Journal of Advances in Medical and Pharmaceutical Sciences

24(4): 28-43, 2022; Article no.JAMPS.86613
ISSN: 2394-1111

Curative and Toxic Effects of Selected Medicinal Plants on Internal Organs of *Rattus albus* Experimentally Infected with Human Intestinal Parasites

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/JAMPS/2022/v24i430295

Open Peer Review History:
This journal follows the Advanced Open Peer Review policy. Identity of the Reviewers, Editor(s) and additional Reviewers, peer review comments, different versions of the manuscript, comments of the editors, etc are available here: https://www.sdiarticle5.com/review-history/86613

Original Research Article

Received 27 March 2022
Accepted 05 May 2022
Published 14 June 2022

ABSTRACT

Curative and Toxic effects of selected plant extracts: (*Napleonaea imperalis, Sida acuta* and *Vernonia amygdalina*) on *Rattus albus* experimentally infected with human intestinal parasites was studied from October 2014 to March 2016. Two hundred and thirty (230) male and female patients presenting with symptoms of gastroenteritis and abdominal discomfort at GOPD, IMSUTH, Orlu, were selected for the study. Three hundred and twenty two uninfected laboratory animals used for the study were innoculated with 7 human intestinal parasites: *Ascaris lumbricoides*, *Necator americanus, Hymenolepsis nana*, *Trichuris trichiura, Entamoeba histolytica,Taenia* species and *Trichomonas hominis.*Eight weeks after, the experimentally infected *Rattus albus* were treated with 3 selected plant extracts: *Vernonia amygdalina, Sida acuta* and *Napoleonae imperialis* and observed for 3 weeks. The active principles of the selected medicinal plants were extracted with ethanol at 78^{o}C using soxhlet extraction method. Fifty four laboratory animals of about 150g body

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weight each were used for toxicity test. The different treatment groups and controls were selected for histopathological studies using paraffin wax embedding method. The results showed that all 3 selected plant extracts contain Tanins, Saponins, Alkaloids, Flavonoids, Cardiac Glycosides, Phytate, Oxalate, Phenol, Steroids, Terpenoids and Cyanide. Curative tests showed that all the plant extracts exhibited anti – parasitic effects (Curative dose) against human intestinal helminthes and protozoa at 10µg/150g body weight. Toxicity test showed that *Napoleonaea imperialis* and *Vernonia amygdalina* extracts exhibited toxicity resulting to death of laboratory animals (LD_{50}) at 50µg/150g while *Sida acuta* extract exhibited toxicity resulting to death of laboratory animals (LD_{50}) at 40µg/150g. Three types of impairment: inflammatory changes, degenerative changes and distortion 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. This study has shown that *Napoleonae imperialis, Sida acuta* and *Vernonia amygdalina* extracts exhibit curative effects on human intestinal parasites at low concentrations. At higher concentration, they exhibit toxic effects on host organs: intestine, kidney and liver.

Keywords: Toxic effects; medicinal plants; organs; human intestinal parasites; flavonoids; alkaloids.

1. INTRODUCTION

Medicinal plants play central roles in the health care system of large proportion of the world's population especially in developing countries where herbal medicine has a long and uninterrupted history of use [1]. Herbal medicine is still the main stay of about 75 -80% of the world population, mainly in the developing countries for primary health care [2].

Moreso, recognition and development of medicinal and economic benefits of these plants are on the increase in both developing and industrialized nations [3]. Many synthetic drugs originated from plant sources, thus, most of the few effective drugs were plant -based [2].

Also, Yedjou et al*.,* [4] estimated that 80% of the population of Africa depend on medicinal plants to satisfy their health care requirement.

The limited availability and affordability of pharmaceutical medicines means that the majority of the world's population depends on traditional medical remedies [5].

Parasitism is well known as a limiting factor responsible for losses in health and productivity [6]. Parasitic diseases remain a major public health problem affecting hundreds of millions of people, particularly in tropical developing countries [5]. Human infections caused by endoparasites, including protozoa, nematodes, trematodes and cestodes, affect more than 30% of the human population [7].

Intestinal helminths are one of the most common causesS of infections in humans, especially in tropical and subtropical countries [8]. These intestinal diseases cause enormous hazards to the health of people, particularly of children, by contributing to malnutrition, anaemia and retarded growth [9]. Human gastrointestinal infections are of frequent occurrence in Nigeria. Quite a number of medicinal plants are used in the treatment of these conditions [10].

Napoleonaea imperialis P.Beauv is one of the plants employed in ethnomedicine in Nigeria [11].

Napoleonaea imperialis extract posseses a better wound healing property as compared to the standard antibiotic used as control [12].

Sida acuta medical uses stem largely from relatively high levels of alkaloids and flavonoids, facilitating medicinal uses such as treatment of wounds or use as an antipyretic [13,14].

The leaves of *Sida acuta* are considered to possess demulcent, diuretic, antihelminthic and wound healing properties and are used for rheumatic affections [15].

Vernonia amygdalina contains not only the active drug molecules but also other substances that are necessary for maintaining health and physiological functions of the body without manifestation of toxicity [16].

Also, the young leaves are used in folk medicine as anti-helminthic, anti-malarial, laxative/purgative, enema, expectorant, worm expeller, and fertility inducer in subfertile women [17,18].

One of the most common medicinal uses of *Vernonia amygdalina* is as a treatment against intestinal worms including nematode [19].

The use of visible light microscopy to observe vital organs such as liver and kidney of laboratory animals treated with the plant extract reveals signs of toxicity [20].

2. MATERIALS AND METHODS

2.1 Materials

2.1.1 Study area

This study was carried out in Orlu, Imo State, South-Eastern Nigeria. Imo State shares boundary in the North with Anambra State, in the South and West with Rivers State and in the East with Abia State. Orlu is one of the three senatorial zones in Imo State comprising of 12 Local Government Areas in the State.

Majority of the inhabitants are students, civil servants, bankers and business men and women. Hence, 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.

2.1.2 Test organisms

The organisms used for this study were intestinal parasites: *Necator americanus, Ascaris lumbricoides, Trichuris trichura, Hymenolepis nana, Entamoeba histolytica, Trichomonas hominis* and *Taenia* spp isolated from stool samples of selected patients presenting with symptoms of gastroenteritis and abdominal discomfort at GOPD, IMSUTH, Orlu.

2.1.3 Plant materials

The medicinal plants used for this study were: *Napoleonaea imperialis* P.Beauv, *Sida acuta* and *Vernonia amygdalina*. Roots of *Napoleonaea imperialis* P.Beauv were purchased from Orie market, Umuna, Orlu. Leaves of *Sida acuta* and *Vernonia amygdalina* were collected from the uncultivated farm land at Amaifeke, Orlu.

2.2 Identification of Plant Materials

Sida acuta and *Vernonia amygdalina* and *Napoleonaea imperialis* P.Beauv were identified and voucher specimens of the plants were deposited in the herbarium, 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 *Rattus albus* of 2 to 3 months old and body weights 80 to 180g.

Collection and Quarantine of Laboratory Animals: A total of four hundred (400) male and female Wistar strain *Rattus albus* aged 2-3months and weighing between 80 - 180g were used for the study. The Albino rats were purchased from accredited animal house. The animals were quarantined and allowed to acclimatize to the laboratory conditions for a period of two weeks. They were fed with a commercial pelleted poultry grower's mash- diet. Potable water was also given at intervals.

2.3 METHODS

2.3.1 Selection of patients

A total of 230 male and female patients aged 18-50 years and presenting with gastroenteritis and abdominal discomfort, were selected from the GOPD, IMSUTH, Orlu, between October, 2014 and March, 2015. They were given a structured questionnaire to complete and indicate their willingness in writing. They were later given specimen containers for the collection of their stool samples.

The stool samples were analyzed using direct wet mount and stool concentration methods (floatation and sedimentation techniques) as described by Chessbrough, [21].

Also, the Harada-Mori cultural method was used to isolate and differentiate between hookworm species*, Necator americanus* and *Ancylostoma duodenale* present in the stool samples [22].

The isolated parasites were washed in physiological saline by centrifugation. The faecal sediments containing the isolated parasites were transferred into screw capped bijou bottles. These bijou bottles were stored in the refrigerator at 8^{o}C until required for innoculation into the Albino rats and further studies.

2.3.2 Processing of plant materials

The roots of *Napoleonaea imperialis*, leaves of *Sida acuta* and *Vernonia amygdalina* were dried

under the shade and finally in thermostatically controlled hot air oven at 40^{o}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.

2.4 Extraction of Active Principles of the Plant Materials

2.4.1 Soxhlet Extraction

The active principles of the selected plant materials were extracted with ethanol at 78^{0}C using soxhlet extraction method as in Harborne, [23], Obiajuru and Ozumba, [24]. The extract was recovered and stored at +8^{0}C in screw capped MacCarteny bottles until required for use.

Qualitative and quantitative analysis of the phytochemical composition of the crude plant extracts were carried out using standard procedures according to the methods of Trease and Evans, [25] and Harborne, [23].

2.5 Pre-inoculation Management and Examination of Laboratory Animals

Selection criteria:

A. Inclusion Criteria
Health: HealthyWistar strain *Rattus albus* aged 2 - 3months and without any sign or symptom of cardiac or renal diseases were selected for the experiement

B. Exclusion criteria
Infection: *Rattus albus* with heavy infection of intestinal parasites were not experienmentally infected. Also those with markers of cardiac and renal diseases when tested were excluded in the research.

2.6 Experimental Design

Out of 400 apprently healthy Laboratory animals used in the study, 78 were infected and 322 were not infected. The uninfected laboratory animals were selected for parasite inoculation and to determine the curative and toxic effects of the selected plant extracts.

The 322 uninfected animals were divided into 3 groups comprising:

- 110 male and female animals with body weights equal to or less than 100g (≤ 100g).
- 108 male and female animals with body weights ranging from 101 to 150g (101 – 150g).
- 104 male and female animals with body weights equal to or greater than 151g (≥151g).

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

Group 1: 56 male laboratory animals aged 2 months with mean body weight 86.67g.
Group 2: 54 female animals aged 2months with mean body weight 88.33g.
Group 3: 54 male animals aged 2 months with mean body weight 113.45g.
Group 4: 54 female laboratory animals aged 2 months with mean body weight 120.2g.
Group 5: 53 male animals aged 3 months with mean body weight 152.6g.
Group 6: 51 female laboratory animals aged 3 months with mean body weight 156.3g.

2.6.1 Innoculation of human intestinal parasites on laboratory animals

Forty eight uninfected laboratory animals were selected from each group for innoculation with human intestinal parasites. Each group of 48 animals was divided into 6 sub – groups of 8 laboratory animals and innoculated with 1 human intestinal parasite isolated from selected patients. The laboratory animals were infected by oral innoculation using the cyst, trophozoites, ova or larvae of human intestinal parasites harvested from stool samples of selected patients, using the method of Yadav and Temjenmongla, [8]. The innoculated animals were fed and observed in separate single shelve compartments of laboratory animal cages for a period of 12 weeks. Those that died were replaced from the uninnoculated reserved ones.

2.6.2 Post – innoculation management of the laboratory animals

At the end of the 12 weeks period after innoculation of the laboratory animals with human intestinal parasites**,** the crude extracts of the selected plant extracts and reference drug solutions were prepared using physiological saline. Different concentrations of the 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 infected 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.

The animals were observed for physical effects of the chemotherapeautic agents.

2.6.3 Determination of Curative Dose (CD) and Lethal Dose (LD)

The Curative dose (CD) and lethal dose (LD) of the plant extracts were determined using the method of Rim et al., [26].

2.6.4 Toxicity testing of plant extracts

A total of 54 laboratory animals of about 150g body weight each were selected for toxicity test. Eighteen were infected with *Necator americanus,* another 18 laboratory animals were infected with *Hymenolepsis nana* and another 18 were infected with *Entamoeba histolytica*. They were observed for 8 weeks after which they were separated into 6 (A, B, C, D, E and F) sub-groups comprising of 1 laboratory animal infected with *Necator* americanus, 1 infected with *Hymenolepsis nana* and another 1 infected with *Entamoeba histolytica*. Three sets of these groups were separated. Laboratory animals in the first 6 Groups (A, B, C, D, E and F) were administered with 5µg, 10µg, 20µg, 30µg, 40µg and 50µg of *Napoleanae imperialis* extract respectively. Laboratory animals in the second 6 Groups (A, B, C, D, E and F) were administered with 5µg, 10µg, 20µg, 30µg, 40µg, and 50µg of *Sida acuta* extract respectively and the other laboratory animals in the third 6 Groups (A, B, C, D, E and F) were administered with 5µg, 10µg, 20µg, 30µg, 40µg, and 50µg of *Vernonia amygdalina* extract respectively. They were observed for 1 – 2 weeks and their internal organs (intestine, kidney and liver) were examined for abnormalities.

2.6.5 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 liver, kidney and intestine of each animal, were dissected and placed into labelled specimen bottles containing 10% formol saline as fixative. These samples were used for histopathological studies. The Albino rats which died 24hours and beyond after administration of chemotherapeautic agent were cleaned with 75% alcohol, dissected and preserved in 10% formol saline and taken to the Histopathology Laboratory, IMSUTH, Orlu for post mortem studies. The animal tissues were fixed to prevent post mortem changes (putrefaction and / or autolysis).

2.6.6 Tissue processing

The tissues of laboratory animals for Histopathology examination were processed and embedded in paraffin wax as described by Winsor, [27]; and Kiernan, [28].

2.6.7 Sectionning 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 methods of Winsor, ([27] and Barker, Silverton and Pallister [29] were applied.

2.6.8 Stanning technique

The fixed sections of the various laboratory animal tissues used for the study were dewaxed, dehydrated and stained using routine Haematoxylin and Eosin stainning technique as in Winsor, [27] Barker, Silverton and Pallister [29]; and Kiernan, [28]. The stained slides were labeled, stored in slide boxes until required for examination.

2.6.9 Microscopy and photomicrography

Stained films of the laboratory animal tissues sections were examined microscopically using x10 eye piece and x40 objective. The assistance of a Consultant Histopathologist at Federal Medical Centre, Owerri was sought for microscopic examination of the slides. Reporting was done, comparing the slide observations of test samples with control groups and standard Histopathology atlas [30]. Photomicrographs of the tissues were taken using digital microscope eye piece (Leica Camera Microscope) (x400).

2.7 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 ± SD for some groups. A p value ≤ 0.05 was considered significant.

3. RESULTS

Table 1 shows the post – parasite – innoculation stool analyses of the laboratory animals. Out of 48 laboratory animals innoculated with *Ascaris lumbricoides,* 3 male animals with mean body weight 86.67g died in the first week and were replaced from the uninnoculated reserve, out of 48 laboratory animals innoculated with *Necator americanus,* 2 females with mean body weight 131.7g and 95.30g respectively died within the first week and were replaced from the uninnoculated reserved ones. Out of 48 laboratory animals innoculated with *Hymenolepsis nana*, 2 males with mean body weight 128.50g, 1 male with mean body weight 152.2g and 1 female with mean body weight 133.60g died within the first week and were replaced from the uninnoculated reserved ones. Out of 48 innoculated with *Entamoeba histolytica,* 1 male with mean body weight 149.80g, 1 male with mean body weight 92.30g and 1 female with mean body weight 93.70g died within the first week and were replaced from the uninnoculated reserved ones. All 12 laboratory animals innoculated with *Taenia* species died between the 5^{th} and 6^{th} week. Autopsy examination of their stool samples did not reveal any ova of *Taenia* nor other parasites. Stool analyses of all survived 288 laboratory animal innoculated with *Ascaris lumbricoides, Necator americanus, Trichuris trichuira, Hymenolepsis nana, Entamoeba histolytica* and *Trichomonas hominis* revealed presence of their respective ova, cysts or trophozoites.

3.1 Results of the Toxicity of the Plant Extacts

The findings of the toxicity testing of the plant extracts is as shown in Tables 2 to 4. All laboratory animals administered with 5µg of the different plant extracts per 150 ± 5g body weight, survived. Stool analysis of their faeces showed presence of the different parasites innoculated. Animals administered with 10 µg of *Napoleanae imperialis* extract per 150 ± 5g to 40 µg/150 ± 5g survived. Examination of their stool samples showed total clearance of the different parasites innoculated. All animals administered with 50 µg of *Napoleanae imperialis* extract per 150 ± 5g died (Table 2). Animals administered with 10 µg of *Sida acuta* extract per 150 ± 5g to 30 µg/150 ± 5g survived. Examination of their stool samples showed total clearance of the different parasites innoculated. Animal administered with 40 to 50 µg of *Sida acuta* extract per 150 ± 5g died (Table 3). Animals administered with 10 µg of *Vernonia amygdalina* extract per 150 ± 5g to 40 µg/150 ± 5g survived. Examination of their stool samples showed total clearance of the different parasites innoculated. All laboratory animals administered with 50 µg of *Vernonia amydalina* extract per 150 ± 5g died (Table 4).

Generally, 12 (22.2%) out of 54 laboratory animals used died. At 10 µg/150 ± 5g, all the plant extracts were able to clear the different parasites innoculated on the laboratory animals, giving their curative doses (CD) as 10µg of extract per 150 ± 5g body weight respectively. At 50 µg/150 ± 5g all laboratory animals administered with *Napoleonae imperialis* extract or *Vernonia amygdalina* extract died, giving their lethal doses (LD) as 50 µg of extract per 150 ± 5g body weight respectively. At 40 to 50 µg/150 ± 5g, all laboratory animals administered with *Sida acuta* extract died, giving the lethal dose as 40 µg of extract per 150 ± 5g body weight.

3.2 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 to 3, while Plates 4 to 16 show the impairments caused on laboratory animal organs (intestine, kidneys and liver) by plant extracts and innoculated intestinal parasites. 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), degerenative 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 degernerative changes (Plate 13), necrosis (Plate 14) and distortion of hepatocytes, (interwoven) (plate 15),

Table 1. Post-parasite innoculation stool analyses of laboratory animals

Age of Lab. Animal (Months)	Body Weight (g)	Sex	Total Number Exam	Pre Infection Mean Body Weight (g)	8 weeks Post Innoculation Stool Examination						
					Innoculated Intestinal Parasites (8 Lab Animals per Group)						
					Ascaris lumbri	***Necator America***	***Trichuris trichiura***	***Hymenolepnana***	***Entamoeba histolytica***	***Trichomonas hominis***	***Taenia* species**
2	≤ 100	M	56	86.67	+	+	+	+	+	+	-
		F	55	88.33	+	+	+	+	+	+	-
		M	54	113.45	+	+	+	+	+	+	-
	101 –	F	53	120.20	+	+	+	+	+	+	-
3	150	M	53	142.60	+	+	+	+	+	+	-
		F	51	156.30	+	+	+	+	+	+	-
	≥ 151										
Total			322	-	48	48	48	48	48	48	12

Key: + = Parasite (egg, larva or cysts) recovered. - = No Parasite (egg, larva or cysts) recovered.

Table 2. Curative effect and toxicity of *Napoleonae imperialis*

Parasite Innoculated	Concentration of Extract administered (µg/150g)					
	5	**10**	**20**	**30**	**40**	**50**
Necator americanus	Survived Parasite recovered	Survived No Parasite recovered	Survived No Parasite recovered	Survived No Parasite recovered	Survived No Parasite recovered	Died
Hymenolepsis nana	Survived Parasite	Survived No Parasite	Survived No Parasite	Survived No Parasite	Survived No Parasite	Died
Entamoeba histolytica	recovered Survived Parasite recovered	recovered Survived No Parasite recovered	recovered Survived No Parasite recovered	recovered Survived No Parasite recovered	recovered Survived No Parasite recovered	Died

CD = 10µg/150 ± 5g LD = 50 µg/150 ± 5g

Table 3. Curative effect and toxicity of *Sida acuta*

Parasite Innoculated	Concentration of Extract administered (µg/150g)					
	5	**10**	**20**	**30**	**40**	**50**
Necator americanus	Survived Parasite recovered	Survived No Parasite recovered	Survived No Parasite recovered	Survived No Parasite recovered	Died	Died
Hymenolepsis nana	Survived Parasite recovered	Survived No Parasite recovered	Survived No Parasite recovered	Survived No Parasite recovered	Died	Died
Entamoeba histolytica	Survived Parasite recovered	Survived No Parasite recovered	Survived No Parasite recovered	Survived No Parasite recovered	Died	Died

CD = 10µg/150 ± 5g LD = 40 µg/150 ± 5g

Table 4. Curative effect and toxicity of *Vernonia amygdalina*

Parasite Innoculated	Concentration of Extract administered (µg/150g)					
	5	**10**	**20**	**30**	**40**	**50**
Necator americanus	Survived Parasite recovered	Survived No Parasite recovered	Survived No Parasite recovered	Survived No Parasite recovered	Survived No Parasite recovered	Died
Hymenolepsis nana	Survived Parasite recovered	Survived No Parasite recovered	Survived No Parasite recovered	Survived No Parasite recovered	Survived No Parasite recovered	Died
Entamoeba histolytica	Survived Parasite recovered	Survived No Parasite recovered	Survived No Parasite recovered	Survived No Parasite recovered	Survived No Parasite recovered	Died

CD = 10µg/150 ± 5g LD = 50 µg/150 ± 5g

and (fragmented): (Plate 16) 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 than liver organs.

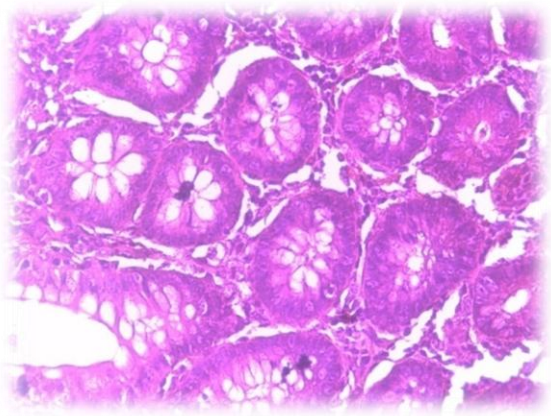

Plate 1. Photomicrograph of a normal intestine
KEY: No Parasite innoculated, No anti parasitic agent administered

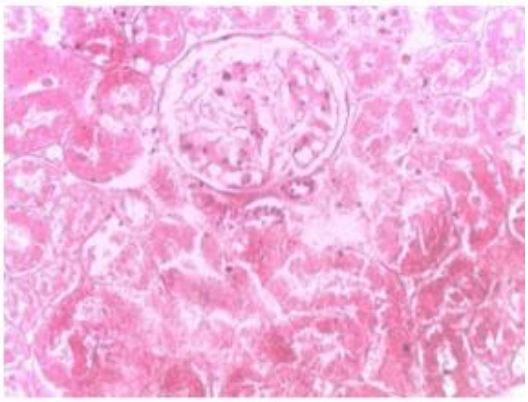

Plate 2. Photomicrograph of a normal kidney
KEY: Parasite innoculated: Hymenolepis nana Extract/drug administered: 10µg Vernonia amygdalin

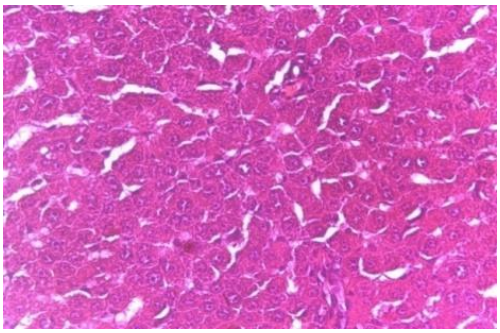

Plate 3. Photomicrograph of a normal liver
KEY: No Parasite innoculated, No anti parasitic agent administetred

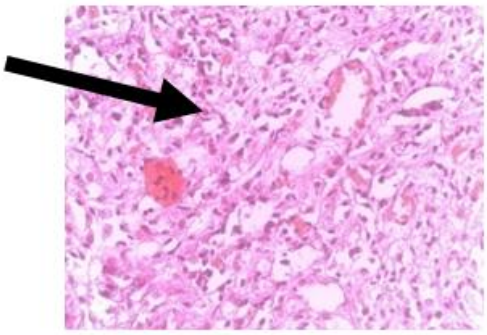

Plate 4. Photomicrograph of the intestine showing inflammatory changes
KEY: Parasite innoculated: H. nana; Extract/drug administered: 30µg Naploeonea imperialis

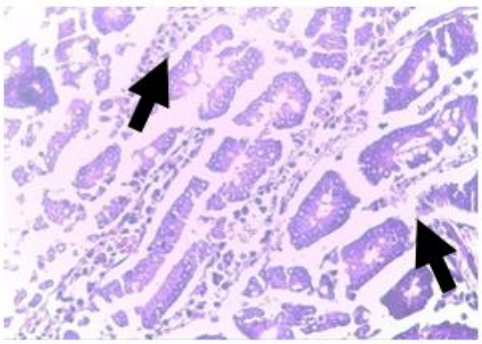

Plate 5. Photomicrograph of the intestine showing degenerative changes
KEY: Parasite innoculated: Necator americanus; Extract/drug administered: 40µg Sida acuta

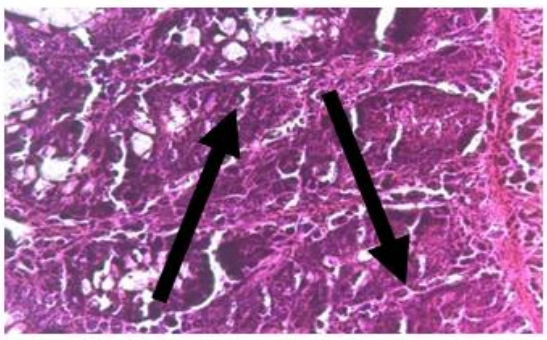

Plate 6. Photomicrograph of the intestine showing distortion of intestine
KEY: Parasite innoculated: Entamoeba histolytica; Extract/drug administered: 40µg Sida acuta

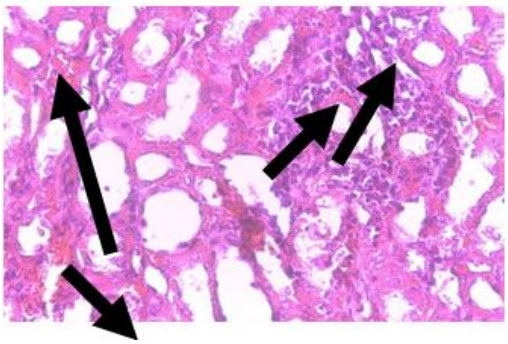

Plate 7. Photomicrograph of the kidney showing lymphocytic infiltration
KEY: Parasite innoculated: H. nana; Extract/drug administered: 50µg Napoleoneae imperialis

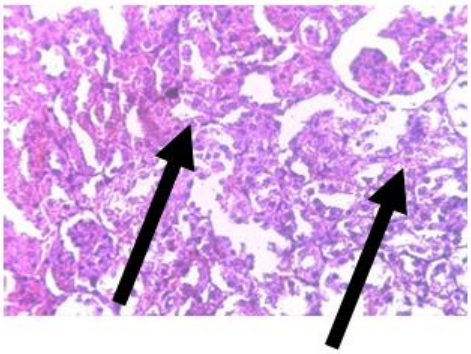

Plate 8. Photomicrograph of the kidney showing degenerative changes
KEY: Parasite innoculated: Necator americanus; Extract/drug administered: 40µg Sida acuta

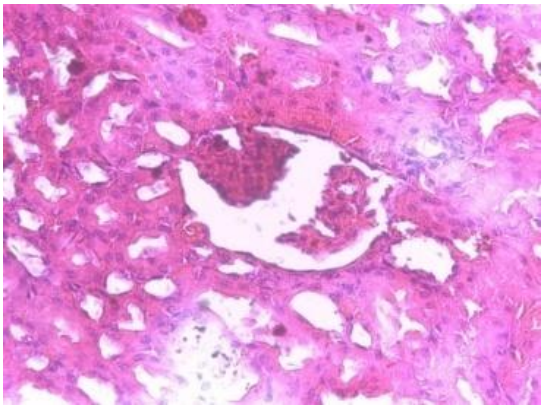

Plate 9. Photomicrograph of the kidney showing necrosis
KEY: Parasite innoculated: Ascaris lumbricoides; Extract/drug administered: 40µg Napoleoneae imperialis

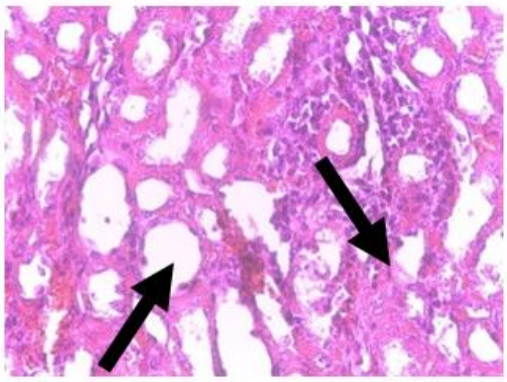

Plate 10. Photomicrograph of the kidney showing vacuolation
KEY: Parasite innoculated H. nana; Extract/drug administered: 50µg Vernonia amygdalina

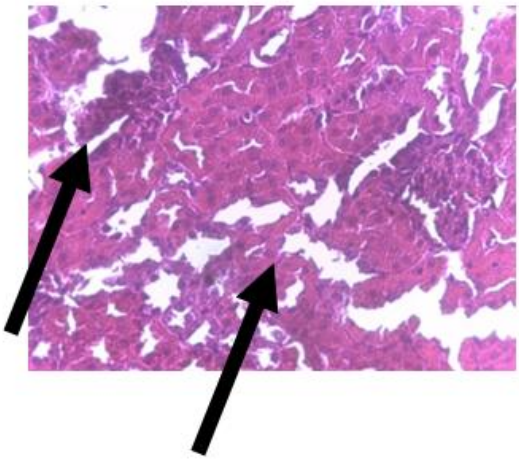

Plate 11. Photomicrograph of the kidney showing distortion of stroma and glomerulus
KEY: Parasite innoculated: Entamoeba histolytica; Extract/drug administered: 40µg Sida acuta

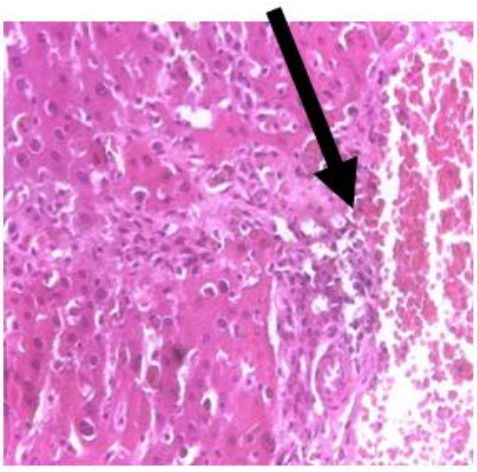

Plate 12. Photomicrograph of the liver showing inflammatory changes
KEY: Parasite innoculated: Necator americanus; Extract/drug administered: 50µg Napoleonea imperialis

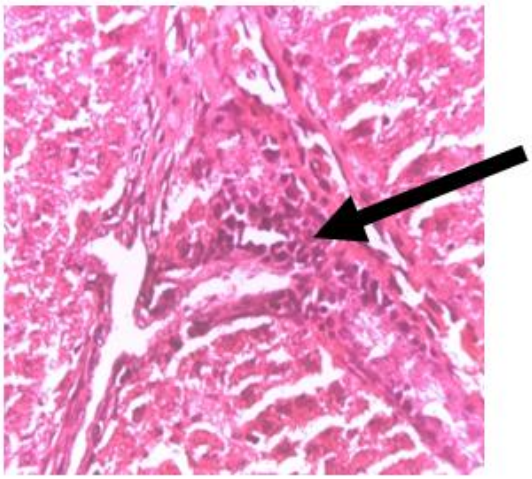

Plate 13. Photomicrograph of the liver showing hepatocytic degenerative changes
KEY: Parasite innoculated: H. nana; Extract/drug administered: 30µg Sida

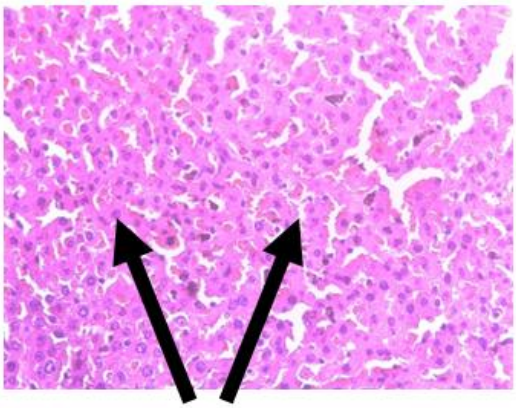

Plate 14. Photomicrograph of the liver showing necrosis
KEY: Parasite innoculated: Necator americanus; Extract/drug administered: 40µg Sida acuta

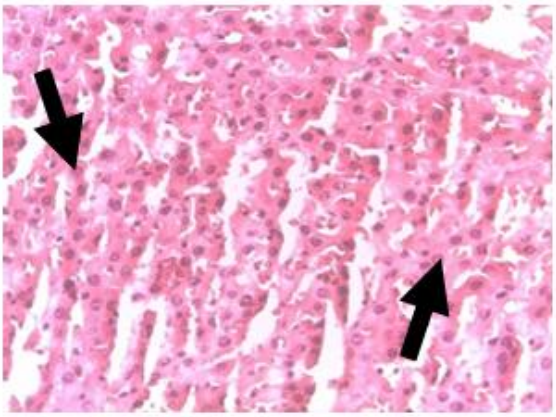

Plate 15. Photomicrograph of the liver showing distortion of interwoven hepatocytes
KEY: Parasite innoculated: Trichomonas hominis; Extract/drug administered: 50µg Vernonia amygdalina

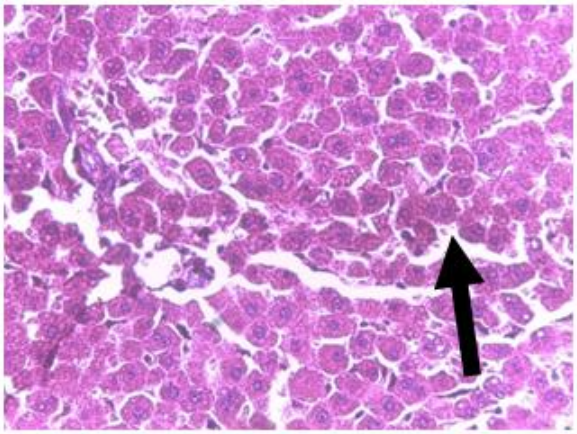

Plate 16. Photomicrograph of the liver showing distortion of fragmented hepatocytes
KEY: Parasite innoculated: Necator americanus; Extract/drug administered: 40µg Napoleonea imperialis

4. DISCUSSION

Several drugs are effective against intestinal helminthes, however, about one - third of the world's population still lack regular access to essential drugs [3]. Whereas some people are aware of the health challenges of intestinal parasite infections, not many of them can afford the drugs due to the cost and limited availability, especially in rural communities. Furthermore, the increasing cases of drug resistant infectious organisms constitutes a major challenge in treatment and management of intestinal parasites infection. The present study is an effort geared towards finding an affordable, safe and relatively cheap remedy for treatment of intestinal parasites infections without adverse effects on the internal organs especially in resource poor communities like Orlu and Imo State generally.

The phytochemical and proximate analysis of the selected medicinal plants (*Vernonia amygdalina, Sida acuta* and *Napoleonoea imperialis*) showed that the active principles (Alkaloids, Flavonoids, Cardiac Glycosides, Tanin, saponin, Terpenoid, Oxalate, phytate, Phenolic compound and steroids) are known phytochemical compounds useful in production of chemotherapeautic agents and other industrial products.

Plant extracts used in this study exhibited remarkable curative effects on human intestinal

parasites (helminthes and protozoa). This finding agrees with the report of previous workers [32,33] who reported that *Vernonia amygdalina* exhibited anti-helminthic property on intestinal helminthes experimentally infected on laboratory animals. This finding suggests that these plants could be sources of novel anti – parasitic drugs or analogues of existing anti – parasitic drugs. In resource poor communities where chemotherapeutic drugs are not readily available or affordable, patients infected with intestinal helminthes can take advantage of this study and seek possible treatment with these plants. *Vernonia amygdalina* in particular is an edible plant, commonly used in cooking soup and other food among the Igbos of South Eastern Nigeria. The leaves can be obtained readily from farms and gardens around the homes. Persons suffering from intestinal parasitic infections can take the leaves, wash them in running water and chew them.

All plant extracts used in this study exhibited curative effects at 10µg/150g body weight on laboratory animals infected with human intestinal parasites without inflicting impairment on the organs of laboratory animals. This finding agrees with previous reports [34] which reported that leaves, stem, roots and root bark of *Vernonia amygdalina* water extract effectively killed cestodes of *Hymenolepis diminuta* after 24hours of treatment. In another study, Abdul et al., [35] suggested that dissolving *Vernonia amygdalina* water extract in potash (Potassium carbonate) is valuable for treatment of worm infection.

The concentration of crude extracts of the plants that effected cure was appreciably low, 10µg/150g body weight. If the extracts are purified, the concentration of the active ingredient that will be required to compound drugs for treatment and cure of intestinal parasitic infection will be small. Animals administered with 10 µg of *Napoleanae imperialis, Sida acuta* and *Vernonia amygdalina* extracts per 150 ± 5g body weight survived with total parasite clearance of the different parasites inoculated. This finding shows that at 10µg/ 150 ± 5g body weight, these plant extracts are effective remedies for intestinal parasites. Elsewhere, other workers ([6,36] reported that many medicinal plants exhibited anti – parasitic effect against a wide range of parasites at minimal concentrations with little or no side effects. These medicinal plants are now available for treatment and control of parasitic infections. Previous workers Ojiako and Nwanjo, [37] reported that *Vernonia amygdalina* is safe to consume and is good for health unless it is consumed in very large quantities. According to these workers, the potential danger of consuming this plant is much lower than that of other common vegetables. Iwu, [16] stated that it contains not only the active drug molecules but also other substances that are necessary for maintaining health and physiological functions of the body without manifestation of toxicity.

The number of laboratory animals that died: 12 (22.2%) out of 54 animals used for the Toxicity test showed that apart from the curative effects of these plants, they have toxic effects on the laboratory animals. However, when the chemotherapeutic index (CI) of the extracts [Lethal Dose (LD) – the Curative Dose (CD)], was considered, it appears that the danger resulting from over dose is minimal. Given that at 10 µg/150 ± 5g, all the plant extracts produced curative effects against helminthic and protozoan parasites and at 50 µg/150 ± 5g *Napoleonae imperialis* extract or *Vernonia amygdalina* extract killed the laboratory animals, and at 40µg/150 ± 5g, *Sida acuta* extract killed the laboratory animals, the chemotherapeutic index of *Napoleonae imperialis* and *Vernonia amygdalina* is 50 – 10 = 40µg while that of *Sida acuta* extract is 40 – 10 = 30 i.e 4 times and 3 times the curative dose respectively.

At the curative dose of 10µg, no impairment was observed in their intestine, kidneys and liver of the laboratory animals treated with *Napoleonae imperialis, Sida acuta* or *Vernonia amygdalina*. At 20µg to 40µg, the number and nature of organ impairment was minimal for laboratory animals treated with *Napoleonae imperialis,*or *Vernonia amygdalina* extract. At lethal dose of 50µg, organ impairment of laboratory animals treated with *Napoleonae imperialis,* or *Vernonia amygdalina* extract became pronounced. Similarly, organ impairment of laboratory animals treated with *Sida acuta* at lethal dose of 40µg/150g body weight, were pronounced.

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 50µg) for *Sida acuta* and *Napoleonoea imperialis* while *V. amygdalina* showed no impairment even at 20 µg on any of the organs (intestine, kidney and liver). The presence of human intestinal parasites

that were experimentally innoculated into the Albino rats and their toxic waste products of metabolism also contributed to the impairments observed in the intestine.

The histopathological changes observed in the kidney sections showed 5 types of impairment: lymphocytic infiltration (Plate 7), degerenative changes (Plate 8), necrosis (Plate 9), vacuolation (Plate 10) and distortion of stroma and glomerulus (Plate 11), which are signs of renal toxicity.The waste products of metabolism of the intestinal parasites in addition with the active principles in the medicinal plants such as alkaloids and oxalates are responsible for the impairmens observed especially at higher concentrations.These findings agree with the report of Mebratu et al., [20] who reported that at 800mg/kg, kidney sections of mice showed increased cellularity of glomerulus, urinary space obliteration and enlarged macula densa. These pathological features observed on sections from animals administered with 800mg/kg of *Vernonia bipontini vatke* plant extract, were interpreted as toxic effect of the plant extract.

Also, the report of [38] showed similar alterations in the structure of glomerulus as a result of treatment with different toxic substances (piroxicam).

On the liver, 4 types of impairment: inflammatory changes (Plate 12) hepatocytic degernerative changes (Plate 13), necrosis (Plate 14) and distortion of hepatocytes (interwoven) (Plate 15), and (fragmented) (Plate 16) were observed.

Liver, the key organ of metabolism and excretion is constantly endowed with the task of detoxification of xenobiotics, environmental pollutants and chemotherapeutic agents [39]. The liver is the recognized primary organ of detoxification and biotransformation by virtue of its position and blood supply.

Therefore, the histo-architectural distortions observed in the liver tissues of Albino rats exposed to grades of plant extracts further substantiate the toxic potential of these plants. With the exception of the low concentration of these extracts, every other concentration appeared to be toxic and run a concentration dependent histological disruption. The histoarchitectural damages seen in the liver of these Albino rats could be linked to high presence of some bioactive substances like alkaloids, which is well recognized for inducing hepatic lesions ([40,41].

Mebratu et al., [20] administered 800mg/kg of the methanol leaf extract of *Vernonia bipontini vatke* on laboratory mice and examined the effects on the liver using light microscope and stained sections of the liver. Their findings revealed dilated sinusoids, nuclear enlargement, lots of bi-nucleation of hepatocytes, peripheral cramped chromatin, shrinkages (single cell death) of hepatocytes, fragmentation of hepatocytes while no histopathological changes were observed in liver and kidney of mice treated at 400mg/kg.

However, the liver, intestine and kidney morphological alterations provoked by higher concentrations of the plant extracts in this study could have serious functional consequences especially on the detoxification and biotransformation roles of the liver, intestine and kidney.

5. CONCLUSION

This study has shown that *Napoleonae imperialis, Sida acuta* and *Vernonia amygdalina* extracts exhibit curative effects on human intestinal parasites at low concentrations. At higher concentrations, they exhibit toxic effects on host oragns: intestine, kidney and liver.

DISCLAIMER

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.

CONSENT

It’s not applicable.

ETHICAL APPROVAL

Ethical approval for the study was obtained from the Ethics Committee, Imo State University Teaching Hospital, Orlu.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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Peer-review history:
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