

HISTOPATHOLOGICAL ASSESSMENT OF ORGAN IMPAIRMENT OF *Rattus albus* TREATED WITH SELECTED MEDICINAL PLANTS

ABSTRACT

Histopathological assessment of organ impairment of *Rattus albus* treated with selected plant extracts: (*Napleonea imperalis*, *Sida acuta* and *Vernonia amygdalina*) was studied from October 2014 to March 2016. The aim was to determine the histopathological effects of the plant extracts on *Rattus albus*. The *Rattus albus* were treated with 3 selected plant extracts: *Vernonia amygdalina*, *Sida acuta* and *Napoleonea imperialis* and observed for 3 weeks. The active principles of the selected medicinal plants were extracted with ethanol at 78°C using soxhlet extraction method. A total of 22 male and 23 female laboratory animals were selected and treated with plant extracts and reference drugs. The different treatment groups and controls were selected for histopathological studies using paraffin wax embedding method. They were sacrificed and examined histopathologically for pathological features. The results showed that, all 3 selected plant extracts contain Tanins, Saponins, Alkaloids, Flavonoids, Cardiac Glycosides, Phytate, Oxalate, Phenol, Steroids, Terpenoids and Cyanide. Out of 4 laboratory animals in each group treated with 10µg of crude *Napoleonea imperialis*, *Sida acuta* and *Vernonia amygdalina* extracts, no feature of impairment was observed in the intestine, kidneys and liver of all the laboratory animals used. Out of 4 laboratory animals in each group treated with 20µg of crude *Napoleonea imperialis* extract, the intestine and liver of one each showed features of impairment. Out of 4 laboratory animals treated with 20µg

of crude *Sida acuta* extract, the intestine, kidney and liver of one animal each showed features of impairment. Out of 4 laboratory animals treated with 20 µg of crude *Vernoniaamygdalina* extract, no impairment was observed in the intestine, kidneys and liver of all laboratory animals used. Out of 4 laboratory animals treated with 30 µg of crude *Napoleoneaimperialis* extract, the intestine of 1, the kidney of 1 and livers of 2 showed features of impairment. Out of 4 laboratory animals treated with 30 µg of crude *Sidaacuta* extract, the intestine of 2, kidney of 1 and livers of 2 showed features of impairment. Out of 5 laboratory animals treated with 30 µg of crude *Vernoniaamygdalina* extract, the intestine of 1, the kidneys 1 and livers of 2 showed features of impairment. Organ impairment on laboratory animals due to plant extract shows that, out of 12 animals (6 males and 6 female laboratory animals) treated with *Napoleoneaimperialis* 10 (83.3%) had normal intestine and 2 (16.7%) had abnormal intestine, 11 (91.7%) had normal kidneys, 1 (8.3%) had abnormal kidney, 9 (75%) had normal liver and 3 (25%) had abnormal liver (Table 2). Out of 12 laboratory animals (6 male and 6 female laboratory animals) treated with *Sidaacuta* 9 (75%) had normal intestine and 3 (25%) had abnormal intestine, 10 (83.3%) had normal kidneys, 2 (16.7%) had abnormal kidney, 9 (75%) had normal liver and 3 (25%) had abnormal liver. Out of 13 laboratory animals (6 male and 7 female laboratory animals) treated with *Vernoniaamygdalina*, 12 (92.3%) had normal intestine and 1 (7.7%) had abnormal intestine, 12 (92.3%) had normal kidneys, 1 (7.7%) had abnormal kidney, 11 (84.6%) had normal liver and 2 (15.4%) had abnormal liver. Analysis of the data using chi square showed significant difference ($p < 0.05$) in the frequency of organ impairments between the different organs of the laboratory animals. The highest frequency of impairment was observed on the liver followed by intestine and the least impairment was on the kidneys. Three types of impairment: inflammatory changes, degenerative changes and distortions were observed on the intestine of the laboratory animals. On the kidneys, 5 types of impairment: lymphocytic infiltration, degenerative changes, necrosis, vacuolation, and distortion of stroma and glomerulus were observed. On the liver, 4 types of impairment: inflammatory changes,

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hepatocyticdegenerative changes, necrosis and distortion of hepatocyteswere observed. The nature and types of impairment caused on the different organs differ remarkably. Whereas the frequency of impairment was higher in liver organs 9 (20.0%) than kidneys 4 (8.9%), the types of impairment observed were higher in kidneys than liver organs. This study has shown that *Napoleonaeimperialis*, *Sidaacuta*and *Vernoniaamygdalina*extracts at higher concentration, exhibit pathologiceffects on host organs: intestine, kidney and liver.The plant extracts are likely to be sources of novel drugs or analogues of synthetic drugs capable of treating human intestinal parasitic infections.

Keywords: *histopathological assessment, organ impairment, Rattusalbus , medicinal plants*

INTRODUCTION

The use of traditional medicine in Nigeria is as old as the people; and has remained relevant among other types of therapy. More than 50% of all modern clinical drugs are of plant origin (Suffness and Douros, 1982). Plant products therefore play an important role in drug development programs of the pharmaceutical industry (Baker *et al.*, 1995). WHO defined traditional medicine as therapeutic practices that have been in existence for hundreds of years before the development of and spread of modern scientific medicine and are still in use today (Onyegbuleet *al.*,2011).Traditional medicines, based largely on herbs and trees, offer a major and accessible source of health care to people living in developing countries (WHO, 2002). Commonly used medicinal plants in Nigeria include: *Napoleonaeaimperialis*, *Sidaacuta*, *Vernoniaamygdalina*.

Napoleonaeaimperialis P. Beauv is a Nigerian folkloic medicinal plant. It is a small, evergreen tropical West African tree in the family, Lecythidaceae (Badri and Renu, 2011). The bark and the fruit pulp are used as a cough medicine and the raw bark is chewed for this effect (Ojinnaka and Okpala, 2012). *Sidaacuta* is an erect, perennial shrub belonging to the mallow family, Malvaceae (Karouet *al.*, 2007). *Sidaacuta* has many traditional usage that varied from one region to another. The most cited illnesses are fever, headache, skin diseases, diarrhoea and dysentery (Karouet *al.*, 2007). The leaves are considered to possess demulcent, diuretic, antihelminthic and wound healing properties and are used for rheumatic affections (Murugesu, 1998).

Vernoniaamygdalina is a perennial herb belonging to the family, Asteraceae. *V. amygdalina* is commonly known as bitter leaf because of its bitter taste. Also, Ademola and Eloff, (2011), reported that extracts of *V. amygdalina* possess *in vitro* anti – parasitic (anti-helminthic) properties. Thus, it is effective against amoebic dysentery, gastro-intestinal disorders and has anti-microbial and anti-parasitic activities (Alawa *et al.*, 2003; Farombi and Owoeye, 2011).

Phytochemicals (biologically active compounds) such as saponins and alkaloids, terpenes, steroids, coumarins, flavonoids, phenolic acids, lignans, Xanthones, anthraquinones, edotides, and sesquiterpenes have been extracted and isolated from *V. amygdalina*, *N. imperialis*, and *Sidaacuta* (Alabiet *al.*, 2005.; Nwanjo, 2005). The medical uses of medicinal plants stem largely from relatively high levels of alkaloids and flavonoids, facilitating medicinal uses (Karouet *al.*, 2007). However, increasing problems of development of resistance in helminths against anti-helminthics have led the proposal of screening medicinal plants for their anti-helminthic activity (Coles, 1997).

The use of living laboratory animals as biological agents have provided knowledge to better understanding of both physiological and pathological processes in both man and other animals (Dillehay *et al.*, 1990). The plants *N. imperialis*, *Sidaacuta* and *V.amygdalina* have been used extensively in Igbo traditional medicine for treating intestinal discomfort. This underscores the need for Histopathological Assessment of Organ Impairment on *Rattusalbus* treated with these Medicinal Plants.

MATERIALS AND METHODS

MATERIALS

Study Area

This study was carried out in Orlu, Imo State, South-Eastern Nigeria. Imo State shares boundary with Anambra State in the North, Rivers State in the South and West and Abia State in the East. The standard of living is average and most of the populace depend on locally prepared herbs as an alternative medicine for their ailments since they are readily available and affordable.

Plant Materials

The medicinal plants used for this study were: *Napoleonaeaimperialis*P.Beauv, *Sidaacuta* and *Vernoniaamygdalina*. Roots of *Napoleonaeaimperialis*P.Beauv were purchased from Orié market, Umuna, Orlu. Leaves of *Sidaacuta* and *Vernoniaamygdalina* were collected from uncultivated farm land at Amaifeke, Orlu. The plants were identified in the Department of Plant Science and Biotechnology, Imo State University, Owerri.

Laboratory Animals:

The laboratory animals used for this study were male and female Wistar strain of *Rattusalbus* of 2 to 3 months old and body weights of 80 to 180g.

METHODS

Processing of Plant Materials

The roots of *Napoleonaeaimperialis*, leaves of *Sidaacuta* and *Vernoniaamygdalina* were dried under the shade and finally in thermostatically controlled hot air oven at 40°C until each maintained constant weight. Each was ground into fine powder using a warren blender machine and sieved using 1mm mesh sieve. The powdered plant materials were stored in labeled screw capped bottles and stored in the fume cupboard until required for extraction.

Extraction of Active Principles of the Plant Materials

Soxhlet Extraction

The active principles of the selected plant materials were extracted with ethanol at 78⁰C using soxhlet extraction method as in Harborne, (1998), Obiajuru and Ozumba, (2009).The extracts were recovered and stored at +8⁰C in screw capped MacCartenybottles until required for use.

Examination of Laboratory Animals

Inclusion Criteria:Healthy *Rattusalbus* without any sign or symptom of cardiac or renal diseases were selected for the study

Exclusion criteria: *Rattusalbus* with heavy infection of intestinal parasites were excluded. Also those with cardiac and renal diseases markers when tested were excluded in the research.

Experimental Design

Four hundred(400) apparently healthy Laboratory animals were used for the study, 78 were infected and 322 were not infected. The uninfected laboratory animals (322) were selected for Histopathologicaleffects of the selected plant extracts.

The selected Laboratory animals were separated into 6 groups according to age, gender and body weight of the Albino rats

Innoculation of Crude Extract of the Medicinal Plants

The uninfected Laboratory animals (Albino rats) were allowed to getacclimated to the environment for a period of 4 weeks. At the end of the 4th week, crude extracts of the selected plant and reference drug solutions were prepared using physiological saline. Different concentrations of crude plant extracts were processed by 2 fold double dilutions to obtain $1/10^{-1}$, $1/10^{-2}$ and $1/10^{-3}$ concentrations ($^{W/V}$). The different concentrations of the plant extracts and reference drugs were administered to the laboratory animals (Albino rats) orally using automatic pipette. 0.5ml of the crude extracts and reference drugs were administered on the laboratory animals once per week for a period of three weeks.

Different colour codes were used to identify the plant extracts or reference drugs administered on the laboratory animals (Albino rats) in each treatment group.

Histological Studies

Two weeks after the 3rd dose of chemotherapy, a male and a female Albino rats were selected from each of the different treatment groups and controls. The selected Albino rats were anesthetized by placing on a wire gauze with cotton wool soaked in Diethyl ether placed below the gauze in a transparent glass dessicator. Within 34 to 57 seconds (average= 48.7secs) each of the Albino rats were sedated to sleep. When each animal was sedated, a sterile surgical blade and scapel were used to make an incision on the mid line of the ventral surface of the rat, the liver, intestine and kidney were excised.

The liver, kidney and intestine of each animal, were dissected and placed into labelled specimen bottles containing 10% formal saline as fixative. These were used for histopathological studies.

Tissue Processing

The tissues of the laboratory animals (Albino rats) for Histopathology examination were processed and embedded in paraffin wax as described by Winsor, (1994) and Kiernan, (2009).

Sectioning of Laboratory Animal Tissues

Paraffin wax embedded tissue blocks were sectioned in the Histopathology Laboratory, Imo State University Teaching Hospital, Orlu, using Rotary Microtome. The microtome was set to cut sections at 4 microns. Disposable microtome blades were used. Serial sections of the various tissues were floated out on warm water bath, collected with grease – free slide and fixed on clean glass slides using warm hot plates as in Winsor, (1994); Barker, Silverton and Pallister, (2009).

Staining Technique

The fixed sections of the various laboratory animal tissues used for the study were dewaxed, dehydrated and stained using routine Haematoxylin and Eosin staining technique as in Winsor, (1994); Barker, Silverton and Pallister (2009) and Kiernan, (2009). The stained slides were labelled, stored in slide boxes until required for examination. The assistance of a Consultant Histopathologist at Federal Medical Centre, Owerri was sought for microscopic examination of the slides.

Microscopy and Photomicrography

Stained films of the laboratory animal tissues sections were examined microscopically using x10 eye piece and x40 objective. Reporting was done, comparing the slide observations of test samples with control groups and standard Histopathology atlas (Eroschenko, 2008). Photomicrographs of the tissues were taken using digital microscope eye piece (Leica Camera Microscope) (x400).

STATISTICAL ANALYSIS

The data obtained from the study were analyzed using analysis of variance (ANOVA), Chi-square and simple percentage analysis. Frequency distributions and cross tabulation to determine relationship between variables. Results were expressed as mean \pm SD for some groups. A p value ≤ 0.05 was considered significant.

RESULTS

PATHOLOGICAL FEATURES OF LABORATORY ANIMALS TREATED WITH SELECTED MEDICINAL PLANTS

A total of 22 male and 23 female laboratory animals were selected from the uninfected Albino rats and treated with plant extracts and reference drugs. They were sacrificed and examined histopathologically for pathological features. **Table 1** shows the effects of the plant extracts on 3 organs: intestine, kidneys and liver, of the laboratory animals. As shown, out of 4 laboratory animals treated with 10 μ g of crude *Napoleoneaimperialis* extract, the intestine of 4 appeared normal, kidney of 4 and liver of 4 also appeared normal. No impairment was observed in their intestine, kidneys and liver. Out of 4 laboratory animals treated with 20 μ g of crude *Napoleoneaimperialis* extract, the intestine of 3, liver of 3 and kidneys of 4 appeared normal. The intestine of 1 and liver of 1 showed features of impairment. Out of 4 laboratory animals treated with 30 μ g of crude *Napoleoneaimperialis* extract, the intestine of 3, liver of 2 and kidney of 3 appeared normal. The intestine of 1, the kidney of 1 and livers of 2 showed features of impairment.

Out of 4 laboratory animals treated with 10 μ g of crude *Sidaacuta* extract, the intestine of 4, kidneys of 4 and liver of 4 appeared normal. No feature of impairment was observed in the intestine, kidneys and liver of all the laboratory animals used. Out of 4 laboratory animals treated with 20 μ g of crude *Sidaacuta* extract, the intestine of 3, kidneys of 3 and liver of 3 appeared normal. The intestine of 1, kidney of 1 and liver of 1

showed features of impairment. Out of 4 laboratory animals treated with 30 µg of crude *Sidaacuta* extract, the intestine of 2, kidney of 3 and liver of 2 appeared normal. The intestine of 2, kidney of 1 and livers of 2 showed features of impairment.

Out of 4 laboratory animals treated with 10 µg of crude *Vernoniaamygdalina* extract, the intestine of 4, kidneys of 4 and liver of 4 appeared normal. No impairment was observed in the intestine, kidneys and liver of all laboratory animals used. Out of 4 laboratory animals treated with 20 µg of crude *Vernoniaamygdalina* extract, the intestine of 4, kidneys of 4 and liver of 4 appeared normal. No impairment was observed in the intestine, kidneys and liver of all laboratory animals used. Out of 5 laboratory animals treated with 30 µg of crude *Vernoniaamygdalina* extract, the intestine of 4, kidneys of 4 and liver of 3 appeared normal. The intestine of 1, the kidneys 1 and livers of 2 showed features of impairment.

Out of 2 laboratory animals treated with Albendazole, the intestine of 2, kidneys of 2 and liver of 2 appeared normal. Out of 2 laboratory animals treated with Metronidazole, the intestine of 2, kidneys of 2 and liver of 2 appeared normal (positive control). Out of 4 negative control laboratory animals not treated with any chemotherapeutic agent, all the organs appeared normal.

ORGAN IMPAIRMENT DUE TO PLANT EXTRACTS

Organ impairment on laboratory animals treated with plant extracts are summarized in **table 2**. As shown, out of 12 animals (6 males and 6 female laboratory animals) treated with *Napoleoneaeimperialis* 10 (83.3%) had normal intestine and 2 (16.7%) had abnormal intestine, 11

(91.7%) had normal kidneys, 1 (8.3%) had abnormal kidney, 9 (75%) had normal liver and 3 (25%) had abnormal liver. Out of 12 laboratory animals (6 male and 6 female laboratory animals) treated with *Sidaacuta* 9 (75%) had normal intestine and 3 (25%) had abnormal intestine, 10 (83.3%) had normal kidneys, 2 (16.7%) had abnormal kidney, 9 (75%) had normal liver and 3 (25%) had abnormal liver. Out of 13 laboratory animals (6 male and 7 female laboratory animals) treated with *Vernoniaamygdalina*, 12 (92.3%) had normal intestine and 1 (7.7%) had abnormal intestine, 12 (92.3%) had normal kidneys, 1 (7.7%) had abnormal kidney, 11 (84.6%) had normal liver and 2 (15.4%) had abnormal liver. Analysis of the data using chi square showed significant difference ($p < 0.05$) in the frequency of organ impairments between the different organs of the laboratory animals. The highest frequency of impairment was observed on the liver followed by intestine and the least impairment was on the kidneys. Statistical analysis of the data, using two - way ANOVA (0.05) shows strong positive correlation between the concentration of crude plant extract and degree of organ impairment in laboratory animals. As the concentration of crude extract administered increased, the degree of impairment increases. The highest degree of impairment was observed on liver of laboratory animals treated with *Napoleoneaeimperialis* and *Sidaacuta*.

Table 3 shows the sex – related frequency of organ impairment in laboratory animals treated with plant extracts. As shown, out of 22 male laboratory animals treated with plant extracts, 20 (90.9%) had normal intestine and 2 (9.1%) had abnormal intestines, 19 (86.4%) had normal kidneys and 3 (13.6%) had abnormal kidneys, 16 (72.7%) had normal liver and 6 (27.3%) had abnormal liver. Out of 23 female laboratory animals treated with plant extracts, 18 (78.3%) had normal intestine and 5 (21.7%) had abnormal intestines, 22 (95.7%) had normal kidneys and 1 (4.3%) had abnormal kidneys, 20 (87.0%) had normal liver and 3 (13.0%) had abnormal liver. Analysis of the data using two – way ANOVA

statistics showed strong positive correlation on the degree of impairment between male and female laboratory animals ($p < 0.05$). The plant extracts caused higher organ impairments (27.3%) on liver of males than liver of female laboratory animals (13.0%).

Table 1: EFFECTS OF PLANT EXTRACTS ON ORGANS OF LABORATORY ANIMALS

Plant Extract	Conc. of Extract Given (μg)	No of Lab. Animals treated	Effects of plant extracts on Lab. Animal Organs							
			Intestine		Kidney		Liver			
			Normal	Abnormal	Normal	Abnormal	Normal	Abnormal		

UNDER PEER REVIEW

<i>Napoleoneaimperialis</i>	10.0	4	4	0	4	0	4	0
	20.0	4	3	1	4	0	3	1
<i>Sidaacuta</i>	30.0	4	3	1	3	1	2	2
	10.0	4	4	0	4	0	4	0
	20.0	4	3	1	3	1	3	1
<i>Vernoniaamygdalina</i>	30.0	4	2	2	3	1	2	2
	10.0	4	4	0	4	0	4	0
Albendazole	20.0	4	4	0	4	0	4	0
Metronidazole(Positive	30.0	5	4	1	4	1	3	2
Control)	10.0	2	2	0	2	0	2	0
Negative Control	10.0	2	2	0	2	0	2	0
		4	4	0	4	0	4	0
Total		45	38	07	41	04	36	09
			(84.4)	(15.6)	(91.1)	(8.9)	(80.0)	(20.0)

Table 2: FREQUENCY OF ORGAN IMPAIRMENT DUE TO PLANT EXTRACTS

Plant Extract	No of Lab. Animals treated	Impairments on Lab. Animal Organs due to plant extracts					
		Intestine		Kidney		Liver	
		Normal	Abnormal	Normal	Abnormal	Normal	Abnormal
<i>Napoleoneaimperialis</i>	12	10 (83.3)	02(16.7)	11 (91.7)	1 (8.3)	9 (75.0)	3(25.0)
<i>Sidaacuta</i>	12	9 (75.0)	3(25.0)	10 (83.3)	2 (16.7)	9 (75.0)	3(25.0)
<i>Vernoniaamygdalina</i>	13	12(92.3)	01(7.7)	12(92.3)	01(7.7)	11(84.6)	02(15.4)
Albendazole							
Metronidazole (Positive control)	02	02(100.00)	00(00.00)	02(100.00)	00(00.00)	02(100.00)	00(00.00)
Negative control	02	02(100.00)	00(00.00)	02(100.00)	00(00.00)	02(100.00)	00(00.00)
	04	04(100.00)	00(00.00)	04(100.00)	00(00.00)	04(100.00)	00(00.00)
Total	45	38(84.4)	07(15.6)	41(91.1)	04(8.9)	36(80.0)	9(20.0)

$(p < 0.05)\chi^2 = 9.735$

TABLE 3: SEX - RELATED FREQUENCY OF ORGAN IMPAIRMENTS DUE TO PLANT EXTRACTS

Nature of organ	Sex	No examined	No. of organ (s) with normal features (%)	No. of organ (s) with abnormal features (%)
Intestine	M	22	20 (90.9)	02 (09.1)
Kidney			19 (86.4)	03 (13.6)
Liver			16 (72.7)	06 (27.3)
Intestine	F	23	18 (78.3)	5 (21.7)
Kidney			22 (95.7)	01 (04.3)
Liver			20 (87.0)	03 (13.0)
Total		45		

$(p < 0.05)\chi^2 = 8.351$

COMPARATIVE ANALYSIS OF NORMAL AND IMPAIRED LAB. ANIMAL ORGANS

The photo - micrographs of normal organs (intestine, kidneys and liver) of laboratory animals are shown in **plates 1, 2 and 3**, while **plates 4 to 15** show the impairments caused on laboratory animal organs (intestine, kidneys and liver) by plant extracts. As shown, 3 types of impairment: inflammatory changes (**plate 4**), degenerative changes (**plate 5**) and distortion (**plate 6**) were observed on the intestine of the laboratory animals. On the kidneys, 5 types of impairment: lymphocytic infiltration (**plate 7**), degenerative changes (**plate 8**), necrosis (**plate 9**), vacuolation (**plate 10**) and distortion of stroma and glomerulus (**plate 11**) were observed. On the liver, 4 types of impairment: inflammatory changes (**plate 12**), hepatocytic degenerative changes (**plate 13**), necrosis (**plate 14**) and distortion of hepatocytes (**plate 15**) were observed.

The nature and types of impairment caused on the different organs differ remarkably. Whereas the frequency of impairment was higher in liver organs 9 (20.0%) than kidneys 4 (8.9%), the types of impairment observed were higher in kidneys (**5 types**) than liver organs (**4 types**).

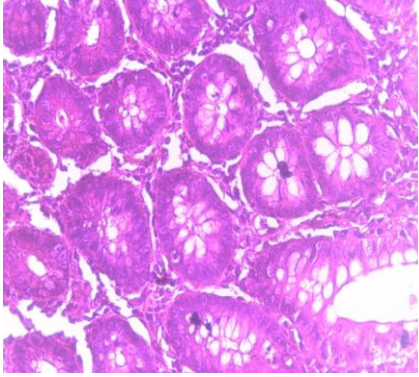


Plate 1: Photomicrograph of a Normal Intestine
KEY: No Extract/drug administered

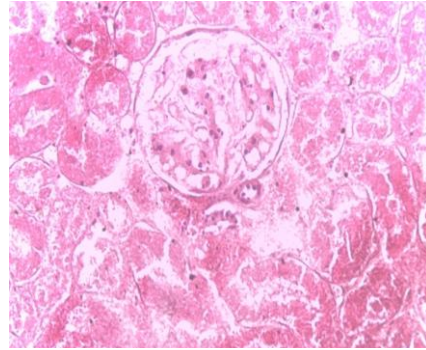


Plate2: Photomicrograph of a Normal Kidney
KEY: Extract/drug administered: 10 μ g
Vernoniaamygdalina

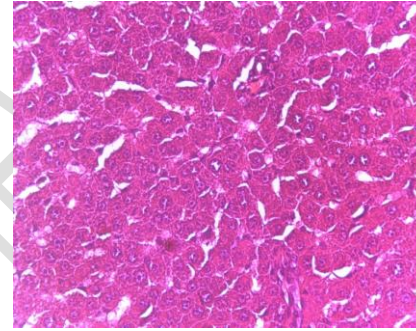


Plate 3: Photomicrograph of a Normal Liver
KEY: No Extract/drug administered.

UNDER PEPER H/VE

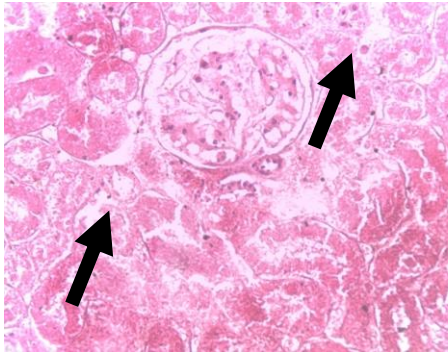


Plate 4: Photomicrograph of the Intestine Showing Inflammatory Changes
KEY: Extract/drug administered: 20 μ g *Sidaacuta*

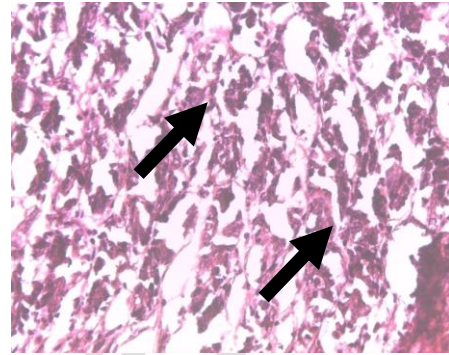


Plate 5: Photomicrograph of the Intestine Showing Degenerative Change
KEY: Extract/drug administered: 30 μ g *Vernoniaamygdalina*

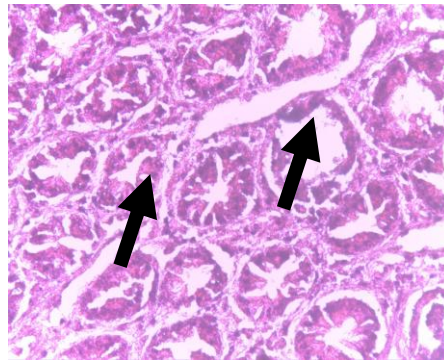


Plate 6: Photomicrograph of the Intestine Showing Distortion of Intestine
KEY: Extract/drug administered: 50 μ g *Napoleoneaeimperialis*

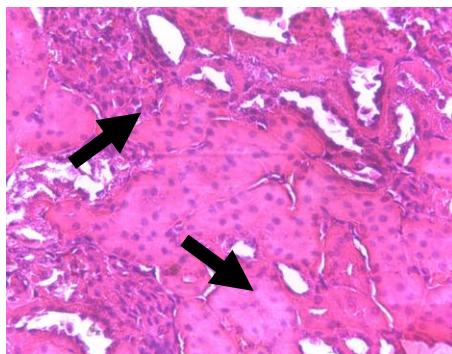


Plate 7: Photomicrograph of the Kidney Showing Lymphocytic infiltrate
KEY: Extract/drug administered: 30µg *Sidaacuta*

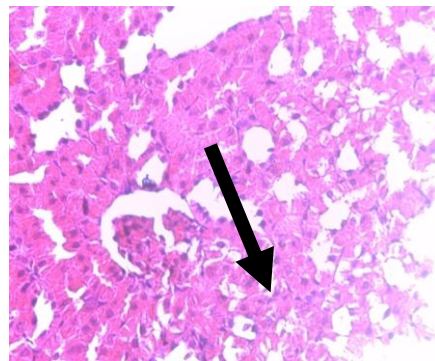


Plate 8: Photomicrograph of the Kidney Showing Degenerative Changes
KEY: Extract/drug administered: 20µg *Sidaacuta*

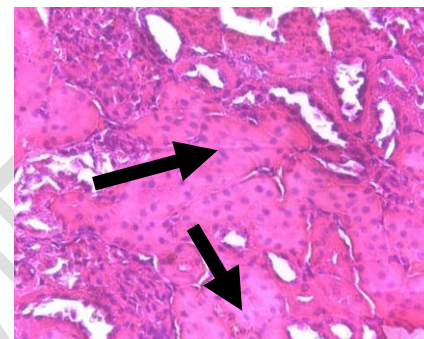


Plate 9: Photomicrograph of the Kidney Showing Necrosis
KEY: Extract/drug administered: 30µg *Sidaacuta*

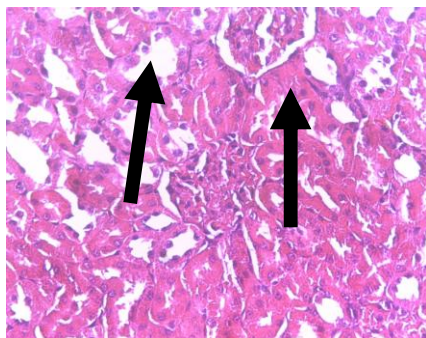


Plate 10: Photomicrograph of the Kidney Showing Vacuolation
KEY: Extract/drug administered: 30µg *Napoleoneaimperialis*

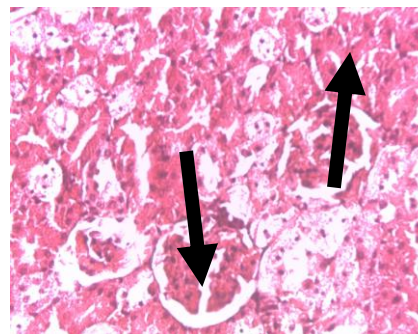


Plate 11: Photomicrograph of the Kidney Showing Distortion of Stroma and Glomerulus
KEY: Extract/drug administered: 50µg *Vernoniaamygdalina*

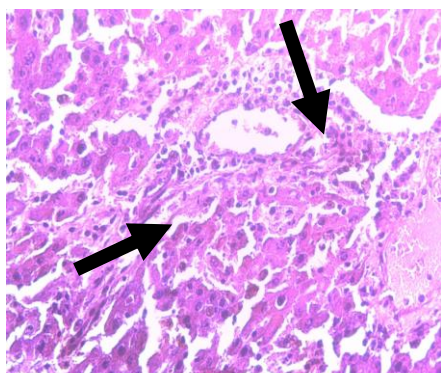


Plate12: Photomicrograph of the Liver Showing Inflammatory Changes

KEY: Extract/drug administered: 30 μ g *Napoleoneaimperialis*

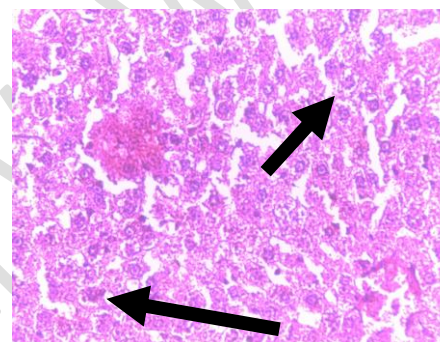


Plate13: Photomicrograph of the Liver Showing Hepatocytic Degenerative Changes

KEY: Extract/drug administered: 40 μ g *Sidaacuta*

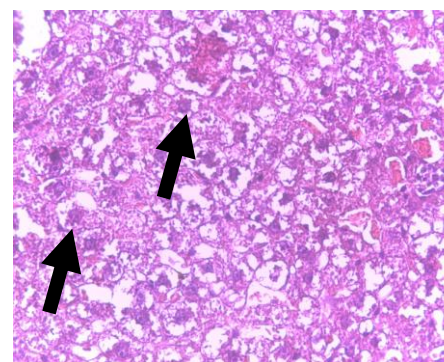
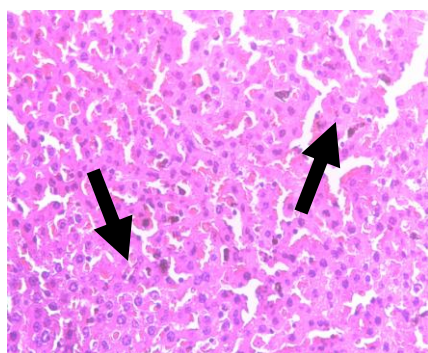


Plate14: Photomicrograph of the Liver Showing Necrosis
KEY: Extract/drug administered: 30µg *Sidaacuta*

Plate15: Photomicrograph of the Liver Showing Distortion of Hepatocytes
KEY: Extract/drug administered: 40µg *Vernoniaamygdalina*

DISCUSSION

Traditional use of medicinal plants in the treatment of animal and human diseases is gaining global interest very fast (Naluleet *al.*, 2013). It was in this light that the World Health Assembly, in 1989, adopted among its resolutions, the support of national traditional medicine program, drawing attention to herbal medicines as being of great importance to the health of individuals and communities (Nwanjo, 2005).

Traditional medicines are used by about 60% of the world population in both developing and developed countries where modern medicines are predominantly used while an estimated 60-80% Africa's population depends solely on herbal remedies for its primary health care needs (Adesuyiet *al.*, 2011).

The phytochemical and proximate analysis of the selected medicinal plants (*Vernoniaamygdalina*, *Sidaacuta* and *Napoleonoaimperialis*) showed that the active principles (Alkaloids, Flavonoids, Cardiac Glycosides, Tanin, Saponin, Terpenoid,

Oxalate, Phytate, Phenolic compound and Steroids) are known phytochemical compound useful in production of chemotherapeutic agents and other industrial products.

Table 1 shows the effects of the plant extracts on 3 organs: intestine, kidneys and liver, of the laboratory animals. As shown, all the laboratory animals treated with 10µg of crude extracts of *Napoleoneaimperialis*, *Sidaacuta* and *Vernoniaamygdalina*, had no impairment in their intestine, kidneys and liver.

At 30 µg, crude extracts of *Napoleoneaimperialis*, *Sidaacuta* and *Vernoniaamygdalina* showed varying features and degrees of impairments.

This shows that at higher concentration (30 µg), more impairments occur in the organs especially in the liver. This may be as a result of the high content of phytochemical agents present in the plant extracts (*Vernoniaamygdalina*, *Sidaacuta* and *Napoleoneaimperialis*) used in this study. The potency of these extracts may have been amplified on account of increased concentration or build up of specific phytochemicals.

Organ impairment on laboratory animals due to plant extract showed that, *Sidaacuta* caused the highest number of organ impairment, 3 (25%) had abnormal intestine, 2 (16.7%) had abnormal kidney, and 3 (25%), followed by *Napoleoneaimperialis*, 2 (16.7%) had abnormal intestine, 1 (8.3%) had abnormal kidney, and 3 (25%) had abnormal liver and the least by *Vernoniaamygdalina*, 1 (7.7%) had abnormal intestine, 1 (7.7%) had abnormal kidney, and 2 (15.4%) had abnormal liver. (**Table 2**). The rate of abnormalities is higher in animals treated with *Sidaacuta* extracts and may be as a result of the high content of Oxalate present in the leaf of the plant.

Table 3 shows that plant extracts used in this study exhibited more organ impairments on male than female laboratory animals. This finding may be due to difference in response to foreign bodies between male and female animals. Very little organ impairment was observed on kidneys of female laboratory animals whereas, more impairments were observed on kidneys of male laboratory animals. This finding may be because male animals retain urine for longer periods than females. The plant extracts were administered orally. The female animals may have excreted large quantities of the extracts via urine within a short time, retaining very little quantities that could not have exhibited much organ impairments as observed in this study. In contrast, male laboratory animals probably retained all the extracts administered for fairly long period leading to absorption of higher concentrations of the extracts, hence higher organ impairments observed.

The photo - micrographs of normal organs (intestine, kidneys and liver) of laboratory animals are shown in plates 1, 2 and 3, while plates 4 to 15 show the impairments caused on laboratory animal organs (intestine, kidneys and liver) by plant extracts.

Generally, this study observed organ impairments on the liver, kidneys and intestine of the laboratory animals. As shown, 3 types of impairment: inflammatory changes (plate 4), degenerative changes (plate 5) and distortion (plate 6) were observed on the intestine of the laboratory animals. These organ impairments occurred at higher concentrations (20 to 40 μg) for *Sida acuta* and *Napoleonoeaimperialis* while *V. amygdalina* showed no impairment even at 20 μg on any of the organs (intestine, kidney and liver)

All these evidences revealed that *V. amygdalina* is a very important plant that can be exploited for cyto-protective purposes. The cyto-protective mechanism may be by mopng up free radical there by protecting the cells of the organs from any type of

assaults. Studies have shown that anti-oxidants significantly strengthen the gastric walls and protect tissue from oxidative damage (Martin, 1996). Several findings showed that *V. amygdalina* has strong antioxidant activity corresponding to mitigation of the generation of hydroxyl radicals (Nwangwu *et al.*, 2011).

On the liver, 4 types of impairment: inflammatory changes (plate 12) hepatocytic degenerative changes (plate 13), necrosis (plate 14) and distortion of hepatocytes (plate 15), were observed.

The acute exposure of the Albino rats to concentrated grades (30 µg) of *Vernonia amygdalina*, *Sida acuta* and *Napoleonoa imperialis* induced progressive striking histological alterations in the liver (inflammatory changes, hepatocytic degenerative changes, necrosis and distortion of hepatocytes).

Retention of water inside hepatocyte resulting in cell enlargement (inflammatory changes) may be due to reduction of energy necessary for ion regulation in the cells.

Some bioactive substances such as alkaloids, flavanoids, tannins, saponins and phenol are present in the extracts of the plants in definite quantities. Previous studies (Rodriguez *et al.*, 2008) reported that phenolic compounds (e.g. flavonoids), nitrogen compounds (e.g. alkaloids), saponins, and tannin present in the plant extracts have antiradical activities. Free radicals setup a chain reaction that can cause biological damage by stimulating glycation of protein, inactivation of enzymes and alteration in the structure and function of collagen basement and other membranes.

The histopathological changes observed in the kidney sections showed 5 types of impairment: lymphocytic infiltration (plate 7), degenerative changes (plate 8), necrosis (plate 9), vacuolation (plate 10) and distortion of stroma and glomerulus (plate 11).

Kebeet *al.*, (2013) reported that the increase in the luminal spaces in the treated rats might be an indication that the extract has adversely affected the renal clearance which may consequently lead to diuresis.

The nature of impairment caused on the different organs differ remarkably. The highest frequency of impairment was observed on the liver followed by intestine and the least impairment was on the kidneys.

CONCLUSION

The secondary metabolite contents of the plant extracts were high and may be responsible for the organ impairment in the Albino Rats.

This study has shown that *Napoleonaeimperialis*, *Sidaacuta* and *Vernoniaamygdalina* extracts exhibit organ impairment on host organs: intestine, kidney and liver only at higher concentration.

COMPETING INTERESTS DISCLAIMER:

Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

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