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Anthelmintic efficacy of the aqueous crude extract of *Euphorbia hirta* Linn in Nigerian dogs

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ABSTRACT

The anthelmintic efficacy of the aqueous crude extract of *Euphorbia hirta* Linn was studied in 20 Nigerian dogs that were naturally infected with nematodes. They were randomly divided into 4 groups, A, B, C and D. Groups A and B each comprised 4 dogs while groups C and D comprised 6 dogs each. Group A animals were untreated, while those in group B were treated with proprietary anthelmintic (Mebendazole). Groups C and D animals were treated with aqueous crude extracts of *E. hirta* using intramuscular and oral routes, respectively. Two weeks after treatment, blood and faecal samples were collected to evaluate haematological values and faecal egg counts, respectively. The procedure was repeated two weeks later. Results of this study show that the aqueous crude extracts of *E. hirta* after its administration into local dogs produced a significant increase ($P< 0.05$) in PCV, RBC, Hb conc., TWBC and lymphocyte counts. The faecal egg counts also showed a remarkable and significant reduction in the levels of the identified helminths. The reduction in faecal egg counts was more pronounced with the extract administered through the oral route when compared with the intramuscular route. The effects of the plant extracts were broad spectrum in action. The phenol compound present in the plant extract could have caused reduction in worm load through this same mechanism that culminates in exhaustion and death of worms. Since the aqueous crude extract of *E. hirta* significantly reduced the faecal egg count of the helminths, it could serve as an anthelmintic agent.

Key words: *E. hirta*, anthelmintic, mebendazole, helminths, haematology

Introduction

The body of knowledge about plants, herbs and spices, and their respective and collective roles in promoting health is modest (BALLATINE et al., 1999). Herbs have been used as food and for medicinal purposes for centuries. However, the use of medicinal herbs has increased over

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the past few years and research interest has focused on various herbs that possess hypolipidemic, antiplatelet, antitumour or immune stimulating properties that may be useful adjuncts in helping reduce the risk of cardiovascular disease and cancer (CRAIG, 1999).

Much work has been carried out in Nigeria on medicinal plants used in humans but very little has been done on those used in animals. NWUDE and IBRAHIM (1980) compiled information on plants used in traditional veterinary medicine in Nigeria. More of this type of work should be done to identify plants used in various localities to treat animal diseases. These plants should be investigated for their efficacy and toxicity. Those found effective with minimal toxicity should be processed for use in veterinary practice (NWUDE, 1997). For instance, Cassia occidentalis was investigated and confirmed to have purgative, diuretic effects in dogs and galactogogue effects in goats. The methanol extract of *Morinda lucida* and aqueous extract of *Alstonia boonie* bark each play an active role against *Trypanosoma brucei* in mice (ASUZU and CHINEME, 1990; ASUZU and ANAGA, 1991). NOK et al. (1993) also reported on the trypanocidal potentials of *Azadirachta indica* leaf extract against *T. brucei*.

Euphorbia hirta Linn is an anthropogenic herb that is commonly seen occupying open waste spaces, roadsides, pathways, and as a weed of cultivation, occurring widely throughout West tropical Africa, and dispersed pan-tropically and sub-tropically around the world. It is usually erect, up to 40 cm tall, but it can also be seen lying down (BURKILL, 1994). The plant contains relatively abundant white latex (BURKILL, 1994). A number of substances have been detected in the plant; tannins, gallic acid, quercetin, phenols, phyto-sterols, alcohols, alkaloids, etc (DALZIEL, 1937; KERHARO and ADAM, 1974). Its diuretic and purgative action has been well documented (JOHNSON et al., 1999). The purgative action of *E. hirta* has led to postulation and the possibility of its use as an anthelmintic agent (AYENSU, 1979; SOFOWORA, 1993).

The importance of helminthosis can be underscored by the fact of the clinical signs associated with it. These include: lethargy, dullness, inappetence, loss of general body condition, rough or starry hair coat, loss of weight, pallor of visible mucous membrane, depression, anaemia, protein-losing enteropathy leading to hypoproteinaemia, gastroenteritis, etc (THOMPSON, 1988; OKEWOLE and ODUYE, 2001).

This study is aimed at exploring the anthelmintic activity of the plant extract and comparing this with Mebendazole, a proprietary anthelmintic, which belongs to the Benzimidazole group of anthelmintics. The study is also performed to determine effective route of delivery for this extract.

Materials and methods

*Preparation of the plant extrac*t. *Euphorbia hirta* leaves were collected freshly from roadsides and pathways and washed with clean water to remove dirt. The plant was identified

and authenticated at the herbarium of the Forestry Research Institute of Nigeria (FRIN), Ibadan, and the voucher specimen was deposited there.

The leaves were weighed (75 g), and blended into liquefaction in 150 ml. of distilled water. The mixture was then centrifuged at 1500 rpm. The supernatant was filtered through sterile filter papers into a conical flask as the study extract. 1 ml. of the filtrate is expected to contain 0.5 g, i.e. 500 mg/ml.

Proprietary anthelmintic (Mebendazole). The daily dosage of mebendazole for therapeutic use in the Nigerian dogs is 50 mg/kg body weight in divided doses for 3 consecutive days (LIU and WELLER, 1993; GANN et al., 1994). For an animal weighing 8 kg, such will receive 400 mg of the active ingredient per day. This amounted to 4 tablets per days since each tablet of Mebendazole contains 100 mg of active ingredients.

Animals. Twenty local dogs used in this study were procured from the local market in Ibadan, Nigeria. They comprised 12 males and 8 females that were kept in concrete-floored, clean, separate cages in the kennels of the Faculty of Veterinary Medicine University of Ibadan, where adequate water and feed was supplied. The kennel was cleaned and fumigated before the animals were installed.

Haematological and parasitological analysis. Faecal sample of each dog was colleted in labelled sterile universal bottles for identification of the type of helminths present using flotation techniques. Blood samples were also collected from each animal into labelled EDTA bottles for haematology. After 2 weeks, the animals were weighed; average weight was 8 kg. Average age was 7 months. After initial stabilization, the baseline haematological and coprological evaluations were made.

The flotation method, which involved the use of salted (NaCl) water, was used to determine the helminths present in the faecal samples, while the modified McMaster egg-counting technique was used for nematode counts. In determining fluke count, the modified McMaster egg-counting technique as used for nematode counts was employed, except that saturated zinc sulphate solution was used for estimation of fluke egg counts.

Blood samples were collected from the cephalic vein of each animal using 5 ml. syringes and 25-gauge needles into appropriately labelled EDTA bottles. Estimation of haemoglobin (Hb) concentration was by Sahli's method. Erythrocytes and leucocytes were counted manually using Neubau's haemocytometer. Packed cell volume (PCV) was determined by the conventional method, i.e. the microhaematocrit method. White blood cell differential counts were also determined.

Animal grouping and treatment. The dogs were divided into 4 groups: A, B, C and D. Group A consisted of 4 animals which received no medication; Group B also consisted of 4 animals that were treated with mebendazole. Group C consisted of 6 dogs, which were administered with the aqueous crude extract of *E. hirta* intramuscularly, while the 6 dogs in Group D had the extract administered orally. Both the drug and the extracts were

administered for 3 consecutive days. This procedure was repeated after 2 weeks. After a further 2 weeks, the procedure was again repeated.

Statistical analysis. Results are expressed as the mean of parameters ± standard error of the mean (SE). Differences between means were evaluated using the Student's test. ANOVA tests to determine multiple comparisons were also used. Differences are significant at $P<0.05$ (BRADFORD and HILL, 1991).

Results

Faecal egg count. The study showed that all the animals were heavily infested with worms, ranging from hookworms (*Ancylostoma caninum*), Ascarids (*Toxocara canis*), to tapeworms (*Dipylidium caninum* and *Echinococcus granulosus*). Administration of drug and extracts produced a significant reduction in the worm burden and by the second administration, Groups B and D animals were effectively dewormed, but Group C animals

Table 1. Faecal egg counts of dogs in the drug trial

Parameters	Baseline	1st 2 weeks	2nd 2 weeks
Ancylostoma caninum (eggs/gram faeces)	2800 ± 91.3 (Group A)	2500 ± 129.1	2600 ± 182.6
	2250 ± 104.1 (Group B)	400 ± 40.8	
	1867 ± 5.9 (Group C)	1916.7 ± 60.1	200 ± 40.8
	2267 ± 68.1 (Group D)	233 ± 23.3	
Toxocara canis (eggs/gram faeces)	1700 ± 57.8 (Group A)	1750 ± 64.6	2250 ±104.1
	2350 ± 64.6 (Group B)	100 ± 8.1	
	1666.7 ± 223.1 (Group C)	1700 ± 229.5	200.0 ± 40.8
	2300 ± 43.6 (Group D)	333.3 ± 88.2	
Echinococcus granulosus (eggs/gram faeces)	1000 ± 40.8 (Group A)	1450 ± 144.3	2050 ± 64.6
	2150 ± 64.6 (Group B)	250 ± 7.1	
	2333 ± 2.7 (Group C)	2330 ± 85.6	167 ± 2.9
	1467 ± 12.7 (Group D)	600 ± 5.2	
Dipylidium caninum (eggs/gram faeces)	1300 ± 91.3 (Group A)	850 ± 64.6	1400 ± 91.3
	925 ± 278 (Group B)	400 ± 40.8	
	2116 ± 86.1 (Group C)	1833.3 ± 166.7	500 ± 0.00
	1433 ± 81.4 (Group D)	900 ± 40.8	67 ± 1.2

Results expressed as means ± SE; 1st 2 weeks after administration of drug/plant extracts; *2nd 2 weeks after 2nd administration of drug/plant extracts; Note that animals in group A did not receive any treatment.

Table 2. Haemogram of the experimental dogs in the drug trial

Parameters	Baseline	1st 2 weeks	2nd 2 weeks
PCV (%)	14.2 ± 0.5 (Group A)	18.6 ± 0.6	18.3 ± 0.2
	19.9 ± 1.8 (Group B)	29.0 ± 1.0	34.2 ± 0.5
	27.5 ± 1.4 (Group C)	34.4 ± 0.6	36.7 ± 1.1
	24.4 ± 1.1 (Group D)	35.2 ± 1.0	33.9 ±0.5
Haemoglobin concentration (mg/dl)	4.7 ± 0.4 (Group A)	5.3 ± 0.1	5.9 ± 1.2
	6.9 ± 0.7 (Group B)	10.9 ± 0.1	11.9 ± 0.2
	10.2 ± 0.5 (Group C)	11.8 ± 0.2	11.4 ± 0.3
	8.2 ± 0.7 (Group D)	11.9 ± 0.3	11.4 ± 0.3
RBC (x 10^6/µl)	2.6 ± 0.1 (Group A)	2.8 ± 0.3	4.1 ± 0.6
	3.6 ± 0.4 (Group B)	5.4 ± 0.2	5.8 ±0.2
	5.2 ± 0.2 (Group C)	5.3 ± 0.2	5.1 ±0.1
	4.2 ± 0.3 (Group D)	5.4 ± 0.3	5.3 ±0.2
TWBC (x10^3/µl)	9.0 ± 1.8 (Group A)	11.3 ± 0.6	13.1 ± 0.3
	15.1 ± 1.4 (Group B)	11.2 ± 0.4	13.1 ± 0.8
	14.6 ± 1.2 (Group C)	20. 1 ± 1.6	20.8 ± 3.6
	11.5 ± 0.3 (Group D)	5.9 ± 0.9	4.7 ±0.2
Lymphocytes (x10^3/µl)	5.7 ± 1.1 (Group A)	7.3 ± 0.8	7.5 ± 1.0
	6.6 ± 0.5 (Group B)	5.3 ± 0.4	7.2 ± 0.5
	8.1 ± 1.3 (Group C)	11.8 ± 1.7	13.6 ±2.3
	5.2 ± 0.3 (Group D)	1.8 ± 0.2	1.2 ±0.1
Monocytes (x10^3/µl)	3.3 ± 0.5 (Group A)	3.0 ± 0.5	3.6 ± 0.3
	3.0 ± 0.6 (Group B)	3.2 ± 0.1	3.8 ± 0.1
	3.9 ± 0.9 (Group C)	5.4 ± 0.9	4.9 ± 1.3
	2.6 ± 0.2 (Group D)	0.9 ± 0.4	0.8 ± 0.1

Results expressed as means ± SE; 1st 2 weeks after administration of drug/plant extracts; * 2nd 2 weeks after 2nd administration of drug/plant extracts; Note that animals in group A did not receive any treatment

were not effectively dewormed. ANOVA tests showed that when all the identified helminths were compared between groups and within groups, the differences were significant at 0.000 (Table 1).

Haematological reports. Haematological results showed that before treatment, the PCV mean values for animals in Groups A, B, C, and D were 14.2 ± 1.7, 19.9 ± 4.9, 27.7

± 4.2 and 24.6 ± 3.7(%), respectively. After treatment, this parameter showed a significant increase. For haemoglobin, pre-treatment values for Groups A, B, C, and D were 4.8 ± 09, 6.9 ± 2, 10 ± 2.3, and 8.2 ± 2.5, respectively. After treatment, this parameter also underwent significant increase. In the case of the red blood cell (RBC), the mean values for Groups A, B, C and D before treatment were 2.6 ± 0.1, 3.6 ± 1.3, 5.2 ± 0.6 and 4.2 ± 1.1, respectively. Like the other 2 parameters, RBC also showed a significant increase after treatment. In the case of TWBC and the differentials, the parameters showed increasing levels with drug and extracts administration. ANOVA tests showed that when all the haematological parameters were compared between groups and within groups, the differences were significant at 0.000 (Table 2).

Discussion

This study has clearly shown that local dogs are heavily infested with worms except when treated with appropriate anthelmintics. Helminths infection manifested general clinical signs such as lethargy, dullness, inappetence, loss of general body condition, rough or starry hair coat, pallor of visible mucous membrane, loss of weight, etc. necessitated the urgent need for treatment (CAROLL and GROVE, 1986; THOMPSON, 1988; OKEWOLE and ODUYE, 2001).

The *E. hirta* extracts administered in this study caused a significant reduction in the worm burden of the dogs. Group D animals particularly experienced more significant reduction in worm burden than Group C animals. This effect was also noticed after the second administration of the extracts. This observation may be due to the fact that the helminths are found in the gastrointestinal tract. Therefore, there is a direct contact of the extract with the helminths in the intestinal tract leading to uncoupling reaction on the parasites. Extract administered through the intramuscular route, not being in direct contact with the worms may require increased concentration for better effect. The pharmacodynamic nature of the parenteral route might mean that another round of extract administration has to be embarked upon before the worms are completely eliminated. This, however, might expose the animals to toxicity. It thus demonstrated that the oral route of administration should be preferred with respect to this extract. It must be stressed, however, that the effect of the proprietary anthelmintic is more pronounced on the worm than those of the extracts of *E. hirta*.

The reduction of worm load observed with the extracts of *E. hirta* on animals in Groups C and D may be attributed to the presence of phenol compound $C_8H_{18}0_{15}$ in the plant extracts (RICE, 1965). Substituted phenols such as disophenol, niclofolan and nitroxynil are established anthelmintics which act by uncoupling the mitochondria reaction involved in electron transport-associated event from ATP generation. This is very lethal to blood-sucking helminths (BEHNKE et al., 1991). The phenol compound present in the plant extract

could have caused reduction in worm load through the same mechanism that culminates in exhaustion and death of worms.

Administration of the drug and extracts resulted in a remarkable improvement in the haematology of dogs in Groups B, C and D because the worms that were responsible for reduction in the levels of these haematological parameters have been removed to some extent. It thus becomes natural that through haemopoeisis the parameters will begin to appreciate with time (HALTON, 1974; OYERINDE, 1980; OMAMEGBE and UCHE, 1985; BARRIGA and OMAR, 1992).

Reduction of worm load by the extracts of *E. hirta* in this study is a positive and welcome development in our local helminths struggle, because the plant, *E. hirta* is available all-year round in Nigeria. The easy access to this plant and its availability might mean that the cost of medication would have been drastically reduced. The plant extracts exhibited a high degree of broad spectrum, which implies that total reliance on proprietary drugs, which in most cases are imported, will be reduced. It also means that the risk of drug resistance could to some extent be avoided.

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SAŽETAK

Anthelmintička učinkovitost vodenog iscrpka biljke *Euphorbia hirta* istražena je u 20 nigerijskih pasa prirodno invadiranih nematodima. Životinje su bile svrstane u skupine A, B, C i D. U skupini A i B bile su četiri životinje, dok su skupine C i D sadržavale šest životinja. Psi u skupini A nisu bili liječeni dok su oni u skupini B bili liječeni mebendazolom. Psi u skupini C bili su liječeni vodenim iscrpkom biljke i to intramuskularnom primjenom. Skupina D bila je liječena peroralnom primjenom vodenog iscrpka. Dva tjedna nakon liječenja svim psima izvađena je krv te sakupljene izmetine. Parazitološka pretraga izmetina bila je ponovljena nakon dva tjedna. Rezultati istraživanja pokazuju da vodeni iscrpak biljke *Euphorbia hirta* značajno ($P<0,05$) povećava broj krvnih stanica, eritrocita, leukocita, limfocita kao i koncentraciju hemoglobina. Promjene su dokazane i u nalazu parazitskih jaja. Smanjeni broj parazitskih jaja bio je izraženiji u skupini koja je iscrpak dobivala peroralno u odnosu na intramuskularnu primjenu. Pretpostavlja se da je upravo fenol kao komponenta iscrpka odgovoran za učinak na endoparazite. S obzirom da se njegovom primjenom značajno smanjio broj parazitskih jaja može ga se preporučiti i kao anthelmintik.

Ključne riječi: *E. hirta*, anthelmintik, mebendazol, helminti, hematologija