

Some of the Effects of Aqueous Leaf Extract of *Cnidoscolus aconitifolius* (Euphorbiaceae) on the Morphology and Histology of the Kidney and Liver of Sprague Dawley Rat.

DA Adekomi, AA Tijani, TD Adeniyi and JO Olajide

Department of Anatomy, Faculty of Basic Medical Sciences, University of Ilorin, Ilorin, Nigeria.

Abstract

Cnidoscolus aconitifolius belongs to a group of arborescent shrubs. It has continued to be used as food, medicine and ornamental plant till date. A wide variety of claims have been made for its medicinal efficacy as a treatment for numerous ailments ranging from its ability to strengthen fingernails and darken gray hair to curing alcoholism, insomnia, gout, and scorpion stings. This study was to elucidate the effect of aqueous leaf extract of *Cnidoscolus aconitifolius* commonly used as food supplement on the kidney and liver of Sprague Dawley rats.

Thirty-two Sprague Dawley rats of both sexes (with an average weight of 235g) were randomly assigned into one control group (n=8) designated as A, while the extract treated groups consists of 3 subgroups and were designated as B, C, and D. the animals in the extract treated groups B, C and D were administered 600mg/kg, 400mg/kg and 200mg/kg bwt of the extract respectively, while the control group received equal volume of phosphate buffered saline (PBS). The administration was done orally using an orogastric tube for seven days (7d). Twenty-four hours after the last administration, all the animals were sacrificed by cervical dislocation. Laparotomy was performed and the liver and kidney excised, trimmed free of fat, rinsed in cold phosphate buffered saline solution. The liver was quickly fixed in 10% formolsaline, while the kidney was fixed in Bouin's fluid for histological processing. The histological findings after Hematoxylin and Eosin (H&E) staining showed that the extract treated sections the Kidney and Liver has no compromising effect on the cellular integrity of the studied organs. There were no degenerative changes and no intercellular vacuolations in the renal cortex and liver parenchyma. These findings indicate that the administration of aqueous leaf extract of *Cnidoscolus aconitifolius* has no adverse effects on hepatic and renal morphology of the treated Sprague Dawley rats. It is therefore recommended that further studies at corroborating these observations be carried out.

Key words: - *Cnidoscolus aconitifolius*, Kidney, Liver, Hematoxylin and Eosin, Laparotomy.

Introduction

The use of plant for nutritional and therapeutic purposes has been dated back to the 'old world' where trial and error methods were employed as the pharmaceutical means to treating ailments and diseases. For animals, man, and plant to survive, they all need food which is either taken (in animals and man) as cooked, raw, or as supplements. In plant, most of the needed nutrients are obtained from the soil. Most of animal's and man's nutrition are derived from plants. *Cnidoscolus aconitifolius* has been used in folklore treatment and management of myriad of ailments and the leaves are widely consumed within many localities in Nigeria.

The most challenging diseases in today's healthcare system involve complex cascading mechanisms. In contrast to current combination therapies, plant based drugs contains a mixture of multiple components thereby saving considerable time and expense^[1].

Nowadays, many herbs and plants have been researched and some of their attributed empirical and folklore properties proved to be true. Various nutritional supplements from plants have been implicated as causing deleterious effects on the consumer (health i.e. clinical status and behavioral pattern)^[2]. Most of these plants often display a wide range of biological and medicinal properties such as anti-inflammatory, anti-hemorrhagic, fertility enhancer, laxative, sexual enhancer, anti-microbial, anti-fungal e.t.c^[3,4].

Cnidoscolus aconitifolius is a native of the East Indies, and found its way to the New World from China. It has spread throughout the tropical world and it is one of the best tropical spinach widely adapted to a variety of soils and climates. It is particularly abundant in India, Malaysia, and the Philippines, but it is also seen throughout tropical Africa, the Caribbean, and tropical South America.

The plant is a common market place product, with its popular leafy shoot often found in vegetable stew. Both the young shoots and stems are eaten. It is an excellent source of calcium and iron; good source of vitamins A, B, and C, with a high roughage value. The roots are employed as rubefacient. Poultice of the leaves are used to reduce local swelling. Sap from the

Correspondence to:

D. A. Adekomi,
Department of Anatomy,
Faculty of Basic Medical Sciences,
P. M. B. 1515, University of Ilorin, Ilorin, Nigeria.
+23480-3844-1671/ +23480-7656-3746
titemitope@yahoo.co.uk

plant is applied to acne eruptions to reduce inflammation. Pulped leaves of the plant when applied to boils and ulcers, hasten suppuration. Leaf-juice of Malabar nightshade mixed with butter is soothing and cooling when applied to burns and scalds^[5].

From the nutritional point of view, it is known that good dietary habit can delay the onset of many cardiovascular diseases and diseases affecting nervous system. Phytochemical analysis of *Cnidioscolus aconitifolius* shows that its plant contain saponins A, B, C, and D, oleanane-type triterpenes oligoglycosides, together with betavulgaroside 1, spinacoside C, and momordins IIb and IIc^[5]. Demand for nutritional food supplements and the act of self-medication calls for extensive study into the nutritional and medicinal properties of the plant alongside its possible side effects.

Liver is the largest gland in the mammalian body. It has both macro-and micro- structures. The micro-morphology is highlighted by the lobules and their sinusoids that are lined with active phagocytes known as Kupffer cells, central veins and hepatocytes plates that are closely associated with the terminal lymphatics and bile canaliculi^[6, 7, 8, and 9]. These morphological features have enabled the liver to carry out a very vast physiological functions such as: production of bile, which helps carry away waste and break down fats in the small intestine during digestion, production of certain proteins for blood plasma, production of cholesterol and special proteins to help carry fats through the body, conversion of excess glucose into glycogen for storage (glycogen can later be converted back to glucose for energy), regulation of blood levels of amino acids, which form the building blocks of proteins, processing of hemoglobin for use of its iron content (the liver stores iron), conversion of poisonous ammonia to urea (urea is an end product of protein metabolism and is excreted in the urine), clearing the blood of drugs and other poisonous substances, regulating blood clotting, resisting infections by producing immune factors and removing bacteria from the bloodstream. The hepatocytes have metabolic functions that deal with very essential processes such as detoxification, deamination, transamination, removal of ammonia in the form of urea, biosynthesis and release of the non-essential amino acids and plasma proteins with the exception of immuno gamma globulins, gluconeogenesis, storage of glycogen, conversion of carbohydrates and proteins into lipids, synthesis of lipoproteins, phospholipids and cholesterol, oxidation of fatty acids, storage of iron in the form of ferritin as well as storage of vitamins A, D and B₁₂. The hepatocytes are efficient in uptaking blood bilirubin to conjugate it mainly with glucuronic acid and then excrete it in the bile. Potassium and sodium salts of the conjugated bile acids are also excreted into

the bile^[10, 11, 12, 13, and 14]. About 15 % of the adult life erythropoietin is synthesized by the hepatic Kupffer cells and hepatocytes^[15, 16, and 13].

The kidneys are dark-red, bean-shaped organs. The functional and structural unit of the kidney is the nephron. The kidneys are the main source (about 85%) of the circulating amount of erythropoietic hormone. The kidney is capable of carrying out several essential functions. These include: maintenance of body fluids volumes and their electrolytes within normal limits. It also participates in regulation of acid-base balance and blood pressure, excretion of the non-protein nitrogenous compounds, such as urea and uric acid, elimination of endogenous toxic waste agents. Through the kidneys the organism can get rid of hazardous compounds such as the endogenously produced creatinine and many metabolites of hormones as well as ingested and administered chemicals (e.g. drug). Formation of the active form of vitamin-D takes place in the kidney. Gluconeogenesis, that is synthesis of glucose from non-carbohydrate source, is one of the kidney functions most especially under conditions of food deprivation and/or starvation^[16, 12, 14, and 17].

Although, there hasn't been any report on the harmful effect of the highly consumed plant on the body, it was known from laboratory testing, that high intake of food supplements and additive from certain plants source may have deleterious effect on the body^[2]. In view of the reputed efficacies of this vegetable plant, the aim of this study was to elucidate some of the implications of *Cnidioscolus aconitifolius* on the histology of the kidney and liver in albino Sprague Dawley rats.

Materials And Methods

Collection of plant and preparation of plant extracts

Fresh leaves of *Cnidioscolus aconitifolius* were collected from the Botanical garden of the University of Ilorin, Ilorin, Kwara State, Nigeria and were authenticated at the Department of Plant Science University of Ilorin, Nigeria. The leaves of the plant were air-dried at 50°C. The dried plant material were measured using Gallenkomp (FA2104A, England) electronic weighing balance and grinded with Blender/Miller III, (model MS -223, China). Sixty grams (60g) of the grinded plant sample were later soaked in 600 ml of phosphate buffered saline (PSB) for 24 hours (Emeka and Clement, 2005)^[18] at room temperature with constant shaking on a shaker (Stuart Scientific Orbital Shaker, UK), and then filtered through silk cloth (Mostofa et al, 2007)^[19], the filtrate was concentrated using a rotary evaporator (Rotavapor® R-210) at 42- 47°C. The residue of the extract obtained was kept in a capped sample bottle and stored in a refrigerator until used.

Toxicity evaluation:

The method of Lorke (1983)^[20] was modified and used to determine the dose of the extract that would be lethal to 50% of the population of the experimental animals. In a pilot test, three dose (550, 500 and 450 mg/kg) were chosen, from which, doses of 300, 200 and 100mg/kg respectively were administered to one animal per group in the second phase.

Animal care and experimental design:

Thirty-two Sprague Dawley rats of the first filial generation were randomly assigned into three extract treated (n=24) and one control (n=8) groups. Body weights of the animals were taken at day 0 (0dy) prior to *Cnidoscolus aconitifolius* extract treated, and on daily basis thereafter, for 2 weeks using a digital weighing scales (Saltun® EK5055Max).

The animals in the extract treated groups designated as A, B, and C were administered orally once daily (07:00am–07:45am) using plastic syringes attached to metal oropharyngeal cannula with 300mg, 200mg and 100mg per kilogram body weight of the aqueous extract of *Cnidoscolus aconitifolius* for fourteen days (14dys) respectively, while the animals in the control group (group D) were administered with equal amount of phosphate buffer saline (PBS).

All the experimental animals were housed in clean cages of dimensions 33.0×20.5×19.0 cm contained in well ventilated standard housing conditions (temperature: 28–31°C; humidity: 50–55%). Their cages were cleaned everyday. All animals were observed for illness, abnormal behavior and morphological anomalies.

All experimental procedures followed the recommendations provided in the “Guide for the Care and Use of Laboratory Animals” prepared by the National Academy of Sciences and Published by the National Institutes of Health^[21].

The rats were fed with standard rat chow at a recommended dose of 100 g/kg as advised by the International Centre of Diarrheal Disease Research, Bangladesh (ICDDR, B) daily. Drinking water was supplied ad libitum.

Twenty-four hour after the last administration, all the experimental animals were sacrificed using cervical dislocation^[22]. Laparotomy was performed, the kidney and liver were excised, trimmed free of fat, rinsed in cold phosphate buffered saline solution. The liver was quickly fixed in 10% formolsaline, while the kidney was fixed in Bouin's fluid^[23] for routine histological study.

Histological procedure:

After fixing the kidney and liver of both the extract treated and control animals, the tissues were processed for Hematoxylin and Eosin^[24] staining techniques. After fixation, the tissues were embedded in paraffin wax; serial sections of 5µ thick were

produced using Leitz Rotary microtome (Leitz 1512 Microtome). The sections were mounted in DPX and examined with the aid of Olympus (XSZ-107BN, No. 071771) binocular light microscope. The photomicrograph of each slide was taken with a Nikon Digital Camera DXM1200F (Nikon, Japan) for subsequent histological analysis.

Statistical Analysis

Numerical data were subjected to statistical analysis in order to test the significance of differences between means. The test of choice was the student t-test. The tabulated figures are presented as mean ± standard error of mean (SEM). Calculated $P < 0.05$ was considered enough to indicate significance of difference between compared means. Computation of the statistical analysis was carried out with the aid of the Microsoft Excel^[10] program (Microsoft Office XP Professional, Microsoft Corporation, USA).

Results

Behavioral responses of the extract treated animals;

There were neither aggressive nor erratic resistances for handling in the extract treated animals through out the duration of this study. The behavioral responses of the animals in all the extract treated groups were as it were in the control group.

Body weight;

The effects of different doses of the aqueous extract of *Cnidoscolus aconitifolius* leaves on the body weight of the animals in the extract treated groups where compared with that of the animals in the control group at termination of treatment (Fig III). The body weights of the extract treated animals were observed to increase significantly ($p < 0.05$) in comparison with the control animals. Among the extract treated groups, the highest body weight was recorded in the group (A) that was administered with 300mg/kg body weight of the leave extract followed by group (B) administered with 200mg/kg body weight of the leave extract and then group (C) with 100mg/kg body weight. Thus the administration of the aqueous extract of *Cnidoscolus aconitifolius* leaves increases the body weight of the extract treated animals in a dose dependent pattern.

Gross Observation

No gross alterations were observed in the morphology of the kidney and liver of the animals in the extract treated groups and those in the control groups twenty-four hours (24hrs) after the termination of experimental procedure. The kidney and liver (with all the component parts) of the animals in both the extract treated and control groups appeared morphologically normal.

Microscopic Observations of the Liver

When the sections obtained from the histological processing of the liver was viewed under the microscope it was observed that the sections

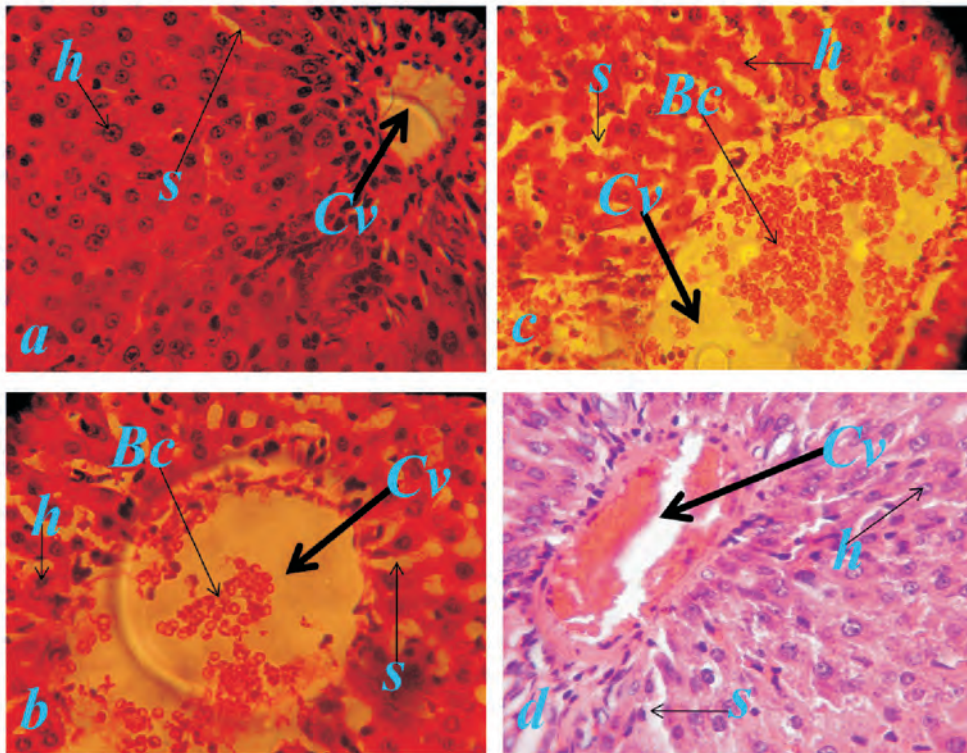


Figure 1; Showing the photomicrograph of the liver in the extract treated groups (a, b, and c) and the control group (d). **Legends;** the hepatocyte (h), the sinusoid (s), the central (centrolobular) vein (Cv) and blood cells (Bc). (H&E x 1200)

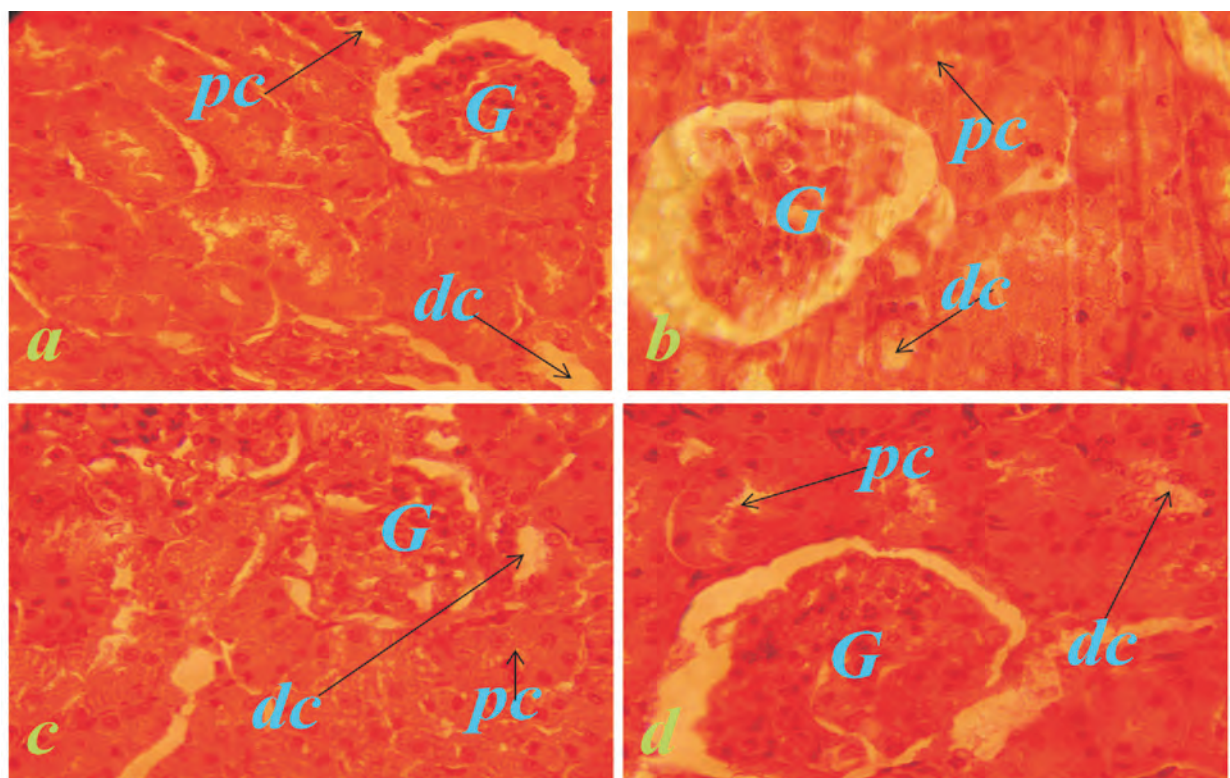


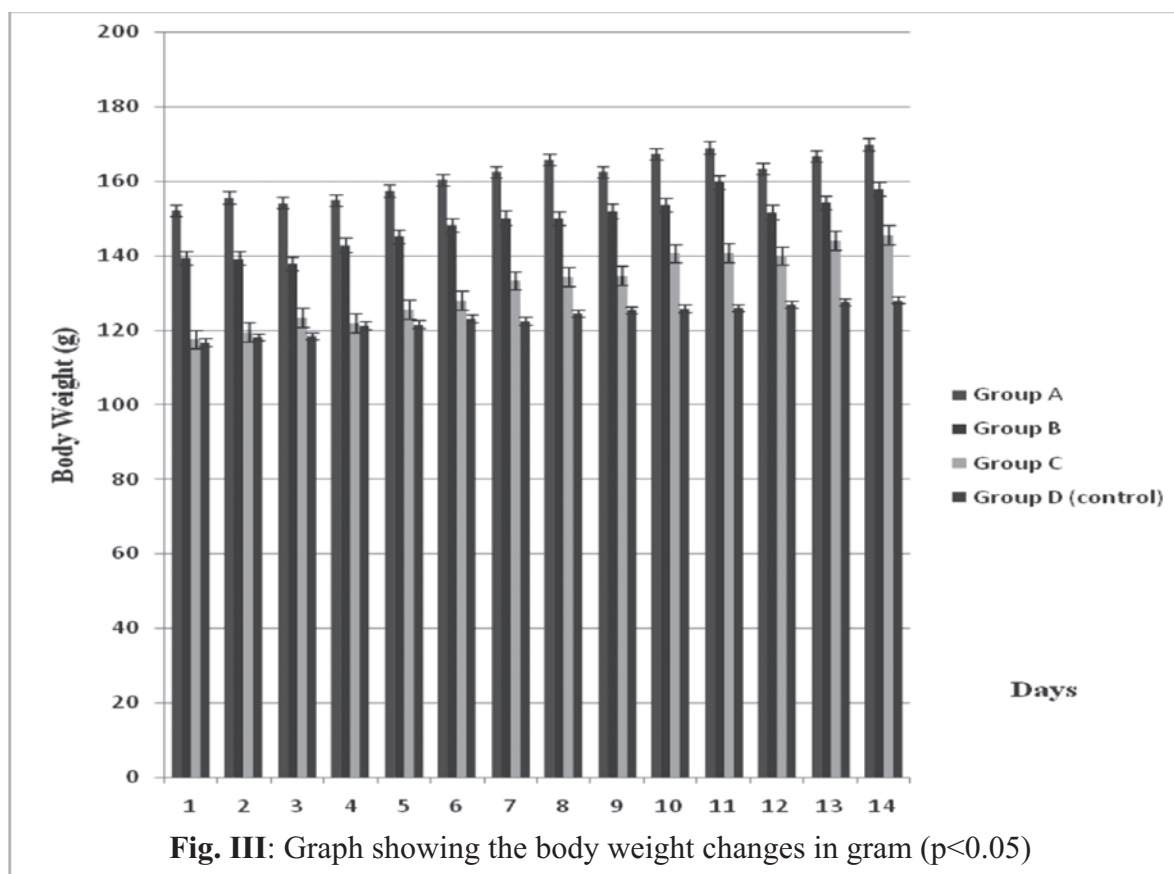
Figure II; Showing the photomicrograph of the cortical portion of the kidney in the extract treated groups (a, b, and c) and the control group (d). **Legends;** G= glomerulus, pc= proximal convoluted tubule, dc= distal convoluted tubule. (H&E x 1200)

conform to normal histological features. The sinusoids in the sections of the treated animals are devoid of occlusions and are not distorted. The extent of conformity to normal histological outline was observed to be higher in the extract treated groups compared with

the control group (Fig 1).

Microscopic Observations of the Kidney

The histological preparations of kidney from the extract treated and control rats showed that the various histological segments of kidney tubules were



well preserved. Abundant glomeruli, nephrons with interspersed blood capillaries were also clearly seen. Various regions of kidney tubules appeared to be normal without any change in mesangial thickening or hyaline deposition. The renal parenchyma showed no evidence of distortion of any kind. It is evident at this magnification ($\times 1200$) that the tubules constitute the bulk of the parenchyma with different shapes, diameters and staining intensity (Fig II).

Discussion

A fourteen days study on the effects of *Cnidioscolus aconitifolius* leaves on the kidney and liver was investigated to highlight the possible histological implications that could occur following its consumption. Using the Olympus binocular light microscope (XSZ-107BN, No. 071771), the results obtained from the sections of the kidney (extract treated animals) stained with hematoxylin and eosin (H & E) revealed that the plant extract is non-toxic and has no negative adverse effects on the histology of the kidney in Sprague Dawley rats. There were no observable cellular injury affecting the epithelial parenchyma and endothelial cell. The renal cortex was devoid of; tubular necrosis and glomerular hemorrhage. There were no histological derangement, degenerative changes, and apoptosis and/or necrosis of the cells within the convoluted tubules of the tubular

system of the sections obtained from the extract treated animals. Also, the histology of the liver sections obtained from the animals treated with the leaf extract was also observed with the same model of microscope and it was observed that the histology of the liver was well preserved. There were no features of cellular degeneration, as the sinusoids in the sections of the extract treated animals were devoid of congestions, occlusions and were not erratically distorted. There were no necroses or oedema of the hepatocytes. There was no evidence of Mallory bodies in the liver parenchyma. The portal tracts were free of inflammations. The hepatocytes did not appear swollen, shrunken or distorted. The central veins were also intact.

Many herbal preparations have been known to exhibit renal tubular necrosis showing extensive interstitial fibrosis and severe tubular loss which are prominent in the outer cortex. Vanherweghem et al. (1993)^[25] and Mengs et al. (1982)^[26] showed that specific acid from herbal preparation are nephrotoxic in female Sprague Dawley rats which rapidly developed renal tubular necrosis and renal failure.

The shapes of the cells within the convoluted tubules of the animals in the control group appeared cuboidal or columnar. These shapes were maintained in the extract treated sections after fourteen days of administration of the aqueous extract of *Cnidioscolus*

aconitifolius leaves (Figs.1A-D). This might enhance the proper functioning of the convoluted tubules in the exchange of fluids and ions, reabsorption of the components of the glomerular filtrate, control of acid-base balance and more importantly, in urine concentration (Steven and Lowe, 2005)^[27].

According to Steven and Lowe, (2005)^[27], biochemical dysfunctions may result from impaired tubular functions as a result of failure of excretion of H^+ and K^+ ions. This then makes the blood to have an increase in the concentration of H^+ ions (acidosis) and K^+ ions (hyperkalemia), coupled with the retention of nitrogenous waste materials as a result of impairment in the function of the glomerulus, this may ultimately lead to renal failure (e.g. acute tubular necrosis, acute and chronic renal failure).

Response of cell to neurotoxins has been implicated as one of the major cause of cell death and this may cause apoptotic death pathway within the kidney and liver cells (Waters, 1994)^[28]. Genetic mechanism has also been implicated in cell death in that cell response to neurotoxins occurring as controlled events involving cascade and/or succession of activated enzymes (Waters, 1994)^[28]. In some instances, the kidney and/or the liver may be damaged as a result of oxidized agents known as free radicals generated in the body by the oxidation of nutrients derived from food substances and other chemical reactions taking place within the cells (Anderson, 2004)^[29]. Cellular degeneration in many organs of the body has been observed to be one of the major causes of cell death, which may occur either as apoptotic and necrotic cell death. These two form of cellular degeneration differs from each other morphologically and/or biochemically (Wyllie, 1980)^[30].

Cell death occurring pathologically or accidentally is regarded as necrotic and could result from extrinsic implications and/or disturbances to the cell and these may include toxic or traumatic effects (Farber et al, 1981)^[31]. Processes involved in cellular necrosis which may lead to cell death include compromise and/or disruption of the structural and functional potentials of the various membranes in and within the cell. Necrosis of the cell is not induced by intrinsic stimuli to the cells as observed in programmed cell death, but by an abrupt environmental disturbances and deviation from the normal physiological conditions, factors and functions (Ito, et al., 2003)^[32]. Gradual loss of the physiological functions of kidney and/or liver over time can lead to chronic diseases (e.g. acute tubular necrosis, acute and chronic renal failure). According to Guyton and Hall, (2000)^[12], one of the main functions of the kidney is to remove metabolic and/or toxic wastes and excess water from the body. In chronic kidney disease, this physiological function is gradually compromised as the clinical condition or

state or status of the chronic kidney disease progressively gets worse over time. In the early stages of chronic kidney disease, there may be no observable symptoms; this is because the loss of function (in the kidney) usually takes months or years to occur. At the final stage of chronic kidney disease known as end-stage renal disease, the kidneys no longer function and the individual may require dialysis or a kidney transplant (Mitch, 2007)^[33]. Several functions of the liver can be compromised if any part of the liver is damaged. This could occur as a result of reactive oxygen species. Reactive oxygen species generation is a component of oxidative phosphorylation and plays a role in normal redox control of physiological signaling pathways. However, excessive generation of reactive oxygen species triggers cell dysfunction, lipid peroxidation, and DNA mutagenesis and can lead to irreversible cell damage or death^[34, 35, and 36].

A report by Razinger et al., (2008)^[37] and Hsu et al., (2007)^[38] suggested that cadmium toxicity in liver may be mediated by the production of reactive oxygen species known to induce necrosis in various rat organs. The most prominent reactive oxygen species are the superoxide anion (O_2^-), hydrogen peroxide (H_2O_2), and the hydroxyl ion (OH) (Turner and Lysiak, 2008)^[39]. Cells also have intrinsic antioxidant systems that counter ROS accumulation. These include enzymes such as catalase, glutathione peroxidases, and superoxide dismutase, and non-enzymatic antioxidants, such as vitamins E, C, beta carotene and ubiquinone (Giordano, 2005; Nordberg and Arner, 2001). The rate of generation of reactive oxygen species under several conditions exceeds the removal and oxidative stress occurs^[36, 40, 41, and 42]. Severe oxidative stress can cause cell death and even moderate oxidation can trigger apoptosis, while more intense stresses may cause necrosis (Lennon et al., 1991)^[43].

Estimation from the use of the modified method of Lorke (1983)^[20], provide a tolerable dose of the aqueous extract of *Cnidioscolus aconitifolius* leaves by the extract treated animals. Dapar et. al (2007)^[44] suggested that, the estimation of tolerable doses of plant extract is of immense importance, in view of the large-scale human consumption of these herbs/plants either in culinary use or in managing or combating certain ailments and diseases and should be a matter of concern.

The plant has been reported to have many beneficial, culinary and medicinal properties and these could have been as a result of the chemical components of the plant. It was observed that the plant has the potentials of enhancing the histological functions of the kidney and liver as the sections obtained from this study showed a better histological organization in the kidneys and livers of the extract treated rats. There is a need to determine if these observations in Sprague

Dawley rats may be applicable to man.

The preservation of the histological components of the kidney and liver observed in the sections obtained from the extract treated animals could be as a result of the phytochemical components of the plant extract.

Conclusion

In this study, the oral administration of the aqueous extract of *Cnidoscolus aconitifolius* significantly increased the body weight of the extract treated animals. The histological outline of the aqueous leaf extract of *C. aconitifolius* on the kidney and liver as seen in the sections obtained from the extract treated rats support this claim as the histology of the studied organs were well preserved. This is indicative of the non-toxic effect of the aqueous extract of *Cnidoscolus aconitifolius* on the histology of the kidney and liver at the doses administered to the animals in this study. In conclusion, data obtained from this study showed that the oral administration of aqueous extract of *Cnidoscolus aconitifolius* has no adverse effects on the kidney and liver morphology. The observations from this study suggested that *Cnidoscolus aconitifolius* was neither hepatotoxic nor was it nephrotoxic. Further studies should be directed towards isolating the

specific biological and/or chemical component(s) of the plant responsible for the positive enhancing effects in order to standardize the plant preparation for maximum culinary and therapeutic benefit.

References

1. Karnath L. The new paradigm of botanical drugs. *Eur. Pharm. Rev.* 2002. 7:19-20.
2. Eweka AO and Om'Iniabohs FAE. Histological Studies of the Effects of Monosodium Glutamate on the Lateral Geniculate Body of Adult Sprague Dawley rats. *The Internet Journal of Nutrition and Wellnes.* 2008.5, Num 1.
3. Nwoha PU, Ojo GB, Ajayi SA, Ofusori DA, Oluwayinka OP, Odukoya SA and Falana BA. *Garcinia kola* diet provides slight protection to mice hippocampal neurons against neurotoxins. *J Environ Neurosci Biomed.* 2007. 1, 125- 136.
4. Okwu DE and Ekeke O. Phytochemical screening and mineral composition of chewing sticks in South Eastern Nigeria. *Global J. Pure and Applied Sci*; 2003. (9): 235-238.
5. www.devilfinder.com; Filipino Herbs Healing Wonders © 2004-