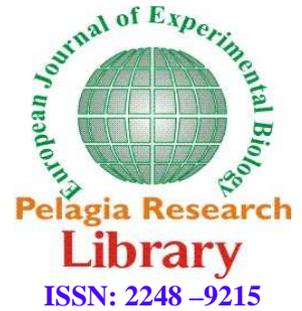




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Some of the Effect of *Crassocephalum crepidioides* on the Frontal cortex, Kidney, Liver and Testis of Adult Male Sprague Dawley Rats: Microanatomical Study

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ABSTRACT

Whenever any plant and/or herb is ingested, the body system interacts with it in an attempt to get rid of any harmful toxins such may contain, especially if the body cannot convert the foreign substance into useful components. This investigation was to evaluate the effects of oral consumption of aqueous leaf extract of *Crassocephalum crepidioides* on the frontal cortex, kidney, liver and testes of Sprague Dawley rats using microanatomical studies. Twenty adult male Sprague Dawley rats weighing between 165-198 g were used. They were divided into 2 groups. The rats in the treated group A received 300 mg/kg body weight of the aqueous leaf extract of *C. crepidioides* for thirty days (30d). 6 rats gained increase in body weight. Histopathological observation of their frontal cortex, liver and testes revealed no significant abnormal alterations apart from alterations observed in the kidneys of 4 rats and these include glomerular distortion, vacuolations and evidence of tubular necrotic bodies. The rats in the control group B received equal volume of phosphate buffered saline (PBS) also for 30d. No histopathological abnormalities were seen in the frontal cortex, kidney, liver, and testes of the rats. Aqueous leaf extract of *C. crepidioides* has histo-toxic effects on the histological profile of the kidneys of the rats. The observed histopathological changes in the kidneys of the treated rats may affect the functional activities of the kidney.

Key words: *Crassocephalum crepidioides*, Cytoarchitecture, Leaf extract, Phosphate buffered saline.

INTRODUCTION

Nearly 80% of the world populations depend on herbal and/or alternative medicine for their primary health care [1]. Nigeria is recognized worldwide for its vast fauna and flora biodiversity, which can be explored in several ways (i.e. culinary, medicinal, therapeutic, nutritional, e.t.c.) for the benefit of mankind. A wide range of plants/herbs (vegetables) species from Nigeria's flora have been used in folklore medicine for the treatment of several maladies both in the "Old and New world" [2]. Green leafy vegetables are known as a main source of vitamins, minerals and fiber for the local consumers. Due to their dietary importance, many scientific studies have been carried out on the potentials of these green leaves [3]. Several investigators have been intensively investigating both the tropical and subtropical plant species with medicinal properties in order to assess the feasibility of developing natural, sustainable, and affordable "natural drugs" [4].

Crassocephalum crepidioides, also called 'Ebolo' (in Yoruba land in Nigeria) is an erect annual slightly succulent herb growing up to 180 cm tall. Its use is widespread in many tropical and subtropical regions, but is especially prominent in tropical Africa where the fleshy mucilaginous leaves and stems are eaten as vegetable and many parts of the plant use for medical purposes [5]. Medicinal folklore use of *C. crepidioides* include treatment of indigestion (in Southern Nigeria), treatment of stomach upset (in DR Congo), treatment for fresh wound (in Uganda), the decoction of the leaf is used in Nigeria for the treatment of headache, in Tanzania a mixture of the leaf sap of *C. crepidioides* and *Cymbopogon giganteus* is used orally and externally for the treatment of epilepsy. Also in Tanzania, the dried leaf powder is applied as a snuff to stop nose bleeding and smoked to treat sleeping sickness. Tannin found in the roots of the plant is used to treat swollen lips [6]. The plant is known to contain a large number of phytochemical compounds which include: tannin, dihydroisocoumarins, pyrrolizidine alkaloids such as jacobine and jacoline and monoterpenes such as myrcene, limonene and α -copaene [6].

Some of the related scientific studies on *C. crepidioides* include chemical composition of the plant [7] and hepatoprotective effect of the plant against CCl₄ liver damage [8]. However there is dearth of scientific information on the effects of *C. crepidioides* on vital organs (brain, kidney, testes e.t.c) of the body.

The evaluation of natural products with biological activity require a proper and adequate study of such product(s) prior to use as it concerns the well-being of the users [9]. Herbal medicines are less damaging than synthetic drugs they have better compatibility thus improving patient tolerance even on long-term use [10]. Contrary to this, it is known that, whenever any plant and/or herb is ingested, the body system interacts with it in an attempt to get rid of any harmful toxins such may contain, most especially if the body cannot convert the foreign substance into useful components. These results into insults which are commonly manifested by changes in enzyme levels and alteration in the cellular make up of various affected organs. The toxicity could as well result in tissue or organ damage. The vital organs that are commonly affected are brain, liver, pancreas, and kidney among others [11]. The objective of this study therefore, was to investigate the effect of the *C. crepidioides* on the histology of the frontal cortex, kidney, liver and testis of Sprague Dawley rats as a marker of toxicity.

MATERIALS AND METHODS

Collection of Plant and Preparation of Plant Extracts

Fresh green leaves of *C. crepidioides* were harvested from the premises of Botanical garden of the University of Ilorin, Ilorin, Nigeria. Identification of the plant was made at the Botany Department of the same University. The authenticated plant material was air-dried at room temperature under standard laboratory procedures. The air-dried leaves were weighed using Gallenkamp (FA2104A, England) electronic weighing balance and were milled with automatic electrical Blender (model MS-223, China) to powdered form.

Three hundred and fifty-five grams of the grinded plant sample was later soaked in 1500 ml of PBS for 48 hours [12] at room temperature, and was later filtered through cheese cloth and then through Whatman #1 filter paper [13], the filtrate was concentrated using a rotary evaporator (Rotavapor® R-210) at 42- 47°C. The approximate analysis of the grinded leaves was determined according to the procedure of AOAC [14].

Laboratory Animals and Feeding:

Twenty presumably healthy adult male Sprague Dawley rats were randomly grouped into a treatment group A (n=10), and control group B (n=10). The body weights of the rats were documented on daily basis using a digital weighing scale (Saltun® EK5055Max).

The rats in the treatment group A were administered orally (through the use of a sterilized orogastric tube) with 300 mg per kilogram body weight of the aqueous leaf extract of *C. crepidioides* for 30d. The rats in the control group B received equal volume of PBS also for 30d.

All the rats were accommodated in clean cages of dimensions 33.0×20.5×19.0 cm situated in well ventilated standard housing conditions (temperature: 28–31°C; humidity: 50–55%). Their cages were properly cleaned everyday. 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 Institute of Health [15]. 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 hours after the last administration, all the rats were sacrificed by cervical decapitation, laparotomy was done and the kidney, liver and testes were excised for biopsies while the frontal cortices were carefully excised from the skulls of the rats and blotted dry on a filter paper. The liver was fixed in specimen bottle containing 10% formol saline, the kidney and testes were fixed in separate specimen bottles containing Bouin’s fluid, and the frontal cortices were fixed in specimen bottles containing 10% formol calcium, for further histopathological studies.

Histological Parameters:

After fixing the frontal cortices, kidneys, liver and testes of both the treated and control rats, the tissues were processed and examined for light microscopy. The stain used was hematoxylin and eosin for the general cytoarchitecture of the respective tissues. The permanent photomicrographs

of each slide was taken with a Nikon Digital Camera DXM1200F (Nikon, Japan) for subsequent histological analysis.

RESULTS

No physical alterations were observed in the morphological outline of the frontal cortices, kidneys, liver and testes of the rats in both the treatment and the control groups as these organs in both groups appeared morphologically normal. There was no statistical significant increase in the body weight gain of the rats in the treatment groups at the termination of this study (fig.5).

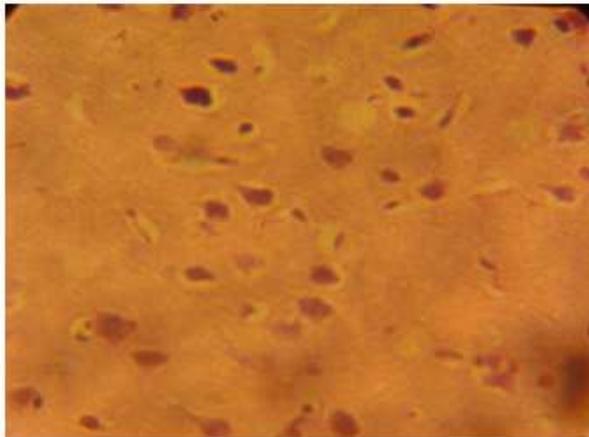


Fig 1A: Photomicrograph of the frontal cortex of the rat in the control group conformed to normal histological features (H&E x480).

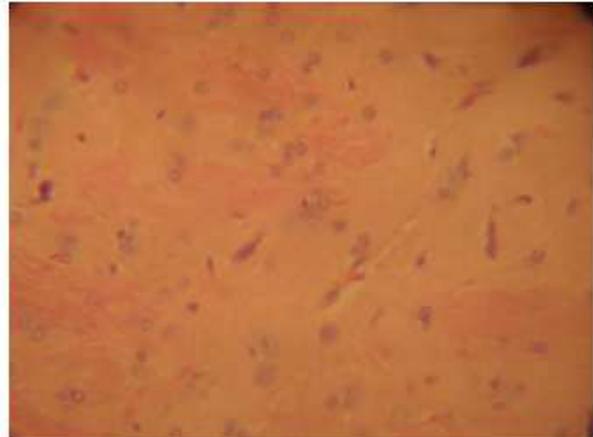


Fig 1B: Photomicrograph of the frontal cortex of the rat in the treated group devoid of degenerative changes such as cytoarchitectural distortions, vacuolations and necrotic bodies (H&E x480).

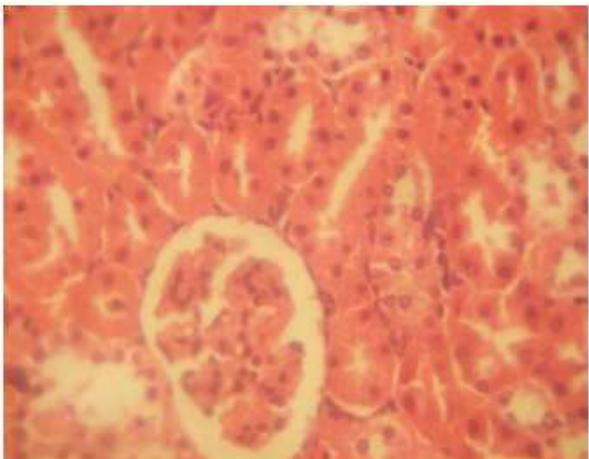


Figure 2A; The kidney section of the rat in the control group with well preserved histological profile (H&E x480).

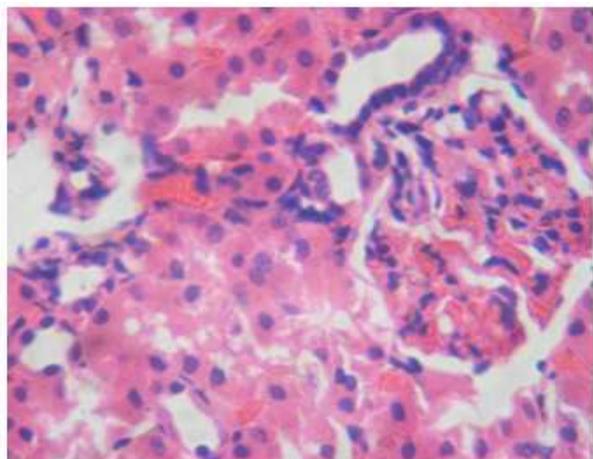


Figure 2B: The kidney section of the rat in the treated group with histological deviations such as glomerular distortion, vacuolations and evidence of tubular necrotic bodies (H&E x480).

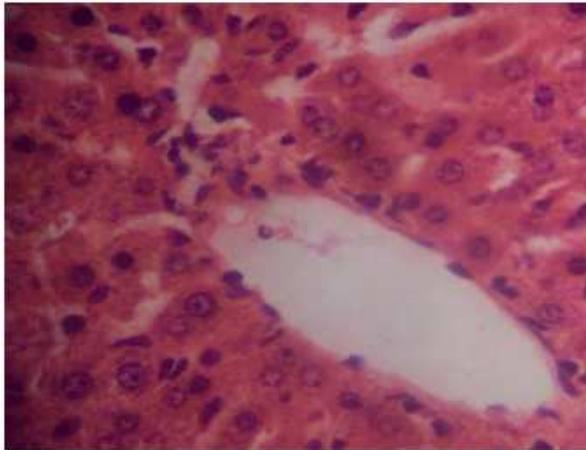


Figure 3A; Histological section of the liver of the rat in the control group with well preserved histological profile devoid of histopathological alterations (H&E x480).

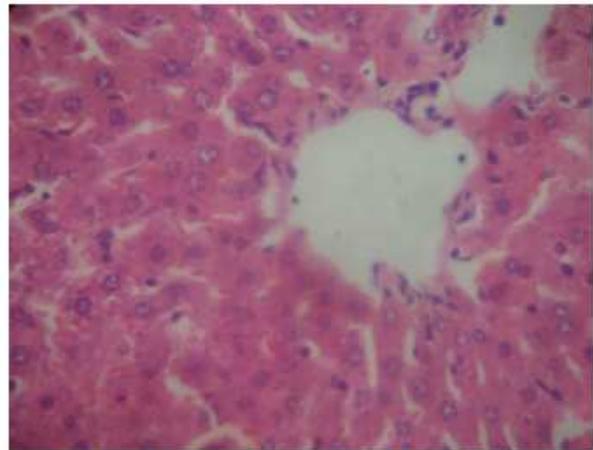


Fig. 3B; Uncompromised histology of the liver of the rat in the treated group with well preserved histological outline devoid of degenerative changes (H&E x480).

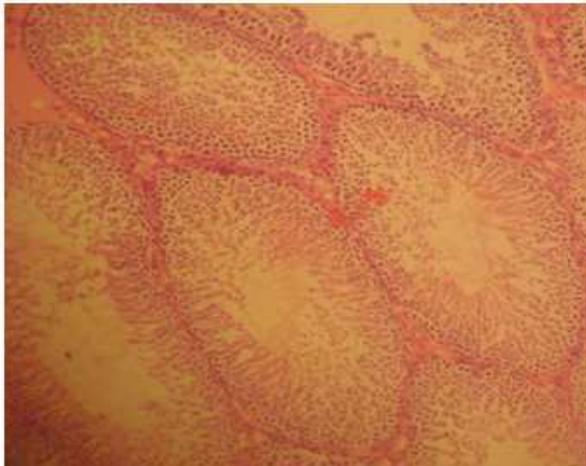


Figure 4A; Normal histological section of the testes of the rat in the control group without histopathological alterations (H&E x480).



Figure 4B; Normal histological section of the testes of the rat in the teated group with no histopathological changes. (H&E x480).

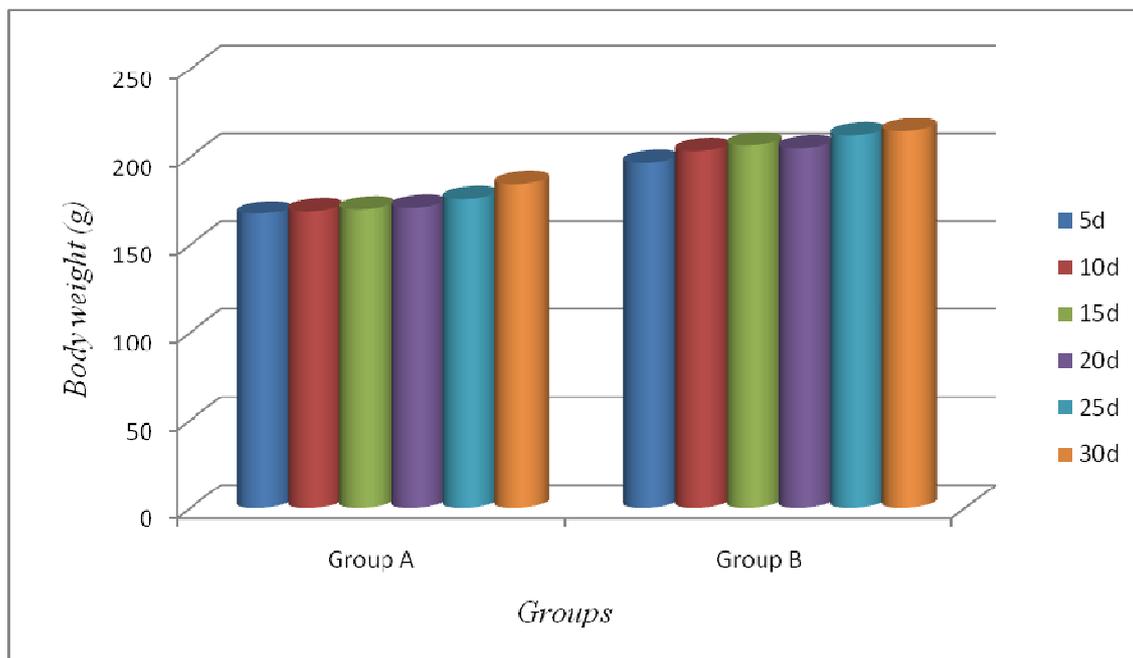


Fig. 5; Showing the average body weight gain of the experimental rats

The neurohistological assessment of the frontal cortices of the rats in the extract treated group displayed normal histological profile, degenerative changes such as cytoarchitectural distortions, vacuolations and evidence of necrotic bodies were absent in the frontal cortices of the extract treated rats. The sections obtained in the control group also conformed to normal histological features (figs. 1 A and B).

Some of the histological alterations observed in the histological sections of the kidneys of the rats in the treated group include; glomerular distortion, vacuolations and evidence of tubular necrotic bodies. These histological alterations were absent in the histological sections of the kidneys of the rats in the control group as they present well preserved histological profile (figs. 2 A and B). The liver of the rats in both the extract treated and the control groups displayed well preserved histological profile. The liver is devoid of any histopathological changes (figs. 3 A and B). The histological section of the testes of the rats in both the extract treated and the control groups were devoid of histopathological abnormalities (figs. 4 A and B).

DISCUSSION

The histopathological effects of aqueous leaf extract *C. crepidioides* on the frontal cortex, kidney, liver and testes of rats have not been studied. In this study, we investigated some of the effects of the aqueous leaf extract of *C. crepidioides* on these organs in order to elucidate some of the possible histo-toxic implications that could occur following its consumption. Using the Olympus binocular light microscope (XSZ-107BN, No. 071771), the histological observations seen in the sections of the frontal cortex, kidney, liver and testis of the experimental rats in the

treated groups stained with H&E revealed that oral administration of the aqueous leaf extract of *C. crepidioides* (at the dose administered in this investigation) has no deleterious effects on the histological outline of frontal cortex, liver and testes as there were no histopathological alterations in the frontal cortex, liver and testes of the rats when compared with the corresponding histological sections obtained from the rats in the control group. However, there were some histopathological alterations observed in the kidneys of the rats in the extract treated group and these alterations include glomerular distortion, vacuolations and evidence of tubular necrotic bodies which may ultimately result to death of the cells in the kidney thereby compromising the functional integrity of the kidney. Evidence from the present investigation showed that the plant extract is nephrotoxic and may confer negative adverse effects on the kidney. This evidence is in support of the claims of Grubben (2004) [5] and Fu *et al.* (2002) [16]. 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 [17]. 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. The type of cell loss and the particular part of the organ affected determines the symptoms associated with individual disease [18].

The histopathological alterations observed in this investigation as characterized by histological damage to kidneys of Sprague Dawley rats could have been as a result of direct toxicity or could have resulted from the release of toxic substances from other organs like the liver. It could also have occurred as a result of the deleterious effects of the phytochemical make up of the plant.

This investigation confirmed that oral administration of the aqueous leaf extract of *C. crepidioides* has no toxic and disruptive interference on cellular characteristics of the frontal cortex, liver and testes of Sprague Dawley rats. To the best of our knowledge, this is the first study reporting the effect of *C. crepidioides* on the histological profile of the selected organs of study in Sprague Dawley rats.

CONCLUSION

In conclusion, data obtained from this study showed that the oral administration of aqueous leaf extract of *C. crepidioides* on the frontal cortex, kidney, liver and testes at the dose administered to the rats has no deleterious effect on the cytoarchitecture of the frontal cortex, liver and testes. Further studies should be directed towards isolating the specific component(s) of the plant responsible for the toxicity in the kidney in order to standardize the plant preparation for maximum culinary and therapeutic benefits.

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