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Histo-morphological Examination of the Visceral Organs of Albino Wistar Rats pre-exposed to *Ocimum gratissimum* Crude Decoction

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ABSTRACT: *Livers and kidneys are the most essential visceral organs in the body. Hence, the present study was aimed to examine the histo-morphological alteration of these organs in white rats pre-exposed to Ocimum gratissimum crude leaf decoction using 30 inbred rats of both sexes, with an average mean weight of 200.12g. The animals were grouped into cages A, B, C, D and a control group E (where n=6). Physical and empirical measurement of the animals were taken prior to and after the experiment. The crude leaf decoction was prepared and administered orally to the animals in order of 400, 600, 800 and 1000mg/kg body weight with regards to 2,450mg/kg L.D$_{50}$ (oral) in rats. At termination, the rats were sacrificed by cervical dislocation. Gross examination was carried out immediately, while excised organs of interest were fixed in 10% buffered neutral formalin. Cut tissue at 3-5mm was processed using paraffin embedding technique while section was cut at 3-5microns for light microscopy. Grossly, visceral organs of animals in groups A-D showed no significant variation in colour, size and consistency while empirical measurement revealed a dose dependent weight loss. Histopathology findings also revealed a dose related offenses when compared to the control. This study showed that indiscriminate consumption of Ocimum gratissimum crude leaf decoction does have cellular deleterious effect in organs of white rats at a higher dose and concentration and may act in a similar circumstance in human. Therefore, it is strongly recommended to put in place, a proper regulatory mechanism to check the dosage and concentration of herbal preparations in Nigeria.*

KEYWORDS: *Scent leaf, changing pattern, visceral organs (Liver and kidney) and decoction.*

I. INTRODUCTION

Ocimum gratissimum is commonly called wild basil, which belongs to the family Lamiaceae and is popularly known as scent leaf in English. It is a well-developed flowering plant with root, stem and leaves systems [1]. The plant which grows among pastures in Nigeria is a perennial plant that is woody at the base, having an average height of 1-3m high. It is a scented shrub with lime-green fuzzy leaves [2]. It is a highly branched shrub with ribbed stem and laborious branches with simple leaves, oppositely arranged, 3-5 centimeters long and 1.2 centimeters wide [3]. It is widely used for treatment of nasal congestion and cough, abdominal pain, well known for its anti-inflammatory antimicrobial and hypoglycemic activity [4]; a detailed description and distribution of the plant have been documented [5].

In view of the multifunctional histomorphological makeup of the liver and kidney respectively, it becomes paramount to critically examine these special and important visceral organs. Therefore, where the liver is especially important for maintaining a normal blood glucose concentration (this is called the glucose buffer function of the liver), synthesis and metabolism of fat and protein metabolism [6]. Reports concerning the toxic effects of herbal medicine are associated with hepatoxicity although the reports of other toxic effects on other organs like the kidney and other systems have been published in the medical literature [7]. The kidneys in turn, are highly vascularized compound tubular glands that function to maintain the composition of body fluids at a constant level and to remove excretory wastes [8]. The kidneys also regulate the fluid and electrolyte balance of the body and are the site of production of hormones as rennin and erythropoietin [9]From the foregoing, examination of the liver and kidney are very important in other to avoid sudden break down of this vital organs at the cellular level. Furthermore, the study of toxic or adverse effect of crude drugs of plant origin is essential so as to prove a guide to their safe usage and eventual standardization.

This is especially pertinent as traditional medicine practitioners often administer such preparations without regards to their possible adverse effects [6]. Nonetheless, *Ocimum gratissimum* is one of those several medicinal plants which has being documented to have antidiarrheal and anti-pyretic activities [6] and is also used as spices and to add flavor to food.In recent times, numerous investigations have proved that the phyto-constituents of plants are responsible for scavenging of the free radical released to the system and there by exhibiting the different beneficial pharmacological properties [10] such as hypoglycemic effect, cardiovascular protective, anti-carcinogenesis, stress depressant and many more to mention [4]. However, there is paucity of information regarding the toxic, adverse and or deleterious effect of repeated or indiscriminate consumption of the decoction made from *Ocimum gratissimum* crude preparation on the visceral organs (liver and kidney) of albino wistar rats. Therefore, this study was to examine the histological indices of the liver and kidney of albino rats at the cellular level, while a secondary goal was to investigate the overall burden of the crude decoction on the morphological pattern of the visceral organs of the test animals.

II. EXPERIMENTAL

Materials, Equipment and Apparatus

Mettler analytical weighing balance (H80-UK), GFL shaker (No 3017 MBH, Germany), Hertz Rotary Microtome (Leica RM2255, Cambridge mode), Water Bath (Gallenkamp), Rotavapor RII0 (Buchi, England) and Pyrex®

Animal grouping and care ethics

Thirty (30) inbred rats of both sexes, weighing 180-220g were randomly selected into five groups of six (6) rats per cage and were labeled as cages A, B, C, D and a control cage E (Note: Physiological status of the animals were not considered). They were housed in plastic cages with saw dust as beddings under standard condition of temperature (25 ± 5ºC) 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. The animal studies were carried out in compliance with ethical policies outlined and prepared by INSA, Animal Welfare Division of the Ministry of Environment & Forest, Council of International Organization of Medical Sciences (WHO/UNESCO), NIH and PHS.

Plant collection and authentication:

The leaf of *Ocimum gratissimum* was obtained in November, 2013 in Oredo Local Government Area, Benin City, Nigeria and was authenticated at the herbarium of the Department of Plant Biology and Biotechnology, Faculty of Life Science, University of Benin, Benin City, Nigeria; where a voucher specimen already exist.

Plant preparation and Decoction

The leaves were washed, air dried, pulverized to uniform powder and sieved to obtain a pure fine powdered particle (Figure 1). Two hundred grams (200g) of the pulverized sieved; fine powdered particle was measured with a weighing balance into a sterile conical flask. One liter (1L) of commercially purchased distilled and de-ionized water was added while the mixture was transferred to the GFL shaker (No 3017 MBH, Germany) and was mechanically agitated for 24hrs. Thereafter, the pure decoction was refrigerated and maintained at 2^{o}C for experimental use only. (Note: Crude decoction of the experimental leaf was used in this study as against the extract mostly used by researchers)

Figure 1: Fresh leaves (A) and pulverized / sieved sample (B) of *Ocimum gratissimum.*

Determination of body weight

The method described by [11] was used to determine body weight of experimental rats. Individual rats were monitored for daily gain/ or loss 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 200.12g.

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.

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

Njoku *et al.* (2011) acute toxicity test ($L.D_{50}$) of *Ocimum gratissimum* was found to be 2,450mg/kg body weight in rats (oral). Therefore, doses up to 1000mg/kg body weight in rats were assumed to be safe for the present study thereby excluding the lethal dose.

Design and Conduct of experiment

Using a hand towel, the animals were picked one at a time and were treated with *Ocimum gratissimum* crude decoction in the order of 400, 600, 800 and 1000mg/kg b.w. (oral) with regards to the $L.D_{50}$ of 2,450mg/kg body weight in rats [12]. Note: *Ocimum gratissimum* crude decoction was dispensed from stock and thawed in a water bath at a temperature of about 37^0C for 10 minutes prior to use on daily basis.

Histo-morphologic Examination

The liver and kidney of the test animals were excised and observed grossly and were processed histologically using automatic tissue processor (Hestion -ATP7000 tissue processor-Germany). Sections of the tissues were obtained using digital rotary microtome (Hestion ERM 4000 Germany). Staining of the section was according to Mayer's Haematoxylin and Eosin staining technique for examination by light microscopy [13].

Location and duration of study

This study was conducted at the animal care unit, Department of Medical Biochemistry and Department of Medical Laboratory Science, School of Basic Medical Sciences, University of Benin, Nigeria. Animal acclimatization, Extract preparation and administration, Grossing, Tissue processing, Sectioning, Staining, Microscopy and Photomicrography lasted for 10weeks (November 28th, 2013 to February 29th, 2014).

Microscopy and Photomicrography

The sections of the liver and kidney were examined using Swift(R) Binocular Microscope with an in built lighting system and white films with an Olympus photomicroscope(R) (Opticshot- 2; Nikon, Tokyo, Japan) at x10 and 40 magnification.

Statistical Analysis

Data was presented in Means ± SD and analyzed using one way ANOVA and Duncan post hoc test while significance was determined at $p < 0.05$ using Statistical package for social sciences (SPSS) version 18.0 (Inc Chicago, Illinois, USA).

III. RESULTS

During the present study, gross examination of the livers and kidneys showed apparently no significant variation in colour, size and consistency. The mean weight of the right kidney in this study was 0.98g, while the left kidney was 0.92g. The mean length, width and thickness of the right kidney were 0.88cm, 0.82 and 0.76 cm respectively while those of the left were 0.86 cm, 0.78 and 0.74 cm. When compared with the control, the mean weight of the right kidneys was 1.0, the left 0.96g and the mean length of right kidney of the rat was 0.98 cm and the left was 0.95cm. Furthermore, the mean weight of the liver of rats was 0.47g while the control was 0.48g. The cut surface of the control liver was smooth and shiny with a reddish brown colour while the cut surfaces of the treated groups (A, B, C and D) were also smooth like the control but glistening. The morphological patterns of all the organs grossly examined were consistent without change in pattern. Histopathology findings in the treated groups (A, B, C and D) were in keeping with normal histology of organs when compared to the control group E (Figure 2 and 3) without evidence of necrosis but with mild deleterious effect at the cellular level which was observed to be dose related offences. Meanwhile, empirical measurement also revealed a dose dependent weight loss in the treated groups; especially in group C and D (Table 1) respectively. Nevertheless, (Table 2) showed scores from histopathological indices.

IV. DISCUSSION

The study of toxic or adverse effect of crude drugs of plant origin is essential in order to prove a guide to their safe usage and eventual standardization. This is especially pertinent as traditional medicine practitioners often administer such preparations without regards to their possible adverse effects [6]. However, the results obtained from Mayer's H and E stained sections in this study showed increased dose related offenses in line with increased dose regimen.

Nevertheless, there was relatively mild portal congestion and dilatation of the central vein in the liver sections from the treated group D compared with the control section of the liver in group E. The portal congestion and dilatation observed is in consonance with the findings reported [6], where it was noted that aqueous extract of *Ocimum gratissimum* orally administered to albino rats; showed varying degrees of dilatations of the central vein of the liver which contained lysed red blood cells in the treatment sections compared with the control. Meanwhile, necrosis was not observed in the present study, this slightly defer from the findings recorded in the work carried out by [14] were necrosis was reported to have adversely affected the lungs of adult wistar rats.

Notwithstanding, [6] revealed that body weights of wistar rats in the treatment groups B, C and D used as experimental rats significantly decreased in relation to the increasing concentration of the extract, which produced a dose and time dependent decrease in body weight over the 3 weeks period of administration of the extract. Hence, the present study is in agreement with the work of [6, 15] where rats in groups (B, C and D) showed a similar decrease in weight of experimental rats. In addition, [16] also reported an increased intake of medicinal herbs among men over a period of 6 months caused a decrease in their body weight.

Furthermore, the mild interstitial congestion observed in rat's kidney section from group D (treated with 1000mg/kg body weight of the crude extract) in this study also confirmed that extract of *Ocimum gratissimum* orally administered to albino rats in higher concentration or dose could affect the nephrons of the kidney at the cellular level. Therefore, in the meantime, this study further support the results obtained [15], who suggested that the regeneration by the tubular epithelial of the kidney might have been due to an earlier insult by the extract at higher dosage and consequently led to the absence of significant change in the level of creatinine (Though this findings is from the biochemical view point). However, the study concludes that ethnomedicinal application of *Ocimum gratissimum* may be quite safe at lower doses but could be hepatotoxic and nephrotoxic at higher doses. From the foregoing therefore, the present study thus align with [15] in which lower doses of the decoction have no deleterious effect at the cellular level but could result in such similar histopathological change in pattern when higher doses are administered.

V. CONCLUSION AND RECOMMENDATION

It was concluded that though grossly, the results from this study revealed a normal morphological representation of the livers and kidneys examined; nevertheless, histopathological examination showed a dose related effect of the crude decoction of *Ocimum gratissimum.* In addition, the acute toxicity observed in the high dose treated animals was also associated with dose related offenses. Hence, the present study showed that oral administration of *Ocimum gratissimum* crude leaf decoction to albino rats; may result in histopathological changes in visceral organs like the liver and kidney of white rats when consumed in a high and unusual concentration especially without proper dose regimen. Therefore, further studies should try to assay for blood chemistry and haematological parameters of the test animals under similar circumstances. While, it is strongly recommended to put in place, a proper regulatory mechanism to check the dosage and concentration of herbal preparations in Nigeria.

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Table 1: Sub-acute toxicity analysis of albino wistar rats pre-exposed to *Ocimum gratissimum* crude leaf decoction for 28 days at 2 days intervals.

Group	Dose in (mg/kg) body weight	Volume in (ml)	Average mean weight before administration	Average mean weight after administration	Physical weight gain /or loss	Activities/ Dullness
A	400	1	189.16 ± 1.2	189.14 ± 1.1	↓	±
B	600	2	196.08 ± 1.4	196.02 ± 1.3	↓	±
C	800	3	212.04 ± 1.3	208.02 ± 1.4	↓↓	+
D	1000	4	218.26 ± 1.2	204.13 ± 1.2	↓↓↓	++
E	-	-	202.04 ± 0.8	216.12 ± 1.1	↑↑	±

Where average mean weight of rats =200.12g, maximum volume of water = 6ml.

Table 2: Histopathological scoring

Group	Dose in mg/kg body weight in rats	Interstitial congestion	Inflammatory cell infiltration	Distortion of central vein	Inflammatory cell / distortion of bile duct	Hepato-cellular necrosis	Portal congestion and dilatation
A	400	-	-	-	-	-	-
B	600	-	-	-	-	-	-
C	800	±	-	±	-	-	±
D	1000	+	-	+	-	-	+
E	-	-	-	-	-	-	-

Key

↑↑ → Increase in weight
\+ → Presence of features
± → Intermediate features
\- → Absence of features
↓ → mild decrease in weight
↓↓ → decrease in weight
↓↓↓ → severe decrease in weight

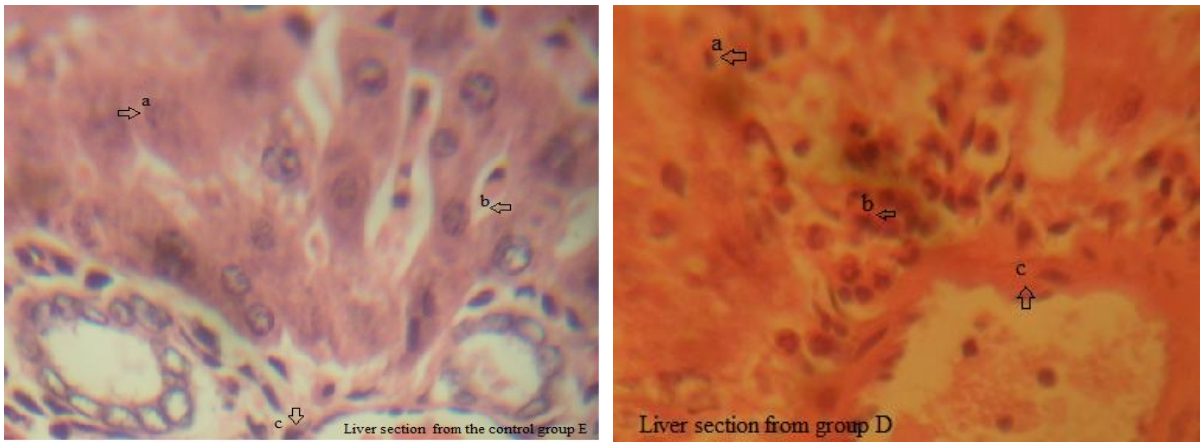

Figure 2: Showed the liver section from the control group E composed of portal triad (c), surrounded by hepatocytes (a) and sinusoids (b); with the liver section from the treated group D composed of relatively mild portal congestion and dilatation (c), moderate activation of periportal lymphocytes B and kupffer cells (a). Stain uptake: Mayer's H&E X40 magnification.

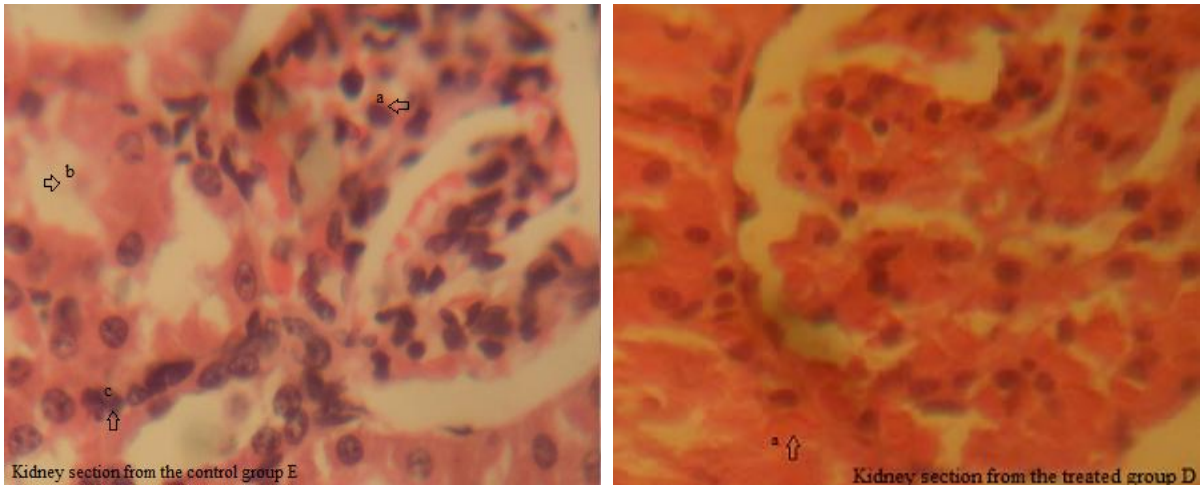

Figure 3: Showed the kidney section from the control group E composed of cortical glomeruli (a), tubules (b) and interstitial space (c); with the kidney section from the treated group D composed of mild interstitial congestion (a). Stain uptake: Mayer's H&E X40 magnification.