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HISTOPATHOLOGICAL PATTERN OF THE LIVER AND KIDNEY OF RATTUS NOVERGICUS ON PROPHYLACTIC CONSUMPTION OF ACALYPHA GODSEFFIANA CRUDE ETHANOLIC EXTRACT

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Abstract

Health concerns over prophylactic abuse of herbal drugs are worrisome. However, histopathological perspectives are not so common. This study was designed to examine the histopathological pattern of the liver and kidney of white rats exposed to prophylactic abuse of *Acalypha godseffiana* crude leaf extract. Thirty (30) albino rats of both sexes, with average mean weight of 199.82g were randomly selected into cages (A, B, C & D) and a control cage (E) where n=6. Cages A-D received 200, 400, 600, and 800 mg/kg body weight of extract respectively. Commercially purchased drinking water and standard top feed[R] were given ad libitum, for 30 days. The animals were weighed before and after extract administration. Dullness and restlessness were observed and was marked on the high dose treated animals. The animals were sacrificed on day 31 by cervical dislocation. Grossing was done and was fixed in 10% buffered neutral formalin. Processed tissues at (3-5mm) were sectioned at 3-5microns. Grossly, all organs showed no apparent changes while histological findings were normal. This study revealed that prophylactic consumption of A. godseffiana leaf extract does not result in any histological changes in the organs (Liver and Kidney) studied. It can therefore be recommended for use.

Introduction

The plant Acalypha wilkesiana 'godseffiana' muell arg. belongs to the family Euphorbiaceae which is a dicotyledonous plant that includes shrubs and trees[1]. In Nigeria, many cultivars are available with different leaf forms and colours[2]. A. wilkesiana 'Godseffiana' has narrow, drooping, green leaves with creamy-white margins[2,3]. Although, there are quite a reasonable number of cultivars worldwide; the macrophylla, hoffamanu, godseffiana, Macafeeana, hispida, marginata and racemosa are peculiar cultivars within Nigeria[5-7]. Meanwhile, during the last two decades, there has been a considerable increase in the study and use of medicinal plants all over the world especially in advanced countries. In some countries, herbal medicines are still a central part of

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the medical system e.g. Ethiopia[8], Argentina[9] and Papua New Guinea[10].

A. wilkesiana 'godseffiana' is used for the treatment of malaria, rheumatism, stomach upset, dermatitis and infantile eczema11 while the efficacy as an antihypertensive agent was demonstrated[12]. Leaf poultice is used for headache, swelling, cold and wound dressing. Chopped pieces of the dried stem and root were steeped in alcohol and used for stomach ache and as worm expellant in man in the Delta region of Nigeria[13,14]. Furthermore, the aqueous extract is also used in the management of fever in infants as well as abnormal sodium and potassium metabolism that accompanies hypertensions[5]. Apparently, lack of scientific proof of efficacies claimed by traditional medical practitioners in Nigeria called for this study. However, there is paucity of information on prophylactic consumption of A. godseffiana from this cultivar; especially, the histopathology point of view.

Therefore, the aim of the study was to examine the histopathological pattern of the liver and kidney of albino wistar rats (Rattus novergicus); under the influence of the crude ethanol leaf extract of Acalypha godseffiana leaves obtained from Benin City, Nigeria.

Materials and methods

Standard histological methods and materials were used asceptically[15].

Location and Duration of Study

This study was conducted at the animal care unit, University of Nigeria Teaching Hospital, Enugu, Nigeria and Department of Medical Laboratory Science, School of Basic Medical Sciences, College of Medical Sciences, University of Benin, Benin City, Nigeria. Animal acclimatization, extract preparation and administration, Grossing, Tissue processing, Sectioning, Staining, Microscopy and Photomicrography lasted for three months (September 2nd to December 1st, 2013).

Design and Conduct of experiment

Oral dose was prepared by dissolving 50g of the yield extract in 250ml of distilled water. Each of the rats in cages A, B, C and D were picked one at a time with a hand towel and appropriate volumes in ml of the extract was administered to the animals at a dose of 200, 400, 600 and 800mg/kg body weight (b.w.) respectively with regards to the L.D50 of > 6,344mg/kg b.w. (Oral) in rats using a 5ml syringe while Cage E served as the control. Each animal was weighed before and after administering the test extract. At the end of the experiment, all animals were sacrificed. The kidney and liver of the animals were excised and observed grossly. Thereafter, it was processed histologically. Sections of the tissues were obtained using the hertz rotary microtome (Leica RM2255, Cambridge mode). Staining of the section was according to Haematoxylin and Eosin staining technique[16].

Animal care ethics

Verbal approval was granted by the animal care unit, University of Nigeria Teaching Hospital, Enugu and was properly documented on 02/09/2013. The rats were obtained and housed in wire gauze cages with saw dust as beddings to acclimatize in the animal care unit for 2 weeks, under standard condition of temperature (25 ± 5°C) and a light/dark periodicity of 12:12 hrs. Enough food (Standard top feed® and commercially purchased UNIBEN table water with NAFDAC Reg. no 01-4597) was provided.

Animal Grouping

The animal studies were carried out in compliance with ethical policies outlined in the 'Guide for the Care and Use of Laboratory Animals', published by the US National Institute of Health (NIH Publication No. 85-23, revised 1996). For this study, 30 albino wistar rats of both sexes were divided into five (5) groups of 6 rats per cage and were labeled as cages A, B, C, D and E. (Where n-6)

Determination of body weight

The method described[17] was used to determine body weight of experimental rats. Individual rat was monitored for daily gain in body weight using digital electronic balance (Gilbertini, Italy). Gain in weight was obtained from the relationship given below: Daily gain in weight= Final day Weight – Initial day Weight, where the average mean weight was 199.82g.

Physical measurement

Behavioral signs of acute toxicity were observed in the high dose experimental rats and were noted; such as: dullness and reduced activities in the first few hours of the dose administered orally.

Collection and Identification of plant

Samples of the fresh A. godseffiana plants (Figure 1) were collected from within University of Benin, Ekewan Campus, Benin City, Nigeria and were authenticated at the Department of Plant Biology and Biotechnology, University of Benin, Nigeria. A sample plant was deposited at the Herbarium, where voucher specimens already exist.

Plant preparation and extraction

The leaves were washed, air dried, pulverized and sieved. 1.4kg of the fine powdered particles using a weighing balance was extracted with 4.5 litres of ethanol, by the soxhlet apparatus. The filtrates were concentrated using rotary evaporator at 40°C which yielded 82.6g of light greenish solid extract and was stored at -4°C.

Figure 1: Fresh plant [2] and pulverized / sieved sample of *A. godseffiana*.

Phytochemical screening

The methods adapted [18] were used to analyze the phytochemical properties of the leaves of A. godseffiana plant and were carried out on the crude ethanol extract to confirm the presence or absence of the following secondary metabolites: alkaloids, flavonoids, steroids, saponins, phenols, tannins, glycosides, reducing sugars, anthraquinones, carbohydrates, resin and cardiac glycosides [2,18].

Acute toxicity test ($L.D_{50}$)

Acute toxicity of A. godseffiana crude ethanol leaf extract was performed according to the modified Lorke's method[28].

Microscopy and Photomicrography

The sections were examined using Swift® binocular microscope with an in built light system and white films with an Olympus photomicroscope (Opticshot- 2; Nikon, Tokyo, Japan) at x40 magnification.

Equipment and Apparatus

Mettler analytical balance H80 (UK), Hertz Rotary Microtome (Leica RM2255, Cambridge mode), Water Bath (Gallenkamp), Rotavapor RII0 (Buchi, England) and Pyrex® Soxhlet extraction apparatus (Sigma-Aldrich-CLS3&$0M)

Data Analysis

Data were presented as Means ± SD and significance was determined at $p < 0.05$ using Statistical package for social sciences (SPSS) version 16.0 (Inc Chicago, Illinois, USA).

Results

(Table 1) showed the distribution of extract doses in mg/kg b.w. in rats, the duration of oral administration as well as the average mean weights of the animals both test and control in cages A-E. Dullness and restlessness were observed amongst moderately treated animals, but were marked in high dose treated animals (Cages C and D) respectively. The low dose treated animals showed little or no weight loss as compared to the severe weight loss observed in the high dose treated animals while the degree of weight loss was prominent and progressive with increase in dose regimen (Table 2).

Meanwhile, Grossing showed no variation in colouration, consistency and appearance. The corresponding histological indices of Liver and Kidney sections were normal (Table 3, Figure 2). Comparison of photomicrograph of the control was matched with the treatment groups for confirmation (Figure 3). Nevertheless, phytochemical screening revealed the presence of the following important secondary metabolites: Tannins, Flavonoids, Steroids, Saponins and Cardiac Glycosides (Table 4).

Furthermore, results from the acute toxicity studies showed that the crude ethanol extract of the leaf of Acalypha godseffiana had no mortality rate at $L.D_{50} \geq 6{,}344$ mg/kg in Rattus novergicus (albino wistar rats) oral routes only.

Table 1: Oral administration of *A. godseffiana* leaf extract on thirty (30) albino wistar rats.

Cages	Number of rats	Average Mean Weight	Dosage in mg/kg Body Weight	Duration of extract administration
A	6	199.18	200	30 days
B	6	199.26	400	30 days
C	6	200.48	600	30 days
D	6	201.16	800	30 days
E	6	199.04	-	30 days

Average mean weight of rats: 199.82g.

Maximum volume of extract: 4ml.

Table 2: Empirical analysis of wistar rats on exposure to prophylactic consumption of *A. godseffiana* leaf extract for 30 days.

Cage	Dose in (mg/kg)	Volume of extract in (ml)	Average mean weight before administration	Average mean weight after administration	Physical weight gain /or loss	Activities/ Dullness
A	200	1	199.18 ã 2.3	198.19 ã 1.6	↓	-
B	400	2	199.26 ã 1.6	198.13 ã 1.2	↓	-
C	600	3	200.48 ã 1.8	198.22 ã 2.3	↓	ã ±
D	800	4	201.14 ã 1.8	189.28 ã 2.2	↓	+
E	-	-	199.04 ã 1.1	206.16 ã 1.8	↑	-

Table 3: Histopathological scoring

Cage	Dose in mg/kg	Increase in urinary pole.	Inflammatory cell infiltration	Distortion of central vein	Inflammatory cell / distortion of bile duct	Hepato-cellular necrosis	vacoulation
A	200	-	-	-	-	-	-
B	400	-	-	-	-	-	-
C	600	ã ±	-	-	-		ã
D	800	ã ±	-	ã ±	-	-	ã ±
E	-	-	-	-	-	-	-

Table 4: Qualitative analyses (phytochemical) of *A. godseffiana* ethanolic crude leaf extract.

Phytochemicals	Presence/or Absence
Resins	Present
Tannins	Present
Flavonoids	Present
Cardiac Glycosides	Present
Steroids	Present
Saponins	Present
Anthraquinones	Absent
Alkaloids	Absent
Terpenes	Absent

Key

↑ → Increase in weight

↓ → Slight weight loss

⏚ → Severe weight loss

\+ → Presence of features

ã± → Intermediate features

\- → Absence of extract /or features

b.w. → Body weight

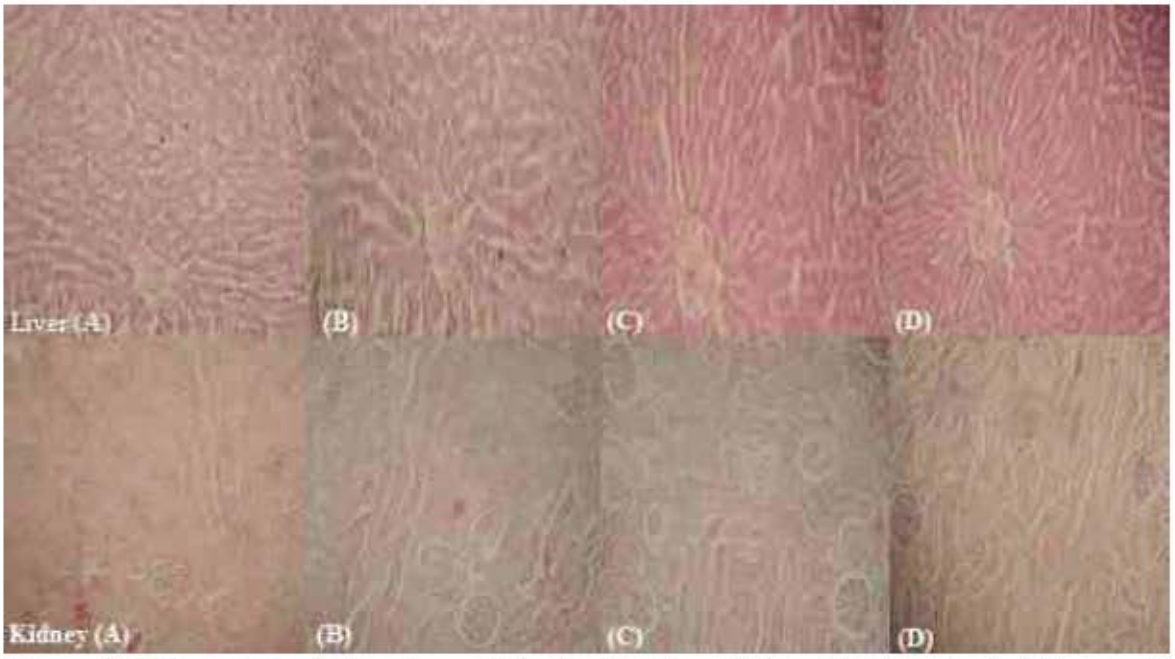

Figure 2: Histopathological pattern of the test sections (*A. godseffiana* treated liver and kidney sections A, B, C and D) showing dose dependent kidney sections (C and D) with negligible signs of increased urinary pole and negligible signs of vacoulation in the liver sections (C and D) respectively. Stain uptake: Mayer's H&E x 40 magnification.

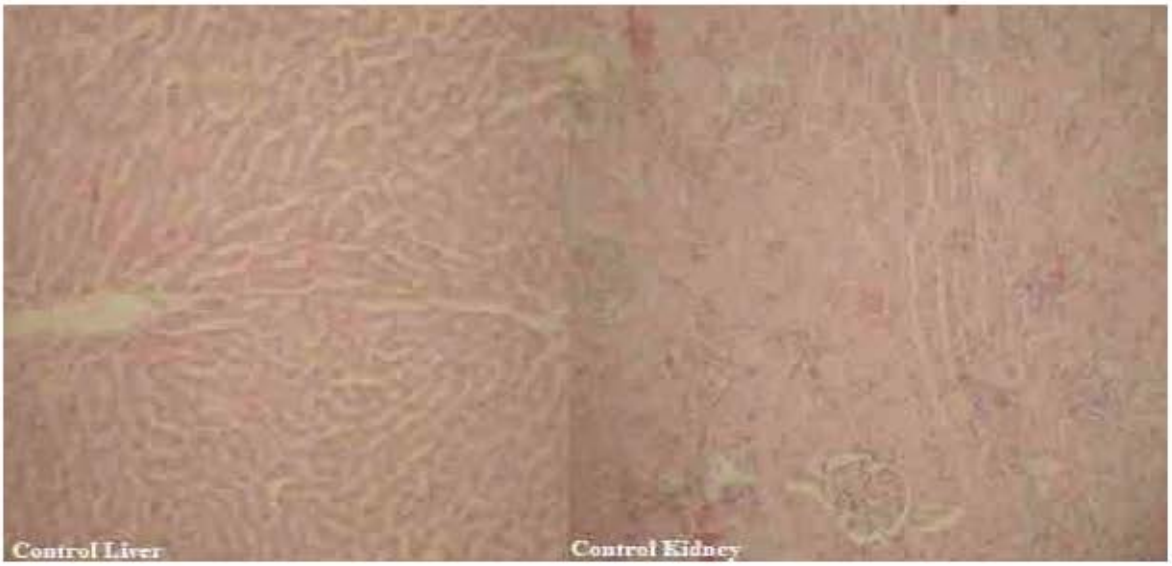

Figure 3: Histological pattern of the control sections (Untreated liver and kidney sections) showing normal histopathological pattern of the liver and kidney respectively. Stain uptake: Mayer?sH&E X40 Magnification. Mayer's H&E x 40 magnification.

Discussion

The study was modeled on ethno-medicinal practices by some local medical practitioners which involves the use of plant materials for treatment of some notable ailment. However, indiscriminate abuse of plants and herbal therapy in Nigeria calls for concern in this study. Table 4: showed the presence or absence of secondary metabolites in the extract of A. godseffiana obtained in Benin City, Nigeria. Revealed that saponins were present in phytochemical analysis of the extract of A. godseffiana. This study showed similarity in the results obtained in[5]; hence the extract contains cardiac glycosides, flavonoids, steroids, tannins and saponins, while alkaloids, anthraquinones and terpenes were absent. This result differs slightly from [20] where the absence of saponins in the extract evaluated was reported. Though, this could be attributed to the different locations where the plants were collected by the various researchers: Jos in North Central Nigeria located at Lat. 9°56' N and Long. 8°53' E, 1,217 m above sea level and enjoys a more temperate climate than much of the rest of Nigeria[29] and the present study; located in Benin City which is a complete different Latitude and Longitude with a more tropical climate.

Meanwhile, [20] also suggested that there is a relationship between chemical composition of a plant and geographical location. In a similar circumstance, [21] reported a variation in the composition of essential oils of Jasminum sambac. (L.) collected from different parts of India while the composition of bee propolis has also been found to depend on geographical source [22]. According to [23], Bee propolis contains flavonoids and phenolic esters in temperate regions but these compounds were absent in propolis obtained from tropical regions, although both exert antibacterial property. More so, the presence of steroid in this study agrees with [23, 24]. Meanwhile, phytochemical analysis revealed that Acalypha species contain high concentrations of polyphenols, terpenoids, and plant sterols [12, 25] which were reported in this study.

Furthermore, the effect of aqueous extract (10%w/v) of the leaf of Acalypha wilkesiana (a popular medicinal plant used in Nigeria for the treatment of fever in infants) was studied in the liver and kidney of albino rats. [26] showed that the administration of the aqueous extract resulted in a significant reduction in the enzymes activities ($p<0.05$) in the liver which was complimented by an increased activity of these enzymes in the serum while the result obtained showed that prolonged usage or overdose of the aqueous extract of Acalypha wilkesiana could exhibit a dose dependent toxicity. In contrast, histological indices were of normal representation and there were no significant histopathological changing pattern in the liver and kidney sections examined (Figure 2) as compared to that of the control (Figure 3) in the present study. However, opinions from [26] were drawn from the accessement of liver enzymes and may also be dose dependent. Note: physiological status of the animals e.g. pregnant female rats were not considered in this study, therefore further studies should try to look at those areas including the biochemical and haematological parameters and to further quantify and demonstrate the levels of concentration of each compound found to be present in the phytochemical analysis of the leaf of A. godseffiana.

Conclusion

In this study, Acalypha wilkesiana "godseffiana" ethanolic crude extract had no significant effect on the histology of the liver and kidney examined. This implies that the extract does not interfere with the histological architecture of the liver and kidney of the test animals at the cellular

level. Meanwhile, further studies should try to assay for blood chemistry and haematological parameters of the test animals under similar circumstances. Therefore, this study showed that prophylactic consumption of A. godseffiana leaf extract is in keeping with normal histopathological pattern of the liver and kidney of albino rats; nevertheless, there is need to throw caution on the dose regimen.

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