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IN VIVO TESTING OF THE EFFECTS OF MEDICINAL PLANTS ON ALBINO RATS EXPERIMENTALLY INFECTED WITH INTESTINAL PARASITES

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ABSTRACT

Anti – Parasitic effects of selected plant extracts: (*Napleonaea imperalis, Sida acuta* and *Vernonia amygdalina*) on Wista rats (*Rattus albus*) experimentally infected with human intestinal parasites was studied. The aim was to determine the anti-parasitic effects of the plant extracts on *Rattus albus* experimentally infected with human intestinal parasites. Two hundred and thirty (230) male and female patients presenting with symptoms of gastroenteritis and abdominal discomfort at GOPD, Imo State University Teaching Hospital (IMSUTH), Orlu were selected for the study. Stool samples were collected from the patients and examined for intestinal parasites, using stool concentration techniques and direct wet mount. The Harada-Mori cultural method was used to isolate and differentiate between hookworm species, *Necator americanus and Ancylostoma duodenale*. The most prevalent intestinal parasite in Orlu was *Necator americanus* (23.90%) while the least prevalent was *Hymenolepsis nana* (1.74%). Statistical analysis of the data using Chi square showed significant difference ($p < 0.05$) in the prevalence of infection between different age groups of patients. The isolates were inoculated on healthy, uninfected *Rattus albus.* A total of 672 laboratory animals were used for the study. Eight weeks after, the infected *Rattus albus* were treated with 3 selected plant extracts: *Vernonia amygdalina, Sida acuta* and *Napoleonae imperialis* and observed for 3 weeks. The results showed that *Rattus albus* were successfully infected with different human intestinal parasites. This study has shown that *Napoleonae imperialis, Sida acuta* and *Vernonia amygdalina* extracts exhibit anti – parasitic effects on human intestinal parasites at low concentrations.

KEYWORDS: Medicinal - Plants Albino Rats Infected Intestinal Parasites

1.1 INTRODUCTION

Parasites have been the subjects of most exciting discoveries in the field of infectious diseases. The gastrointestinal tract of man and other animals harbour more parasites than any other part of the body. Parasites found in the human gastrointestinal tract comprise of protozoa, helminthes and others. People of all race, age and gender are infected by different parasites. However, highest rates of Infection are in children living in sub-Saharan Africa, followed by Asia and Latin America and the Caribbean. While some parasites are acquired by behavioral, social and biological changes during evolutionary history (Araújo *et al*. 2000) others such as *Enterobius vermicularis* may be inherited from parents or ancestors (Hugot *et al*. 1999).

Medicinal plants have been used for centuries before the advent of orthodox medicine. Leaves, flowers, stems, roots, seeds, fruit, and bark have been constituents of herbal medicines. The medicinal values of these plants lie in their component phytochemicals, which produce the definite physiological actions on the human body. The most important of these phytochemicals are alkaloids, tannins, flavonoids and phenolic compounds (Hill, 1952). Plants used for medicinal purposes are also used for various other reasons in different cultures For instance, the stem and root of *Vernonia amygdalina* divested of the bark are used as chewsticks in many West African countries like Cameroon, Ghana and Nigeria (Hamowia, 1994). The leaves and bark in Ethiopian local medicine are used as purgative against menstrual pain and wound dressing (Uhegbu & Ogbuchi, 2004). Its antibacterial properties have been evaluated by Akujobi *et al.* (2006) and it has been shown to contain cardiac glycosides, saponins, tannins and alkaloids.

Infectious diseases with increasing trends of drug resistant microorganisms have been common global problem posing enormous public health concerns (Iwu *et al*., 1999).

Much work has not been done in Nigeria on the effect of medicinal plants on experimentally infected laboratory animals. The present study was carried out to determine the anti – parasitic and histopathological effects of some selected medicinal plants: *Napoleonaea imperialis, Sida acuta* and *Vernonia amygdalina*) on Albino rats experimentally infected with human intestinal parasites. The study specifically aims:

1. To determine the infectivity of human intestinal parasites on albino rats
2. To determine the anti-parasitic effects of selected medicinal plants on human intestinal parasites experimentally infected to Albino rats.
3. To determine the histopathological effects of the selected medicinal plants on albino rats infected with human intestinal parasites

1.2 Materials and Methods

1.2.1 Materials

Study Area

This study was carried out in Orlu, Imo State, South Eastern Nigeria. Imo State lies on the latitude 5^0 29'N and 7^0 2'E. and shares boundary in the North with Anambra State, in the South and West with Rivers State and in the East with Abia State. It comprises of three senatorial zones: Owerri, Orlu and Okigwe. Orlu senatorial zone comprises of 12 Local Government Areas. Based on the estimated census figure of 2006 (National Bureau of Statistics, 2006), Imo State has a teaming population of 3,934,899 men and women of various ages engaged in all walks of life: students, civil servants, artisans, farmers and traders. The standard of living is high (Nigerian Muse, 2006) and a significant number of people in the State depend on locally prepared herbs as an alternative medicine for their ailments since they are readily available and affordable.

Test Samples

The samples used for this study are stool samples collected from male and female patients aged 18 to 50 years, presenting with symptoms of gastroenteritis and abdominal discomfort at the General Out Patient Department (GOPD), Imo State University Teaching Hospital, (IMSUTH), between October, 2015 and April, 2016.

Test Organisms

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

Test Plants

The medicinal plants used for this study are: *Napoleonaea imperialis* P.Beauv, *Sida actua* and *Vernonia amygdalina* collected from Umuna and Amaifeke in Orlu area.

Laboratory Animals:

The laboratory animals used for this study are male and female Wistar strain of Albino rats of known ages and body weights, purchased from accredited animal house at Emii Veterinary farm, Owerri, Imo State.

1..2.2 METHODS

Ethical Permit

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

Selection of Patients

A total of 250 male and female patients presenting with gastroenteritis and abdominal discomfort , were selected from the GOPD, IMSUTH, Orlu, between October, 2015 and April, 2016. The patients were approached on person to person contact basis. The objectives of the study were explained to them and their willingness to participate in the study was sought for using a structured questionnaire. Out of 250 selected patients, 20 declined and 230 accepted to participate in the study. 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.

Isolation of Human Intestinal Parasites

A total of 150 intestinal parasites comprising of 32 ova of *Ascaris lumbricoides*, 55 ova of *Ancylostoma duodenale*, 20 ova of *Taenia* species, 4 ova of *Hymenolepis nana*, 25 cysts of *Entamoeba histolytica* and 14 trophozoites of *Trichomonas hominis* were isolated from stool samples collected from the selected patients. The parasites were washed in physiological saline by centrifugation. The faecal sediments containing the isolated parasites were transferred into screw capped bijou bottles. The bijou bottles were stored in the refrigerator at 8^oC until required for inoculation into the Albino rats and further studies.

Collection and identification of plant materials

Roots of *Napoleonaea imperialis* P.Beauv were purchased from Orie market, Umuna, Orlu. Leaves of *Sida* acuta and *Vernonia amygdalina* were collected from uncultivated farm land at Amaifeke, Orlu.

Sida acuta and *Vernonia amygdalina* were identified by Botanists in the Department of Plant Science and Biotechnology, Imo State University, Owerri while *Napoleonaea imperialis* P.Beauv was authenticated botanically in the Department Biology, Federal University of Technology, Owerri. The voucher specimen of the plants were deposited in a herbarium,

Department of Plant Science and Biotechnology, Imo State University, Owerri.

Processing of Plant Materials
The roots of *Napoleonaea imperialis*, leaves of Sida *acuta* and *Vernonia amygdalina* were dried partially under diffused sunlight 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 fume cupboard until required for extraction.

Extraction of Active Principles of the Medicinal Plants
The active principles of the selected medicinal plants were extracted with soxhlet extractor using ethanol at 78^{0}C as in Harborne, (1998), Obiajuru and Ozumba, (2009).

Collection and Quarantine of Laboratory Animals
A total of 400 male and female Wistar strain Albino rats aged 2 - 3months and weighing between 80 - 180g were used for the study. The Albino rats were purchased from an animal house, Emii Veterinary Farm, Owerri, Imo State, Nigeria. The animals were quarantined in a separate compartment shelves netted with barb - wire in the animals house, Faculty of Medicine, Imo State University, Orlu Campus. Those that died and those that became physically sick were excluded. A total of 322 apparently healthy ones survived and were used.

Selection of Laboratory Animals
Inclusion criteria
Inclusion criteria was based on age, health and classification of the animals. Exclusion criteria was based on age and infection. Prior to inoculation of the laboratory animals with the isolated human intestinal parasites, they were fed with equal proportion of Vital Grower's Feed for 2 weeks before commencement of the experiment and while the study lasted. The faecal samples of all the selected laboratory animals were collected into universal containers and examined for the presence of intestinal parasites as in Chessbrough, (2002); Obiajuru and Ozumba, (2009). Modified McMaster egg counting technique was used for quantification of helminths eggs in (egg/g faeces) from individual faecal samples (MAFF, 1986; Bondarenko *et al*., 2009).

Inoculation of Human Intestinal Parasites on Laboratory Animals
The uninfected laboratory animals were infected by oral inoculation using the cyst, trophozoites, ova or larvae of human intestinal parasites harvested from stool samples of selected patients as described by Yadav and Temjenmongla, (2011).

Post-Inoculation Management and Examination of the Laboratory Animals The laboratory animals were maintained in separate single shelve compartments of animal cage. Their faecal samples were collected in labeled universal containers and examined for intestinal parasites at 4 weeks interval for 12weeks. Isolated intestinal parasites were identified using their morphological characteristics and compared with standard Parasitology atlas (Jeffrey and Leach, 1985; Karen, 2005). At the end of the 12^{th} week post inoculation of intestinal parasites, the Albino rats were re-weighed and their new weights recorded. This was used to ascertain the impact of intestinal parasites on the body weight of the Albino rats.

Administration of Medicinal Plants Extracts
Crude extracts of selected medicinal plants and reference drug solutions were prepared using physiological saline. They were prepared by 2 fold double dilutions: $^{1}/_{10}$, $^{1}/_{10}{}^{2}$ and $^{1}/_{10}{}^{3}$ concentrations ($^{W}/_{V}$). 0.5ml of these concentrations of the plant extracts and reference drugs were administered to the infected laboratory animals orally using automatic pipette, once per week for a period of three weeks. Different colour codes were used to identify the medicinal plant extracts and reference drugs administered on the laboratory animals in each treatment group. The animals were observed for physical effects of the chemotherapeautic agents and their faecal samples were also collected and examined in the laboratory for presence of intestinal parasites.

PARASITE INOCULATION AND ANTI – PARASITIC EFFECTS OF TEST MEDICINAL PLANTS
Out of 400 Laboratory animals used in the study, 78 were infected and 322 were not infected. The uninfected laboratory animals were used for the study. They were divided into 3 groups of 48 laboratory animals in each group, for inoculation with human intestinal parasites. Each group of 48 animals was divided into 6 sub – groups of 8 laboratory animals and inoculated with 1 human intestinal parasite isolated from selected patients. The inoculated animals were fed and observed in separate laboratory animal cages for a period of 8 weeks. Those that died were replaced from the reserved uninoculated ones. At the end of 8 weeks their stool samples were examined for parasites and their body weights were measured.

Post – Inoculation treatment and Examination of the Laboratory Animals for Anti – Parasitic Effects
At the end of the 8 weeks, the inoculated laboratory animals were administered different medicinal plant extracts orally and examined for 3 weeks. Their stool samples were examined for intestinal parasites and their body weights measured again to determine the impact of infection and post infection medicinal plant extracts and reference drugs treatments on the animals. The ability of the chemotherapeautic agents to reduce the parasite

(cysts, egg and worm) load in the stool of the animals was also studied.

1.3 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.

2.0 RESULTS

2.1 Prevalence of Test Isolates

Table 1 summarizes the prevalence of intestinal parasites amongst the selected patients. As shown, 55 (23.9%) *Necator americanus*, 32 (13.9) *Ascaris lumbricoides*, 20 (8.7%) *Taenia* species, 7 (3.04%) *Trichuris trichiura*, 4 (1.7%) *Hymenolepsis nana*, 25 (10.9%) *Entamoeba histolytica* and 5 (2.3%) *Trichomonas hominis* were isolated. Table 2 shows the gender – related prevalence of infection. Analysis of the data using chi square showed no significant difference ($p > 0.05$) in the prevalence of infections between male and female patients.

Table 1: Prevalence of Intestinal Parasitesamongst Selected Patients

Patient's Age (yrs)	Sex	Number Examined	Parasites Isolated (%)						
			Ascaris lumbricoides	*Necator americanus*	*Trichuris trichiura*	*Taenia* species	*Hymenolepsis nana*	*Entamoeba histolytica*	*Trichomonas hominis*
18 – 25	**M**	16	5	2	2	-	-	4	2
	F	21	6	3	1	-	-	3	3
26 – 30	**M**	28	5	5	2	1	-	5	1
	F	22	4	3	2	3	-	6	3
31 – 40	**M**	29	3	9	-	4	1	2	2
	F	34	4	10	-	6	1	2	2
41 – 50	**M**	36	3	10	-	4	1	1	-
	F	44	2	13	-	2	1	2	1
Total		**230**	**32 (13.90)**	**55 (23.90)**	**7 (3.04)**	**20 (8.69)**	**4 (1.74)**	**25 (10.87)**	**14 (6.09)**

Table 2: Gender – related Prevalence of Infection.

Sex	Number Examined	Number Infected	Parasites Isolated						
			Ascaris lumbricoides	*Necator americanus*	*Trichuris trichiura*	*Taenia* species	*Hymenolepsis nana*	*Entamoeba histolytica*	*Trichomonas hominis*
Male	109	74 (67.89)	16	26	4	9	2	12	5
Female	121	83 (68.60)	16	29	3	11	2	13	9
Total	**230**	**157 (68.26)**	**32 (13.90)**	**55 (23.90)**	**7 (3.04)**	**20 (8.69)**	**4 (1.74)**	**25 (10.87)**	**14 (6.09)**

($p > 0.05$)

2.2 ANTI – PARASITIC EFFECTS OF MEDICINAL PLANTS ON LABORATORY ANIMALS INOCULATED WITH PARASITES

Out of 322 uninfected laboratory animals selected for the study, 110 (34.3%) had body weights equal to or less than 100g, 108 (33.5%) had body weights 101 to 150g and 104 (32.3%) had body weights greater than 150g. Table 3 summarizes the body weights of the selected laboratory animals. As shown, out of 112 male laboratory animals aged 2 months, 57 (50.9%) had body weights equal to or less than 100g and 55 (49.1%) had body weights 101 to 150g. Out of 106 female animals aged 2 months, 53 (50%) had body weights equal to or less than 100g and 53 (50%) had body weights 101 to 150g. Fifty three male animals aged 3 months and 51 female animals aged 3 months had body weights greater than 150g.

Table 4 summarizes the post parasite – inoculation stool analyses of the laboratory animals. As shown, out of 48 laboratory animals inoculated with *Ascaris lumbricoides*, 3 male animals with mean body weight 86.67g died in the first week and were replaced from the uninoculated reserve, out of 48 laboratory animals inoculated 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 uninoculated reserved ones. Out of 48 laboratory animals inoculated 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 uninoculated reserved ones. Out of 48 inoculated 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 uninoculated reserved ones. All 12 laboratory animals inoculated with *taenia* species died between the 5^{th} and 6^{th} week. Autopsy examination of their stool samples revealed no parasites. Stool analyses

of all survived 288 laboratory animal inoculated with *Ascaris lumbricoides*, *Necator americanus*, *Trichuris trichuira*, *Hymenolepsis nana*, *Entamoeba histolytica* and *Trichomonas hominis* revealed presence of the respective ova, cysts or trophozoites. Tables 5 to 7 summarized the body weights of infected laboratory animals, 3 weeks after treatment with medicinal plant extracts and reference drugs. Statistical analysis of the data using Chi square showed significant difference ($p < 0.05$) in the increase in body weights between post inoculation and post treatment times. The laboratory animals gained more weights in 3 weeks post – treatment than in 8 weeks post – parasite inoculation.

EFFECTS OF PARASITE INOCULATION AND MEDICINAL PLANT THERAPY ON THE LABORATORY ANIMALS

Fig 1 and 2 summarized the survival rates of laboratory animals experimentally infected with human intestinal parasites and treated with medicinal plant extracts. As shown, out of 37 laboratory animals inoculated with *Ascaris lumbricoides* and treated with *Napoleonaea imperialis*, 1 (2.7%) died. Out of 38 laboratory animals inoculated with *Ascaris lumbricoides* and treated with *Vernonia amygdalina*, 2 (5.3%) died. Out of 37 laboratory animals inoculated with *Necator americanus* and treated with *Napoleonaea imperialis*, 1 (2.7%) died. Out of 37 laboratory animals inoculated with *Necator americanus* and treated with *Vernonia amygdalina*, 1 (2.7%) died. All 12 Laboratory animals inoculated *Taenia* species died and infection was not established in any one. Out of 37 laboratory animals inoculated with *Hymenolepsis nana* and treated with *Napoleonaea imperialis*, 1 (2.7%) died. Out of 37 laboratory animals inoculated with *Hymenolepsis nana* and treated with *Sida acuta*, 1 (2.7%) died and out of 38 laboratory animals inoculated with *Hymenolepsis nana* and treated with *Vernonia amygdalina*, 2 (5.3%) died. Out of 37 laboratory animals inoculated with *Entamoeba histolytica* and treated with *Napoleonaea imperialis*, 1 (2.7%) died and out of 37 laboratory animals inoculated with *Entamoeba histolytica* and treated with *Vernonia amygdalina*, 1 (2.7%) died. All laboratory animals inoculated with *Trichomonas hominis* and treated with the 3 selected medicinal plants respectively survived. Similarly, all laboratory animals inoculated with *Trichuris trichiura* and treated with the 3 selected medicinal plants respectively, survived.

Table 3: Pre – Innoculation Weights of Uninfected Laboratory Animals.

Age of Lab. Animals (Months)	Sex	Number Examined	Weight of Laboratory Animals (g)		
			≤ 100	101 - 150	≥ 151
2	M	112	57	55	-
	F	106	53	53	-
3	M	53	-	-	53
	F	51	-	-	51
Total		322	110	108	104

Table 4: Post - Parasite Inoculation 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 lumbrico	*Necator America*	*Trichuris trichiura*	*Hymenolep nana*	*Entamoeba histolytica*	*Trichomonas hominis*	*Taenia* species
2	≤ 100	M	56	86.67	+	+	+	+	+	+	-
		F	55	88.33	+	+	+	+	+	+	-
	101 – 150	M	54	113.45	+	+	+	+	+	+	-
		F	53	120.20	+	+	+	+	+	+	-
3	≥ 151	M	53	142.60	+	+	+	+	+	+	-
		F	51	156.30	+	+	+	+	+	+	-
Total			**322**	**-**	**48**	**48**	**48**	**48**	**48**	**48**	**12**

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

Table 5: Mean Body Weights of Lab Animal 3 weeks Post Medicinal Plants Treatment

Lab Animal Age (Months)	Parasite Infected	Treatment Administered	Sex of Lab Animal	Mean Body Weight (g)		$W_2 - W_1$
				Pre-Treatment (W_1)	Post TREATMENT (W_2)	
2 (≤ 100g)	*Ascaris lumbric*	*Napoleaon*	M	95.20	103.20	8.10
			F	98.55	105.40	6.85
		Vernonia amygdalina	M	93.70	107.20	13.50
			F	96.35	104.50	12.15
		Sida acuta	M	94.70	102.90	8.20
			F	96.50	108.40	11.9
		Albendazole	M	94.80	105.30	10.50
			F	95.30	109.20	13.90
	Necator america	*Napoleaon*	M	92.50	102.51	10.01
			F	95.30	109.60	14.3
		Vernonia amygdalina	M	97.80	111.50	13.70
			F	99.60	112.30	12.70
		Sida acuta	M	91.30	103.60	12.30
			F	93.70	106.30	12.60
		Albendazole	M	93.40	102.40	9.00
			F	94.10	104.20	10.10
	Trichuris trichura	*Napoleaon*	M	90.50	98.80	8.30
			F	94.20	103.40	9.20
		Vernonia amygdalina	M	94.50	108.40	13.90
			F	96.50	108.60	13.10
		Sida acuta	M	91.20	96.30	5.10
			F	93.10	102.70	9.60
		Albendazole	M	91.20	97.10	5.90
			F	92.60	99.40	6.80
	Hymenol nana	*Napoleaon*	M	90.20	96.10	5.90
			F	92.60	99.70	7.10
		Vernonia amygdalina	M	94.60	103.20	8.60
			F	97.10	106.60	9.50
		Sida acuta	M	93.30	98.90	5.60
			F	95.50	101.40	5.90
		Albendazole	M	91.40	95.70	4.90
			F	93.50	98,40	3.8
	Entamoe histolysti	*Napoleaon*	M	91.40	95.20	5.20
			F	92.20	100.40	5.20
		Vernonia amygdalina	M	93.80	99.30	5.50
			F	95.30	104.30	9.00
		Sida acuta	M	92.30	96.90	4.60
			F	94.50	100.10	5.60
		Metronidazole	M	90.70	94.50	3.80
			F	91.90	97.20	5.30
	Trichom hominis	*Napoleaon*	M	90.20	94.70	4.50
			F	94,50	99.10	4.60
		Vernonia amygdalina	M	93.50	98.50	5.00
			F	95,10	102.30	7.20
		Sida acuta	M	90.50	95.40	4.90
			F	92,70	98.30	5,60
		Metronidazole	M	90.40	94.70	4.30
			F	92,40	100.40	8.00

Table 6: Mean Body Weights of Lab Animal 3 weeks Post Medicinal Plants Treatment

Lab Animal Age (Months)	Parasite Infected	Treatment Administered	Sex of Lab Animal	Mean Body Weight (g)		$W_2 - W_1$
				Pre-Treatment W_1	Post TREATMENT W_2	
2 (101 – 150g)	*Ascaris lumbric*	*Napoleaon*	M	130.20	142.50	13.30
			F	136.67	145.30	8.63
		Vernonia amygdalina	M	140.40	154.10	13.7
			F	138.70	153.70	15.0
		Sida acuta	M	132.40	140.20	7.80
			F	135.60	146.70	11.7
		Albendazole	M	132.30	145.40	13.1
			F	141.40	150.60	9.20
	Necator america	*Napoleaon*	M	127.50	134.30	6.80
			F	131.70	143.50	11.80
		Vernonia amygdalina	M	129.10	136.20	7.10
			F	134.50	141.50	7.00
		Sida acuta	M	126.80	135.20	8.40
			F	133.30	146.40	13,10
		Albendazole	M	128.30	138.50	10.20
			F	133.20	145.30	12.10
	Trichuris trichura	*Napoleaon*	M	125.60	137.10	11.50
			F	128.20	136.50	8.30
		Vernonia amygdalina	M	127.10	138.50	11.40
			F	129.50	136.40	6.90
		Sida acuta	M	124.20	135.40	11.20
			F	126.50	139.50	13.00
		Albendazole	M	124.30	138.40	14,10
			F	125.10	134.60	9.50
	Hymenol nana	*Napoleaon*	M	128.50	135.60	9.10
			F	133.60	140.20	6.60
		Vernonia amygdalina	M	121.90	134.50	12.60
			F	135.20	149.30	14.10
		Sida acuta	M	125.20	138.20	13.00
			F	13670	145.60	8.90
		Albendazole	M	127.60	140.20	12.60
			F	135.50	147.40	11.90
	Entamoe histolysti	*Napoleaon*	M	121.20	135.90	14.70
			F	129.30	138.50	9.20
		Vernonia amygdalina	M	123.40	137.52	14.12
			F	127.70	139.30	11.60
		Sida acuta	M	125.80	137.10	11.30
			F	130.50	141.40	10.90
		Metronidazole	M	123.50	136.24	12.74
			F	126.10	138.70	12.60
	Trichom hominis	*Napoleaon*	M	120.50	134.60	14.10
			F	128.30	140.51	12.21
		Vernonia amygdalina	M	122.30	137.45	15.15
			F	126.60	139.10	12.50
		Sida acuta	M	124.60	138.20	13.60
			F	127.50	136.50	9.00
		Metronidazole	M	121.70	131.70	10.00
			F	128.30	140.60	12.30

Table 7: Mean Body Weights of Lab Animal 3 weeks Post Medicinal Plants Treatment.

Lab Animal Age (Months)	Parasite Infected	Treatment Administered	Sex of Lab Animal	Mean Body Weight (g)		$W_2 - W_1$
				Pre-Treatment W_1	Post TREATMENT W_2	
3 (≥ 151g)	*Ascaris lumbric*	*Napoleaon*	M	159.40	170.55	11.15
			F	164.50	175.34	10.84
		Vernonia amygdalina	M	150.10	162.20	12.10
			F	162.30	175.30	13.0
		Sida acuta	M	158.80	170.10	11.30
			F	161.20	171.80	10.60
		Albendazole	M	158.70	164.45	5.75
			F	163.20	178.10	14.90
	Necator america	*Napoleaon*	M	152.70	160.30	7.60
			F	155.60	169.40	13.80
		Vernonia amygdalina	M	150.50	165.30	14.80
			F	156.60	166.20	10.40
		Sida acuta	M	152.40	162.40	10.00
			F	155.50	165.20	10.20
		Albendazole	M	153.10	165.15	12.50
			F	157.60	167.60	10.00
	Trichuris trichura	*Napoleaon*	M	152.30	162.50	10.20
			F	156.20	167.30	11.10
		Vernonia amygdalina	M	154.10	164.10	10.00
			F	157.30	170.50	13.20
		Sida acuta	M	151.30	161.60	10.30
			F	158.70	169.50	10.80
		Albendazole	M	152.50	163.30	10.80
			F	158.20	168.50	10.30
	Hymenol nana	*Napoleaon*	M	151.00	162.40	11.40
			F	154.30	165.10	10.80
		Vernonia amygdalina	M	153.90	164.50	10.60
			F	156.70	170.10	12.00
		Sida acuta	M	154.50	163.50	10.50
			F	157.80	174.30	12.10
		Albendazole	M	153.10	162.20	10.80
			F	156.10	165.90	10.80
	Entamoe histolysti	*Napoleaon*	M	149.50	156.30	10.50
			F	152.70	161.20	9.50
		Vernonia amygdalina	M	144.40	156.40	12.00
			F	157.20	168.30	11.10
		Sida acuta	M	149.40	160.7	11.30
			F	152.80	167.20	14.40
		Metronidazole	M	145.70	157.60	11.90
			F	159.40	165.20	5.80
	Trichom hominis	*Napoleaon*	M	150.60	156.30	5.70
			F	155.20	161.80	6.60
		Vernonia amygdalina	M	148.30	159.30	11.00
			F	159.50	164.50	5.00
		Sida acuta	M	142.10	152.40	10.30
			F	153.40	166.20	12.80
		Metronidazole	M	145.80	156.50	10.70
			F	154.20	164.40	10.20

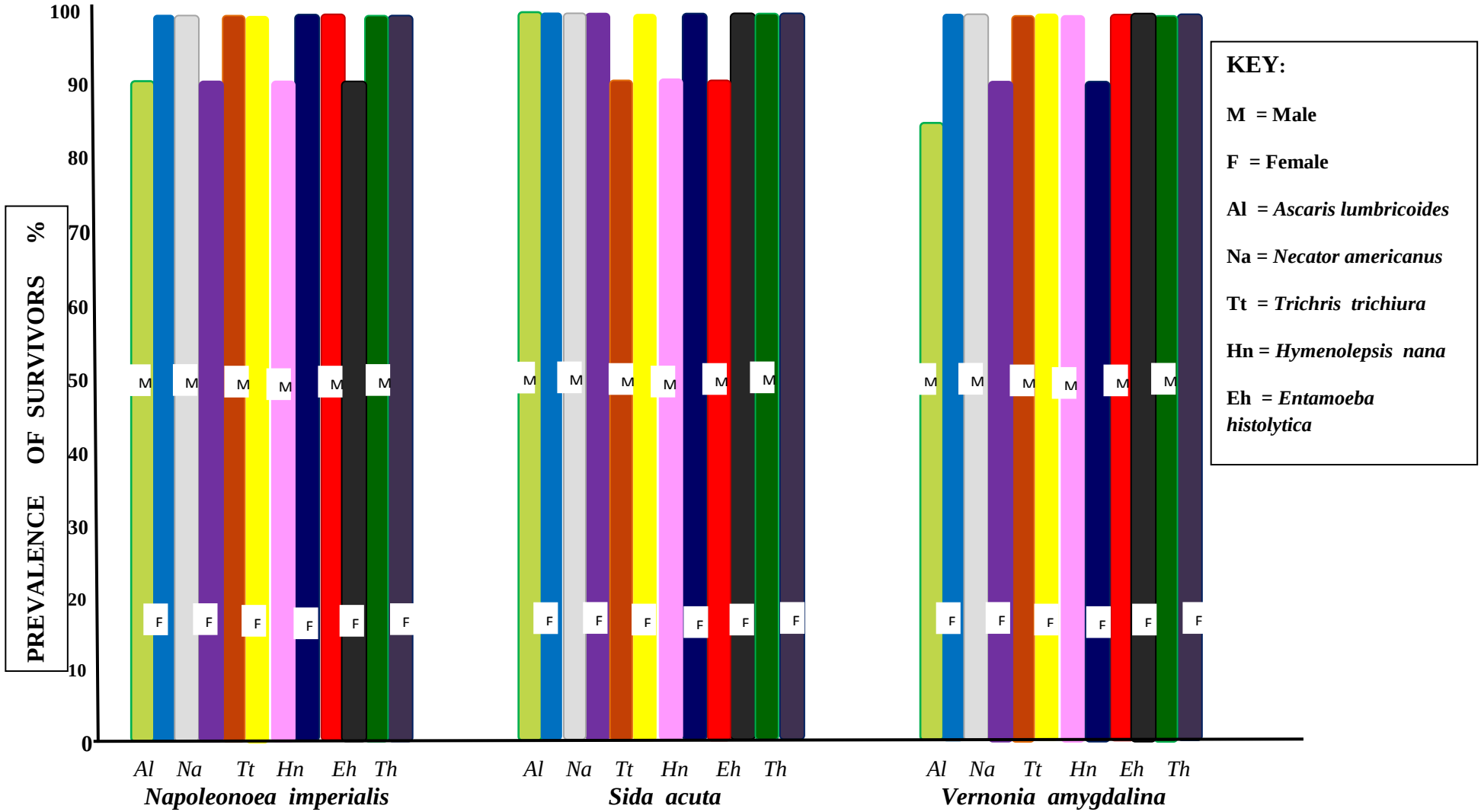

Fig. 1: Survival Rate of 2 Months – old Lab. Animals Infected with Parasites & Treated with Medicinal Plant Extracts.

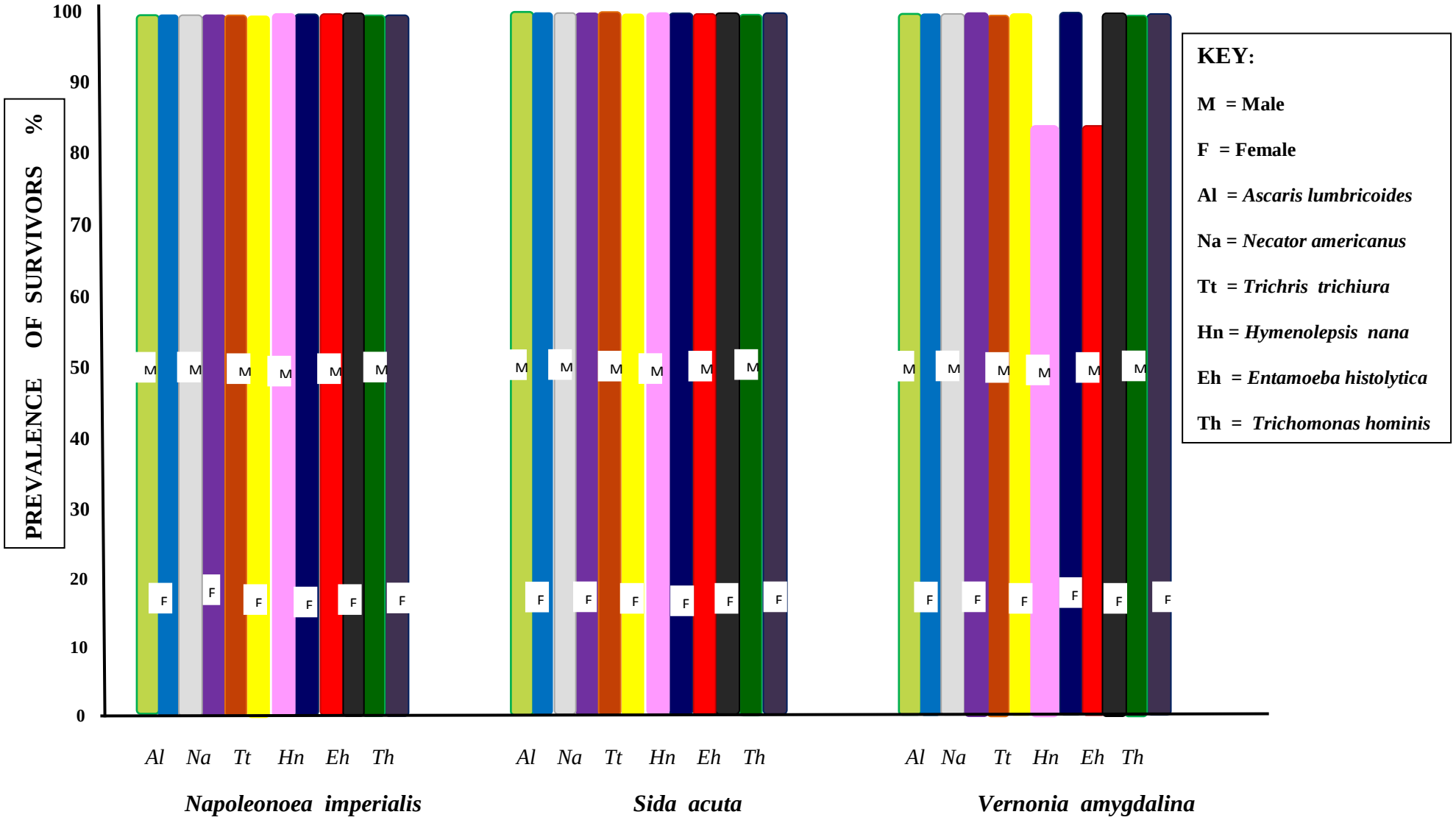

Fig. 2: Survival Rate of 3 Months – old Lab. Animals Infected with Parasites & Treated with Medicinal Plant Extracts.

POST TREATMENT STOOL ANALYSIS OF INNOCULATED LABORATORY ANIMALS

Table 8 summarized the findings of post treatment stool analysis of laboratory animals inoculated with different human intestinal parasites. As shown, *Necator americanus* was isolated from the stool of 50% male and female laboratory animals aged 2 months and treated with *Napoleonaea imperialis.* Also *Hymenolpsis nana* was isolated from stool of 100% male and female laboratory animals treated with *Napoleonaea imperialis. Ascaris lumbricoides* and *Hymenolepsis nana* were isolated from the stool of 50% male and female laboratory animals aged 2months with mean body weight 101 – 150g and treated *Sida acuta. Neactor americanus* was also isolated from stools of laboratory animals aged 3 and treated with *Sida acuta. Entamoeba histolytica* was isolated from stool of 50% male and female laboratory amimals aged 3 months with body weights ≥151g and treated with *Vernonia amygdalina*. Also *Trichomonas hominis* was isolated from stool of 100% male and female laboratory animals aged 3 months with mean body weight ≥ 151g, treated with *Vernonia amygdalina.*

Table 8: Post - Treatment Stool Analyses of Laboratory Animals Innoculated with Human Intestinal Parasites.

Initial Age & Body Weight	Treatment Administered	Sex	Number Examined	Parasites Isolated (%)					
				Ascaris lumbricoides	*Necator americanus*	*Trichuris trichiura*	*Hymenolep nana*	*Entamoeba histolytica*	*Trichomonas hominis*
2 (≤ 100)	*Napoleonaea imperialis*	M	12	-	+ (50)	-	+ (100)	-	-
		F	12	-	+ (50)	-	+ (100)	-	-
2 (101 – 150)	*Sida acuta*	M	12	+ (50)	-	-	+ (50)	-	-
		F	12	+ (50)	-	-	+ (50)	-	-
3 (≥ 151)	*Vernonia amygdalina*	M	12	-	-	-	-	+ (50)	+ (100)
		F	12	-	-	-	-	+ (50)	+ (100)

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

DISCUSSION OF FINDINGS

Previous studies (Confalonieri *et al*. 1985, Fry 1985) report that helminths (nematodes, cestodes, trematodes and acantocephalans) keep their morphological parameters almost unchanged when 0.5% Na_3PO_4 aqueous solution is employed to rehydrate desiccated organic remains Similarly, protozoa cysts are recovered unchanged, however, cysts decay faster, resulting in artificially low estimations of protozoa (Gonçalves *et al*. 2002).The present study showed high prevalence of human intestinal parasites (68.26%) in Imo State. This finding agrees with previous reports (Ikpeama *et al*, 2016), who reported high prevalence of intestinal helminthiasis in Owerri, Imo State. Ikpeama *et al.* (2016) also reported that delay in disposal of refuse around Owerri municipal metropolis led to increasing prevalence of intestinal helminthiasis in the area. In comparison with neighbouring communities, Okolie (2007) reported that prevalence of hookworm infection was statistically higher in Owerri (23.3%) than Port Harcourt (13.3%) ($p<0.005$).Parts of the State with poor drainage and lower standard of hygiene had higher prevalence rates (11.6% , 8%,) than zones with improved standard of sanitation and adequate facilities (3.3% ,1.3%). The intestinal parasites isolated from patients in this study (*Necator americanus, Ascaris lumbricoides, Taenia* species, *Trichuris trichiura, Hymenolepsis nana, Entamoeba histolytica* and *Trichomonas hominis*) were similar to those reported in past years in Imo State by previous workers (Obiajuru and Ogbulie, 2003). This observation suggests that intestinal helminthiasis is major health challenge in Imo State and has not received adequate attention.

Although there are several drugs effective against intestinal helminthes about one - third of the world's population still lack regular access to essential drugs (WHO, 2002). 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 constitute a major challenge in treatment and management of intestinal parasites infection. Increasing cases of drug failures in treatment of infectious diseases in Nigeria (Ohalete *et. al*., 2016) is more worrisome than the increasing prevalence of intestinal parasite infections. A number of factors have been blamed for the rising cases of drug failure in management and treatment of these infections. Research is actively going on in different institutions in the country in search of better remedy for treatment and management of infectious diseases. However, not many of these studies included drugs for intestinal parasites infection. It appears in Nigeria, many people including health workers do not regard intestinal parasite infections as serious health challenge. The present study is an effort geared towards finding an affordable, safe and relatively cheap remedy for treatment of intestinal parasites infections especially in resource poor communities like Orlu and Imo State generally.

EXPERIMENTAL INFECTION OF THE LABORATORY ANIMALS

The study showed that the survival rate of human intestinal parasites in laboratory animal was high (92.5%). This shows that Albino rats can successfully be used for *in vivo* studies of anti parasitic effects of potential remedies. The rate at which the plant extract cleared parasite infected on the albino rats showed that the plants contain useful ingredients that could help produce novel drugs or analogues of existing drugs capable of treating intestinal parasites infection. 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, steroids and cyanide) are likely to be known phytochemical compounds useful in production of chemotherapeautic agents and other industrial products.

Post treatment stool analysis of laboratory animals inoculated with different human intestinal parasites showed that the plant extracts exhibited 100% cure of all laboratory animals infected with *Trichuris trichiura* and varying degrees of cure upto 80%, of laboratory animals infected with *Hymenolepsis nana*, *Necator americanus*, *Ascaris lumbricoides*, *Entamoeba histolytica* and *Trichomonas hominis*. This finding shows that the plant extracts are promising for effective treatment and cure of humans infected with these parasites.

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 (Danquah *et al*., 2012; Nalule *et al*., 2013) 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 shew them.

All plant extracts used in this study exhibited curative effects on laboratory animals infected with human intestinal parasites without inflicting impairment on the organs of laboratory animals. This finding agrees with previous reports (Molgaard *et al*., 2001) which reported that leaves, stem, roots and root bark of *Vernonia amygdalina* extract effectively killed cestodes of *Hymenolepis diminuta* after 24hours of treatment. In other studies, Abdul *et al*., (2000) 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 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 smaller. Animals administered with 10µg 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 crude plant extracts are effective remedies for intestinal parasites. Elsewhere, other workers (Yeap *et al*., 2010; Adediran *et a*l., 2014) 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, (2006) 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, (2002) 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 toxicity and nature of organ impairment caused on the laboratory animals were minimal. 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. It is recommended that histopathological assessment of organ impairment be carried out to determine the toxicity of the plants and their chemotherapeautic index.

This study has shown that human intestinal parasites can be destroyed and removed from the body of infected persons. Since human intestinal parasite remained a major public health challenge in Nigeria and other poor resource countries, these plants which are readily available with little or no money, can be used, especially *Vernonia amygdalina* which used in cooking food in many countries

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