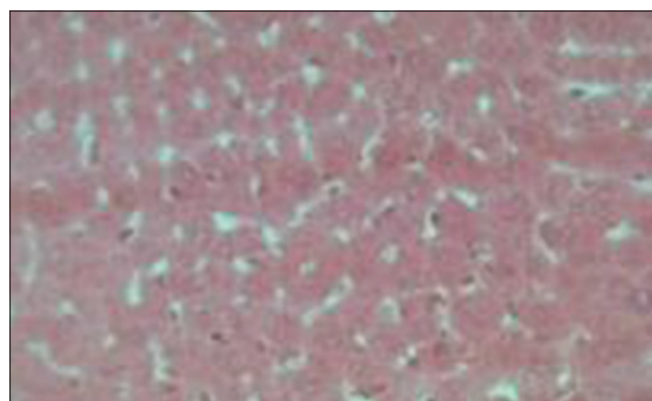


Figure 6: Photomicrograph of liver section from rat administered 100 mg/kg body weight of *Cnidoscopus aconitifolius* leaf extract for 28 days showing normal liver histology (H and E, x400)

and burns [25]. Saponins have both hemolytic and cholesterol binding activities [26]. The presence of tannins and saponins in this plant is in line with the work of Awoyinka *et al.* [6] who also reported the presence of tannins and saponins in both the aqueous and ethanolic extract of *C. aconitifolius*. The level of cyanide (3.00 mg/100 g) in the oven-dried leaves of *C. aconitifolius* [Table 1] can be reduced by boiling to release

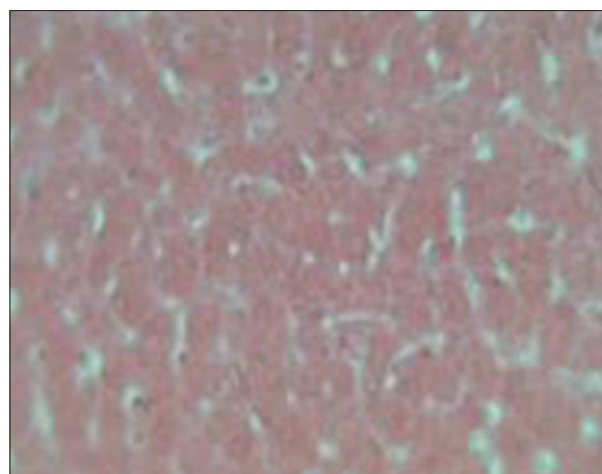


Figure 7: Photomicrograph of liver section from rat administered 200 mg/kg body weight of *Cnidoscopus aconitifolius* leaf extract for 28 days showing normal liver histology (H and E, x400)

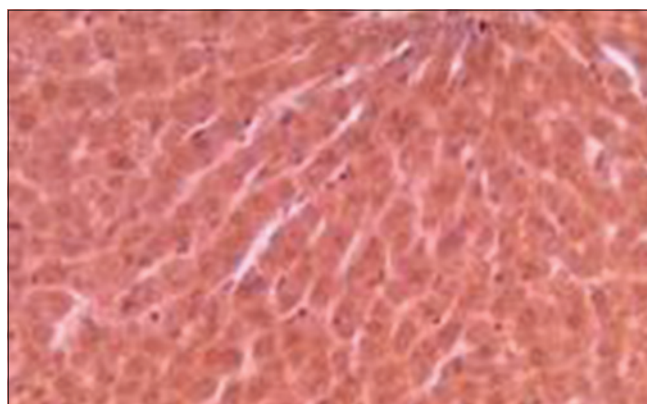


Figure 8: Photomicrograph of liver section from rat administered 400 mg/kg body weight of *Cnidoscopus aconitifolius* leaf extract for 28 days showing normal liver histology (H and E, x400)

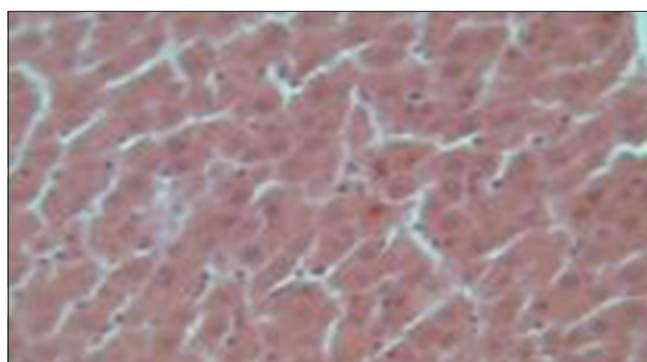


Figure 9: Photomicrograph of liver section from rats in the control group (H and E, x400)

the volatile hydrogen cyanide (HCN) thereby making it safer for consumption. In various species of animal, the lethal dose of HCN are generally reported to be between 0.66 and 15 mg/kg body; the acute lethal dose of cyanide for human beings is reported to be 0.5-3.5 mg/kg weight [28]. This implies that a 70.0 kg human would need to consume 3.06 kg of the oven-dried leaves (at 105 mg cyanide/70.0 kg body weight) at once to be poisoned,

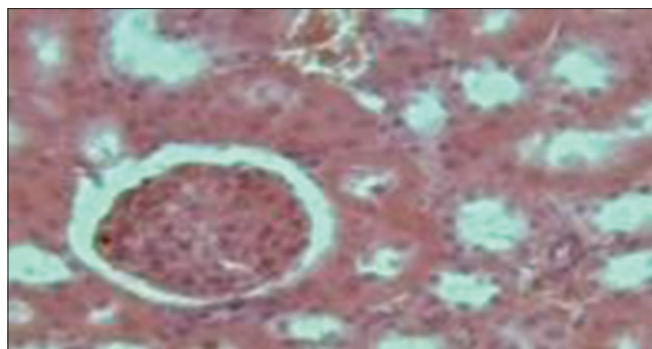


Figure 10: Photomicrograph of kidney section from rat administered 100 mg/kg body weight of *Cnidoscolus aconitifolius* leaf extract for 28 days showing normal kidney histology (H and E, x400)

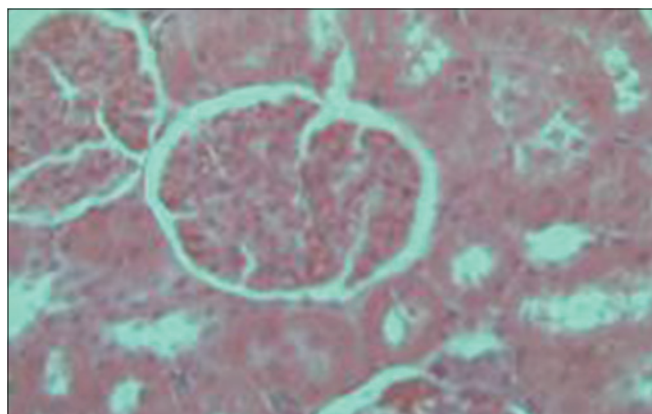


Figure 11: Photomicrograph of kidney section from rat administered 200 mg/kg body weight of *Cnidoscolus aconitifolius* leaf extract for 28 days showing normal kidney histology (H and E, x400)

and this is near impossible to consume (weight of one fresh leaf = 6.55 g and weight of oven-dried = 1.301 g). The presence of alkaloids in the plant suggests that it may have analgesic, antispasmodic and anti-bacterial properties [29]. The presence of phenol in the plant suggests the ability to block specific enzymes that cause inflammation [30]. Flavonoids are antioxidants and free radical scavengers and also show anti-allergic, anti-inflammatory, antimicrobial properties [31]. Steroids develop and control the reproductive tract in humans [32]. The presence of these phytochemicals in the plant has supported its varied uses in traditional medicine practice.

The insignificant ($P > 0.05$) change in the relative liver and kidney weights in this study [Figure 1] may be an indication that the extract did not cause any inflammation at the cellular levels of the organs investigated.

Albumin and globulin are mixtures of molecules that can be used to indicate the integrity of glomeruli and regulation of osmotic pressure, respectively [33]. The significant ($P < 0.05$) decrease in albumin concentration in animals administered 100 mg/kg body weight of extract [Figure 2] may be associated with cirrhosis of liver, nephritic syndrome, malnutrition, and malignancy as reported by Mathotra [34]. Bilirubin, the yellow breakdown product of normal haem catabolism is a useful tool

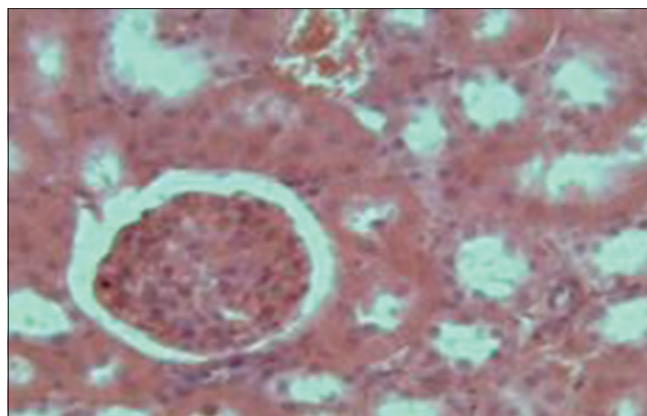


Figure 12: Photomicrograph of kidney section from rat administered 400 mg/kg body weight of *Cnidoscolus aconitifolius* leaf extract for 28 days showing normal kidney histology (H and E, x400)

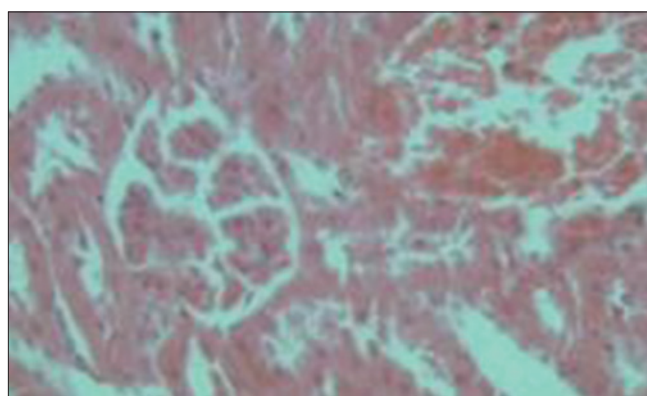


Figure 13: Photomicrograph of kidney section from rats in the control group (H and E, x400)

in the assessment of hemolytic anemia and excretory function of the liver [35]. However, other parameters assayed for in this study did not support any of these disease conditions. The result of the total protein and globulin also suggested that the extract had no adverse effect on the biosynthetic abilities of the liver.

The activities of serum ALP, ALT, and AST were not altered throughout the experimental period [Figure 3]. This suggested that these enzymes carried out their biochemical activities unperturbed.

The significant decrease in serum urea concentration noticed after the administration of the 200 mg/kg body weight of extract [Figure 4] may be due to factors that are beyond the scope of the study. The insignificant change in serum creatinine concentration suggested that the kidneys were not compromised. Renal function indices such as serum urea and creatinine can also be used to evaluate the functional capacity of the nephrons of animals [36].

RBC count was not significantly altered in this study [Figure 5] suggesting that neither the incorporation of Hb into RBCs nor the morphology and osmotic fragility of the RBCs were

affected [36]. The non-significant ($P > 0.05$) effect of the extract on the levels of platelets suggested that the clotting ability of the blood was not compromised.

The levels of WBCs, neutrophils and lymphocytes were not altered during the experimental period, indicating that the immune systems of the experimental rats were not challenged by the extract.

Histopathological examination of all the tissue showed that the extracts did not cause any pathological lesions [Figures 6-13]. This supported the submissions made in Figure 1 where the relative weights of the liver and kidneys were shown not to have been affected by the different levels of the extract.

CONCLUSION

The aqueous leaf extract of *C. aconitifolius* were not toxic at the concentrations orally administered in this study.

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REFERENCES

- Tilburt JC, Kaptchuk TJ. Herbal medicine research and global health: An ethical analysis. *Bull World Health Organ* 2008;86:594-9.
- Lambert JD, Ryden PA, Esikuri EE. Capitalizing on the bioeconomic value of multi-purpose medicinal plants for rehabilitation of dry lands in Sub-Sahara Africa. The International Bank for Reconstruction and Development. Washington, D.C: The World Bank; 2005. p. 11-4.
- Fronhe D. Plant intoxicants – Is there a risk from herbal preparation? *J Phytother* 1999;20:201-2.
- Ross-Ibarra J, Molina-Cruz A. The ethnobotany of chaya (*Cnidoscopus aconitifolius*): A nutritious maya vegetable. *J Ethnobotany* 2003;56:350-64.
- Sarmiento-Franco L, McNab CA, Quijano-Cervera R, Reyes-Ramirez RR. Effect of age of growth on chemical composition of chaya (*Cnidoscopus aconitifolius*) leaves. *J Sci Food Agric* 2003;3:257-69.
- Awoyinka AO, Balogun JO, Ogunnowo AA. Phytochemical screening and *in vitro* bioactivity of *Cnidoscopus aconitifolius* (*Euphorbiaceae*). *J Med Plant Res* 2007;19130:063-5.
- Oladeinde FO, Kinyua AM, Laditan AA, Michelin R, Bryant JL, Denaro F, et al. Effect of *Cnidoscopus aconitifolius* leaf extract on the blood glucose and insulin levels of inbred type 2 diabetic mice. *Cell Mol Biol (Noisy-le-grand)* 2007;53:34-41.
- Oyagbemi AA, Odetola AA, Azeze OI. Ameliorative effects of *Cnidoscopus aconitifolius* on anaemia and osmotic fragility induced by protein energy malnutrition. *Afr J Biotechnol* 2008;11:1721-6.
- Atuahene CC, Poku-Prempeh B, Twun G. The nutritive values of *Chaya* leaf meal (*Cnidoscopus aconitifolius*): Studies with broilers chickens. *Anim Feed Sci Technol* 1999;77:163-72.
- Said O, Khalil K, Fulder S, Azaizeh H. Ethnobotanical survey of medicinal herbs of the Middle Eastern region. *J Ethnopharmacol* 2004;83:251-6.
- Harbone JB. *Phytochemical Methods. A Guide to Modern Techniques of Plant Analysis*. London: Chapman and Hall Limited; 1973. p. 11-21, 49-189.
- Pearson D. *The Chemical Analysis of Foods*. 7th ed. London: Churchill Livingstone; 1976. p. 7-14, 572.
- Bradbury MG, Egan SV, Bradbury JH. Determination of all forms of cyanogens in cassava roots and cassava products using picrate paper kits. *J Sci Food Agric* 1999;79:593-601.
- United State Department of Health, Education, and Welfare. Public Health Service; NIH. *Guide for the Care and Use of Laboratory Animals*. Washington, DC: National Academics Press; 1985, Revised 1996.
- Tietz NW. *Clinical Guide to Laboratory Test*. 3rd ed. Philadelphia: W.B. Saunders Co.; 1995. p. 518-9.
- Cheesbrough M. *District Laboratory Practice in Tropical Countries Part 1*. 2nd ed. Hong Kong: Sheck Wah Tong Printing Press; 2005. p. 310-26.
- DGKC. Recommendation of the Deutsche Gesellschaft für Klinische Chemie (German Society for Clinical Chemistry) optimized standard method for the determination of alkaline phosphatase. *J Clin Chem Clin Biochem* 1972;10:182.
- Reitman S, Frankel S. A colorimetric method for the determination of serum glutamate oxaloacetate transaminase. *Am J Clin Pathol* 1957;28:53-6.
- Jendrassik L, Grof P. *Biochemistry*. London: Churchill; 1938. p. 297-81.
- Weatherbun MW. Measurement of urea level in serum. *Anal Chem* 1967;39:971.
- Bartels H, Böhmer M. Micro-determination of creatinine. *Clin Chim Acta* 1971;32:81-5.
- Ochei J, Kolhatkar A. *Medical Laboratory Science: Theory and Practice*. New Delhi: Tata McGraw-Hill; 2005. p. 281-3.
- Bancroft JD, Stevens A. *Theory and Practice of Histological Techniques*. Edinburgh, London: Churchill Livingstone; 1990. p. 86.
- Farquar JN. Plant sterols, their biological effects in human. *Handbook of Lipids in Nutrition*. Boca Rotan, FL: CRC Press; 1996. p. 101-5.
- Sodipo OA, Akinyi JA. Studies on certain characteristics of extracts from bark of *Pansinystalia macrucas* (*K. Schum*) pierre exbeille. *Glob J Pure Appl Sci* 2000;6:83-7.
- Stray F. *The Natural Guide to Medicinal Herbs and Plants*. London: Tiger Books International; 1998. p. 12-6.
- FSAZ. Cyanogenic Glycoside in Cassava Bamboo Shoots; a Human Risk Assessment. Technical Report, No 28. New Zealand: Food Standard Australia; 2005.
- Mbagwu FN, Okafor VU, Ekeanyanwu J. Phytochemical screening on four edible legumes (*Vigna subterranean*, *Glycine max*, *Arachis hypogaea*, and *Vigna unguiculata*) found in Eastern Nigeria. *Afr J Plant Sci* 2011;5:370-2.
- Cushnie TP, Lamb AJ. Antimicrobial activity of flavonoids. *Int J Antimicrob Agents* 2005;26:343-56.
- Okwu DE. Evaluation of the chemical composition of indigenous spices and flavouring agents. *Glob J Pure Appl Sci* 2001;7:455-9.
- Owolagba GK, Awuoro OG, Oduola T, Adeosun OG, Enaowho TN, Wilson JJ, et al. Histological observation of 24 hrs oral administration of the extract of *S. bicolor* on albino rat. *Plant J Cell Anim Biol* 2009;3:001-3.
- Ganong WF. *Review of Medical Physiology*. 20th ed. London: Lange Medical Books/McGraw-Hill Medical Publishing Division; 2001. p. 414-7.
- Mathotra VK. *Practical Biochemistry for Students*. 3rd ed. New Delhi: Jaypee Brothers Medical; 1989. p. 94-7.
- Yakubu MT, Bilbis LS, Lawal M, Akanji MA. Evaluation of selected parameters of rat liver and kidney function following repeated administration of Yohimbine. *Biokemistri* 2003;15:50-6.
- Adebayo JO, Adesokan AA, Olatunji LA, Buoro DO, Soladoye AO. Effects of ethanolic extract of *Bougainvillea spectabilis* leaves on haematological and serum lipid variables in rats. *Biokemistri* 2005;17:45-50.

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