

Research Article

EVALUATION OF THE EFFICACY OF LEAF EXTRACT OF *MANGIFERA INDICA*+ROOT EXTRACT *NAUCLEA LATIFOLIA* IN LABORATORY RAT: TOWARDS THE CONTROL OF GASTRO INTESTINAL HELMINTHES INFECTION

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ABSTRACT

There are a large number of plants whose anthelmintic activity has been demonstrated under controlled experimentation, either through feeding the whole plant or administering plant extracts to parasitised hosts. However, contrary to traditional expectation, there are also a great number of plants with purported antiparasitic properties, which have not been reproduced under experimental conditions. Studies on the helminthocidal activities of leaf extract of *Mangifera indica*+root extract of *Nauclea latifolia* in mice was designed to determine their efficacies against intestinal helminthes. Sixty Swiss albino mice maintained conventionally were selected randomly from laboratory animal house. The collected faecal samples were examined by simple faecal flotation techniques for isolation of parasitic eggs and/or oocysts. 42 (70.0%) of the mice under study were infected with at least one gastro intestinal parasite. The leaf extract of *Mangifera indica*+root extract of *Nauclea latifolia* was prepared by boiling in tap water. Graded concentrations (100%, 50%, 25% and 12.5%) of stock that was produce in Tetfund Zoology Laboratory of Osun State University was tested against both mice of different weights and sexes for helminthocidal efficacy. LC50 of the extract value obtained was found to be 75 mg/l in both big and small mice. Prevalence of gastrointestinal infections reduced with increased concentration of extract. Statistical analysis using analysis of variance indicated that there was a significant difference in the means of the treatment concentrations and the rates of prevalence of gastrointestinal infections in the sampled mice. The results of this research showed that the leaf extract of *Mangifera indica*+root extract of *Nauclea latifolia* are highly active against intestinal helminths in the sampled mice. The extract action also had significant influence ($p>0.05$) on the time of exposure of mice and the rate of prevalence. The safety of the leaf extract of *Mangifera indica*+root extract of *Nauclea latifolia* on vital organs of humans may need to be investigated to determine its safety on humans.

Keywords: Efficacy; Root extracts; *Mangifera indica*; *Nauclea latifolia*; Swiss albino mice; Gastro intestinal parasitic parasites

INTRODUCTION

Intestinal tract parasites cause significant morbidity and mortality throughout the world, particularly in undeveloped countries where there is limited access to safe drinking water, sanitation, and nutrition (Anosike et al., 2006). They are widely distributed throughout the world causing substantial implication to the public health, economy, and physical and cognitive development particularly among children in developing countries like Nigeria. The poor personal hygiene, poor environmental hygiene, and poor health system commonly observed in developing countries make the prevalence to be highest among these populations (PAHO, 2011).

Intestinal parasites are most prevalent in children of school-going-age, pregnant women and immune-compromised individuals (Anosike et al., 2006). Children with parasite infections may suffer from a wide variety of symptoms including anaemia as well as a myriad of mental and physical problems (Rufai and Awi – Waadu, 2006). As intestinal

parasites survive by removing important nutrition from their human host, children infected with gastrointestinal parasites may have decreased mental and physical development. Thus the parasitic disease may have far greater effects than are immediately apparent and may decrease the learning ability and productivity of infected children. Intestinal parasite infestation in pregnant women is also of particular concern. It may result in anaemia, a weakened immune system and a resulting susceptibility to infections, all of which may affect foetal survival and development (Tsoka-Gwegweni and Ntombela, 2014). Maternal intestinal parasite infestation may result in low birth-weight infants who are also susceptible to infections and have developmental problems. Even postnatal, maternal infestation may cause lethargy and malaise which may adversely affect the mothers' ability to provide adequate care for the infant.

Studies have established that some types of worm are in the intestines of over 75 percent of the world's population[?]. This is a frightening statement. Common intestinal parasites include:

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Roundworm (*Ascaris lumbricoides*), Hookworm (*Necator americanus*, *Ancylostoma duodenal*), Pinworm (*Enterobius vermicularis*), Roundworm (*Toxocara canis*, *Toxocara cati*), Heart worm (*Dirofilaria immitis*), Strongyloides (*Strongyloides stercoralis*), Trichinella (*Trichinella spiralis*) (Mehmet and Oprah, 2015).

The main drugs used to treat human intestinal parasites nowadays are mebendazole, albendazole, pyrantel pamoate, and levamisole for intestinal nematodes, ivermectin (IVM) for onchocerciasis, and DEC alone or DEC-albendazole and IVM-albendazole combination treatments for filariasis (Albonico et al., 1999). Depending on local epidemiology, availability, and cost, these drugs have been widely available in most health care systems for the curative treatment of clinical cases for many years.

In recent years, several reports of apparent failures in the treatment of intestinal parasites and nematodes have been published (De Clercq et al., 1997, Ismail et al., 1996). These have led to an increased awareness of the potential problem of anthelmintic resistance in the treatment and control of human helminths.

Even multiple drug resistance is not uncommon in helminths of veterinary importance. In parts of Paraguay (Maciel et al., 1999), helminths are resistant to all available broad-spectrum anthelmintics and farmers have started to give up sheep farming because of insurmountable problems with anthelmintic resistance (Reynoldson et al., 1997).

Because vaccinations do not work in most instances and the parasites have sometimes become resistant to the available synthetic therapeutics, it is important to search for alternative sources of anti-parasitic drugs. Plants produce a high diversity of secondary metabolites with interesting biological activities, such as cytotoxic, anti-parasitic and anti-microbial properties. These drugs often interfere with central targets in parasites, such as DNA (intercalation, alkylation), membrane integrity, microtubules and neuronal signal transduction. Plant extracts and isolated secondary metabolites which can inhibit protozoan parasites, such as Plasmodium, Trypanosoma, Leishmania, Trichomonas and intestinal worms are discussed. The identified plants and compounds offer a chance to develop new drugs against parasitic diseases. Most of them need to be tested in more detail, especially in animal models and if successful, in clinical trials.

Despite the number of plants with recorded ethnobotanical uses against gastrointestinal worms and helminths, no rigorous studies have yet evaluated the activity of most medicinal plants against specific human intestinal worms. Instead, previous research has relied on the use of model organisms as a substitute for these parasites. Most studies have used the free living (non-parasitic) nematode *Caenorhabditis elegans* as a helminth model to represent human intestinal worms. *Acorus calamus*, *Cotyledon orbiculata*, *Cyathea dregei*, *Melia azedarach* and *Tulbaghia violacea* have all been reported to have

activity against *C. elegans* (Aremu et al., 2012). There are also a large number of plants whose anthelmintic activity has been demonstrated under controlled experimentation, either through feeding the whole plant or administering plant extracts to parasitised hosts. However, contrary to traditional expectation, there are also a great number of plants with purported antiparasitic properties, which have not been reproduced under experimental conditions (Ahmed et al., 2013; Adenubi et al., 2016; Aremu, 2009).

Leaves of *Nauclea latifolia* and roots of *Mangifera indica* are both important medicinal plants which play an important role in management of many pathological conditions in developing countries of the world.

This work therefore studies the helminthocidal activities of leaf extract of *Mangifera indica*+root extract *Nauclea latifolia* in mice for the control of intestinal helminth infections owing to the increasing toxicity and resistance to hitherto widely used parasitic infections' drugs.

MATERIALS AND METHODS

Preparation of stock solution and various concentrations of leaf extract of *Mangifera indica*+Root extract of *Nauclea latifolia* used

100 g of plant root of *Nauclea latifolia* and 100 g plant leaf of *Mangifera indica* were weighed and boiled in 2 litres of clean tap water for thirty (30) minutes and allowed to cool. This was freeze dried in freeze dryer machine to turn the liquid recipe into powdery form (Behnke et al., 2001, Adenubi et al., 2016, Ahmed et al., 2013). The powder was placed in universal bottles containing 10 ml sterile distilled water after which they were filtered into a conical flask using a filter paper and a funnel to produce a clear juice. From this mixture of leaf extract of *Mangifera indica*+root extract of *Nauclea latifolia*, a stock solution was made in universal bottles by adding 0.1 ml of the mixture to 9.9 ml of sterile distilled water followed by agitation for one minute. From the stock solution 100 mg/l, 50 mg/l, 25 mg/l, 12.5 mg/l dilutions were prepared by fetching 1 ml, 0.5 ml, 0.25 ml, and 0.125 ml and added to six beakers each containing 100 ml of water (Table 1). All concentrations were prepared in duplicates. A control beaker was set up having only water (Aremu et al., 2009, Rufai et al., 2017).

Testing for prevalence of gastro-intestinal parasites in laboratory animals

A total of sixty (60) Swiss albino mice weighing between 105-158 g maintained conventionally were selected randomly from laboratory animal house. The (60) Swiss albino mice were divided into a group of five, resulting in 5 groups labelled G0, G1, G2, G3 and G4. The faeces of each group of mice were collected and examined by parasitological methods for the presence of intestinal parasites before extract was fed to them and prevalence and intensities were noted. The animals were housed and bred in the well-ventilated wooden cages with metal wiring and were fed once daily with commercially formulated rat feed and water was given

ad libitum. The research was carried out in accordance with the ethical rules on animals experimentation approved by the ethical committee of Osun State University.

Testing for helminthicidal efficacy of leaf extract of *Mangifera indica*+root extract of *Nauclea latifolia*

Four of the groups of the mice (G1, G2, G3 and G4) were each treated with 100 mg/l, 50 mg/l, 25 mg/l and 12.5 mg/l of extract except G0 group that was not treated but were given 1 ml of distilled water and serve as the control. After one week of extract application, the faeces of the mice were again collected and examined by parasitological methods. After five days, the prevalence and intensity of gastro-intestinal helminthes among the sampled groups was obtained. The recovered helminthes were examined and counted using the compound microscope. Morphological identification was done using perineal patterns for species identification as described by Bundy et al (1998).

RESULTS

The prevalence and intensities of gastrointestinal helminthes parasites in the sixty (60) Swiss albino mice prior to administration of various concentrations of leaf extract of *Mangifera indica*+root extract of *Nauclea latifolia* against helminthes is given in Table 1. 42 (70.0%) of the mice under study had parasitic infection with at least one parasite. Seven genera of parasites were detected in the mice, the three of which were protozoa including *Entamoeba muris*, *Eimeria spp.* and *Cryptosporidium spp.*, and the four other ones were helminths including *Hymenolepis nana*, *Hymenolepis diminuta*, *Syphacia muris* and *Strongyloides ratti*. *E. muris* showed the highest prevalence rate (96%) followed by *S. ratti* (95%). The lowest prevalence rate was observed in infections with *Hymenolepis diminuta* and *Syphacia muris*,

all with 2.5% and 2.3% prevalence respectively. Polyparasitic contamination was observed in the sampled mice. 27 (11.7%) of the total samples were contaminated with two species of parasites, while 8 (13.3%) of the samples with three species of parasites and single parasitic contamination were observed 7 (11.7%) (Table 2).

The prevalence of intestinal helminthes parasites in the sampled population in relation to sex of the mice showed that female had higher prevalence rate 25 (41.7%) than male with 17 (28.3%). Percentage infection rates showed that there was statistically significant association between the sexes and prevalence rate. Age specific prevalence of parasites showed that adult mice had the highest prevalence of 29 (48.3%), while the young mice had the least with 13 (21.7%) out of 42 (70.0%) infected (Table 2).

Table 2 showed the result of the antihelminthic activities of various concentrations of the leaf extract of *Mangifera indica*+root extract of *Nauclea latifolia* against gastrointestinal parasites in the sampled mice. Treatment was observed for 5 days after which prevalence rate was determined. Prevalence rate was seen to have reduced from treatments with higher concentrations to lower concentrations. The highest concentrations of 100 mg/l and 50 mg/l were recorded to have reduced the prevalence rate in G3 from 8 (66.7%) to 0 (0%) and G4, 9 (75.0%) to 0 (0%) while the prevalence in G0 was found to have increased from 8 (66.7%) to 10 (83.3%) (Tables 2 and 3). The intensities of gastrointestinal helminthes in the sampled mice before and after treatment recorded reduction in the intensities of all the parasites (Tables 4 and 5). Statistical analysis using analysis of variance indicated that there was a significant difference in the means of the treatment concentrations and the rates of prevalence of gastrointestinal infections in the sample mice.

Table 1: Various concentrations of leaf extract of *Mangifera indica*+root extract of *Nauclea latifolia* used.

Weight(mg) of the extract (stock solution) per volume of different concentrations					
Solution	Solution 1	Solution 2	Solution 3	Solution 4	Solution 5
Stock solution	1	0.5	0.25	0.125	0
10 ml	100 mg/l	50 mg/l	25 mg/l	12.5 mg/l	0

Table 2: The prevalence of intestinal helminthes in the sampled mice before treatment with leaf extract of *Mangifera indica*+root extract of *Nauclea latifolia*.

Variable	No of rats examined	No (%) infected	Number of parasitic spp		
			One (%)	Two (%)	Three (%)
Group (G)					
G0	12	8 (66.7)	1 (8.3)	5 (41.7)	2 (16.7%)
G1	12	7 (58.3)	1 (8.3)	5 (41.7)	1 (8.3%)
G2	12	10 (83.3)	2 (16.7)	7 (58.3)	1 (8.3%)
G3	12	8 (66.7)	2 (16.7)	4 (33.3)	2 (16.7%)
G4	12	9 (75.0)	1 (8.3)	6 (50.0)	2 (16.7%)
Sex					
Male	34	17 (28.3)	4 (11.8)	10 (29.4%)	3 (8.8%)
Female	26	25 (41.7)	3 (11.5)	17 (65.4%)	5 (19.2%)
Age					
Young	30	13 (21.7)	2 (6.7)	8 (26.7%)	3 (10.0%)
Adult	30	29 (48.3)	5 (16.7)	19 (63.3%)	5 (16.7%)
Overall	60	42 (70.0)	7 (11.7)	27 (45.0%)	8 (13.3%)

Table 3: The prevalence of intestinal helminths in the sampled mice after treatment with leaf extract of *Mangifera indica*+root extract of *Nauclea latifolia*.

Variable	No of rats examined	No (%) infected	Number of parasitic spp		
			One (%)	Two (%)	Three (%)
Group (G)					
G0	12	10 (66.7%)	2 (8.3%)	6	2
G1	12	7	1	5	1
G2	12	3	0	2	1
G3	12	0	0	0	0
G4	12	0	0	0	0
Sex					
Male	34	7	2	5	1 (8%)
Female	26	13	1	8	3
Age					
Young	30	2	0	2	0
Adult	30	18	3	11	4
Overall	60	20	3	13	4

Table 4: The intensities of intestinal helminthes in the sampled mice before and after treatment with leaf extract of *Mangifera indica*+root extract of *Nauclea latifolia*.

Parasites	Mean of the ova/cysts of the parasite per mice	
	Before Treatment	After Treatment
<i>Hymenolepis diminuta</i>	4	1.5
<i>Hymenolepis nana</i>	3.5	0.5
<i>Syphacia muris</i>	3.4	1.5
<i>Strongyloides ratti</i>	7.3	0
<i>Entamoeba muris</i>	10.7	0
<i>Cryptosporidium spp.</i>	9.3	0
<i>Eimeria spp.</i>	12.7	0

Table 5: Sample diagnostic doses and diagnostic times for the prevalence of intestinal helminths.

Extract concentrations	Prevalence of intestinal parasites per group					Diagnostic time (days)
	G0	G2	G3	G4	G5	
0 mg/l	8 (66.7%)	7 (58.3%)	10 (83.3%)	8 (66.7%)	9 (75.0%)	5
12.5 mg/l	7 (58.3%)	7 (58.3%)	7 (58.3%)	7 (58.3%)	7 (58.3%)	5
25 mg/l	3 (25.0%)	3 (25.0%)	3 (25.0%)	3 (25.0%)	3 (25.0%)	5
50 mg/l	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	5
100 mg/l	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	5

The results of this research showed that the leaf extract of *Mangifera indica*+root extract of *Nauclea latifolia* is highly active against intestinal helminths in the sampled mice.

DISCUSSION AND CONCLUSION

Table 4 shows the influence of leaf extract of *Mangifera indica*+root extract of *Nauclea latifolia* treatment levels on the prevalence and intensities of gastrointestinal helminthes in the sampled mice. After 5 days after treatments (5DAT), the data revealed that G0 recorded increase in the prevalence of gastrointestinal helminthes after treatment (Table 4). This may be attributed to growth in the number of parasites due to no hindrance. Prevalence in G1, G2, G3 and G4 reduced consistently with various concentrations with zero prevalence of helminthes recorded in G3 and G4 (Table 4). This can be attributed to the antihelmintic activities of the leaf extract of *Mangifera indica*+root extract of *Nauclea latifolia* treatment. Helminthes mortality was recorded to increase gradually from lower concentrations to higher concentrations. The

lowest prevalence and intensities of parasites were recorded at 50 mg/l and 100 mg/l concentrations and 50 mg/l as the lethal concentration (LC50) determined from the sample diagnostic doses and diagnostic times for the prevalence of gastrointestinal parasites (Table 5). Statistical analysis using analysis of variance indicated that there was a significant difference in the means of the treatment concentrations and the rates of prevalence of gastrointestinal infections in the sampled mice. The results of this research showed that the leaf extract of *Mangifera indica*+root extract of *Nauclea latifolia* are highly active against gastrointestinal parasites in the sampled mice. The extract action also had significant influence ($p > 0.05$) on the time of exposure of mice and the rate of prevalence (Table 5).

The results of this work showed that leaf extract of *Mangifera indica*+root extract of *Nauclea latifolia* is highly active against gastrointestinal helminthes infection. Although there had been practice in some part of the world where leaf extract of *Mangifera indica*+root extract of *Nauclea latifolia* was

used locally against malarial infection, its efficacy against gastrointestinal helminth parasites that was discovered in this study is recommended for the control of gastro-helminth parasites.

CONFLICT OF INTERESTS

The authors declare that there is no conflict of interests regarding the publication of this paper.

AUTHORS' CONTRIBUTION

Rufai Mohammed conceived the study, participated in the study design, data collection, data analysis, and drafted the paper for publication. Akeem Akinboro participated in study design sample collection, laboratory work, data analysis and interpretation. All authors have read and approved the final copy

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