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Anthelmintic Potency of Pawpaw (*Carica papaya*) Seeds in West African Dwarf (WAD) Sheep

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Absract: The anthelmintic potency of the aqueous and crude extract of *Carica papaya* seeds was studied in 40 WAD sheep naturally infected with nematodes. They were randomly divided into 4 groups, A, B, C and D with 10 animals per group. Group A animals were untreated, while those in group B were treated with proprietary anthelmintic (*Mebendazole*). Groups C and D animals were treated with powdery and aqueous forms of *C.papaya* via 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 showed that the powdery and aqueous *C.papaya* after its administration produced a significant increase ($P< 0.001$) in PCV, RBC, Hb conc. and lymphocyte counts and significant decreased in eosinophil 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 aqueous extract than crude extract administered through the oral route. The effects of the *C.papaya* seed extracts were broad spectrum in action. The papain compound present in the *C.papaya* seed extracts could have caused reduction in worm load through this same mechanism that culminates in exhaustion and death of worms. Since the aqueous and crude extract of *C.papaya* significantly reduced the faecal egg counts of the helminths, it could serve as an anthelmintic agent.

Key words: *Carica papaya* · Anthelmintic · Mebendazole · Helminths · Haematology

INTRODUCTION

West African Dwarf (WAD) sheep and goats are the small ruminants which are ubiquitous in villages throughout the Nigerian rainforest and the derived savannah [1]. These animals have great economic potential due to high fertility, fecundity, prolificacy, early maturity and their adaptability to the humid environment [2]. However, the economic benefits obtained from these animals are far below expectation due to low productivity as a result of worms infestation. Helminthosis is the infestation of the body with parasitic worms most especially *Haemonchus contortus* which caused anaemia [3,4]. The most important nematode parasites of ruminants implicated in production losses are principally those found in the gastro-intestinal tract. The body of knowledge about plants, herbs and spices and their respective and collective roles in promoting health is modest [5]. Herbs have been used as food and for medicinal purposes for centuries. However, the use of medicinal herbs has increased over 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 [6]. Scientific studies have proven that a number of plants used in human ethnomedical practice have pharmacological activities and may also be useful as ethno-veterinary remedies [7-9]. 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 [10] compiled information on plants used in traditional veterinary medicine in Nigeria. Thirteen plant species were used as anthelmintics and nine to combat worm infestation in livestock [11] and these include *Carica papaya*, *Allium sativum, Areca catecha,*

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Ananas comosus Nicotiana tabacum, Cucurbita moschata [11]. Among these botanical specie, *Carica papaya,* (pawpaw) may be preferred to others as an ethno-veterinary remedy in this part of the tropics because of its adaptability, agro-ecological considerations and availability [12]. Efforts in many countries to control helminthosis in ruminants result from the development of resistance by parasite populations to certain chemical anthelminthic drugs which are costly and therefore, there is a need for cheaper and more easily available alternatives. Pawpaw *(Carica papaya*) seeds have been evaluated as an anthelminthic against gastro intestinal worms but this has not been fully substantiated in WAD sheep. This work investigated anthelminthic potency of Carica papaya seeds in powdery and aqueous forms in WAD sheep naturally infected with gastro-intestinal worms.

MATERIAL AND METHODS

Preparation of the Carica Papaya Seeds: The seeds were collected freshly from ripe pawpaw fruits and washed with clean water to remove dirt. The seeds were sundried and later grinded into powdery forrms.

The pawpaw seed powders 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 sheep is 12.5 mg/kg body weight in divided doses for 3 consecutive days . For an animal weighing 10 kg, such will receive 125 mg of the active ingredient per day [13]. This amounted to 1¼ tablets per day since each tablet of Mebendazole contains 100 mg of active ingredients.

Animals: Forty sheep used in this study were procured from the local market in Ogbomoso, Nigeria. They were kept in concrete-floored, clean, separate pens in the Ladoke Akintola University of Technology Teaching and research farms, where an adequate water and feed was supplied. The pen was cleaned and fumigated before the animals were installed.

Haematological and Parasitological Analysi: Faecal sample of each sheep was collected in labelled sterile universal bottles for identification of the type of helminths eggs present using flotation techniques[13]. Blood samples were also collected from each animal into labelled EDTA bottles for haematology. After 2 weeks, the animals were weighed; average weight was 10 kg. Average age was 12 months as determined by dentition. 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 jugular 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 and Leucocyte differential counts were also determined [14,15].

Animal Grouping and Treatment: The sheep were divided into 4 groups with 10 animals per group: A, B, C and D. Group A received no medication; Group B was treated with mebendazole. Group C had the extract administered at 300mg per day as feed additives [16], while Group D were drenched with the aqueous crude extract of C. papaya at 1:10 ml of water . 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 $P<0.05$ [17].

RESULTS AND DISCUSSION

Faecal Egg Counts: The study showed that all the animals were naturally and heavily infested with worms, ranging from *Haemonchus contortus,Trichostrogylus spp, Strongyloides and Ostertagia spp* [18,19] . Administration of drug and *Carica papaya* powder and aqueous extracts produced a significant reduction in the worm burden and

Table 1: Faecal Egg Counts of Experimental Sheep during Trial

Haemonchus contortus(epg)	Groups	Baseline	1st 2 weeks	2nd 2 weeks
	A	2700 ± 0.06	2800 ± 0.4	2800 ± 0.5
	B	2100 ± 0.7	-	-
	C	2300 ± 0.3	500 ± 0.5	200 ± 0.3
	D	2600 ± 0.3	-	-
Trichostrongylus spp(epg)	Groups	Baseline	1st 2 weeks	2nd 2 weeks
	A	2400 ± 0.4	2500 ± 0.6	2500 ± 0.2
	B	2200 ± 0.3	-	-
	C	2250 ± 0.3	600 ± 0.5	400 ± 0.4
	D	2350 ± 0.5	-	-
Strongyloides spp (epg)	Groups	Baseline	1st 2 weeks	2nd 2 weeks
	A	1600 ± 0.7	1800 ± 0.5	1800 ± 0.4
	B	1400 ± 0.5	-	-
	C	1400 ± 0.4	200 ± 0.5	-
	D	1500 ± 0.2	-	-
Ostertagia spp(epg)	Groups	Baseline	1st 2 weeks	2nd 2 weeks
	A	2100 ± 0.5	900 ± 0.4	2200 ± 0.3
	B	1500 ± 0.6	-	-
	C	1700 ± 0.5	600 ± 0.2	-
	D	1700 ± 0.2	-	-

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

Table 2: Haematological Parameter of Sheep during Trial

PCV (%)	Groups	Baseline	1st 2 weeks	2nd 2 weeks
	A	19.00 ± 0.6	20.00 ± 0.5	20.00 ± 0.4
	B	24.50 ± 0.8	28.50 ± 1.2	29.50 ± 1.1
	C	23.00 ± 0.6	27.00 ± 1.4	28.00 ± 1.1
	D	24.00 ± 1.2	29.50 ±1.4	30.00 ± 1.5
HB(g/dl)	Groups	Baseline	1st 2 weeks	2nd 2 weeks
	A	6.6 ± 0.2	6.8 ± 0.4	6.8 ± 0.7
	B	8.3 ± 0.4	9.5 ± 0.2	9.6 ± 0.4
	C	7.4 ± 0.3	9.0 ± 0.3	9.2 ± 0.7
	D	7.5 ± 0.4	9.7 ± 0.2	10.1 ± 0.3
RBCx10^6/mm^3	Groups	Baseline	1st 2 weeks	2nd 2 weeks
	A	6.7 ± 0.1	6.9 ± 0.2	6.9 ± 0.4
	B	8.2 ± 0.5	9.4 ± 0.5	9.5 ± 0.2
	C	7.2 ± 0.3	9.2 ± 0.2	9.3 ± 0.4
	D	7.3 ± 0.3	9.5 ± 0.2	9.6 ± 0.4
Lymphocyte (%)	Groups	Baseline	1st 2 weeks	2nd 2 weeks
	A	52 ± 0.3	48 ± 0.2	46 ± 0.4
	B	52 ± 0.5	56 ± 0.1	60 ± 0.1
	C	50 ± 0.5	54 ± 0.1	58 ± 0.2
	D	51 ± 0.3	57 ± 0.2	62 ± 0.3
Eosinophils (%)	Groups	Baseline	1st 2 weeks	2nd 2 weeks
	A	2.1 ± 0.3	2.2 ± 0.4	2.2 ± 0.2
	B	2.0 ± 0.3	1.8 ± 0.2	0.0
	C	2.2 ± 0.2	1.8 ± 0.3	1.5 ± 0.2
	D	2.2 ± 0.2	1.0 ± 0.2	0.0

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

by the second administration, Groups B and D animals were effectively dewormed, but Group C animals 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 ($P<0.001$) (Table 1).

Haematological Reports: Haematological results showed that before treatment, the PCV mean values for animals in Groups A, B, C and D were 19.00 ± 0.6, 24.50 ± 0.8, 23.00 ± 0.6 and 24.00 ± 1.2. After treatment, this parameter showed a significant increase. For haemoglobin, pre-treatment values for Groups A, B, C and D were 6.6 ± 0.2, 8.3 ±0.4, 7.4 ± 0.3 and 7.5 ± 0.4, 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 6.7 ± 0.1, 8.2 ± 0.5, 7..2 ± 0.3 and 7.8 ± 0.3, respectively. Like the other 2 parameters, RBC also showed a significant increase after treatment. Lymphocyte values showed increasing levels while eosinophil values declined with drug and powder and aqueous extracts administration. ANOVA tests showed that when all the haematological parameters were compared between groups and within groups, the differences were significant at ($P<0.001$) (Table 2).

The powder and aqueous extracts administered in this study caused a significant reduction in the worm burden of the sheep. 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 powder and aqueous extracts. This observation may be due to the fact that animals may not pick entire doses applied in additive forms unlike oral doses that were drenched directly into the gastrointestinal tract where helminthes were located. Therefore, there is a direct contact of the aqueous extract with the helminths in the intestinal tract leading to total reaction on the parasites. 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 powdery and aqueous extracts of *C.papaya*.

The reduction of worm load observed with the powdery and aqueous extracts of *C.papaya* on animals in Groups C and D may be attributed to the presence of papain in seeds of *C.papaya*

Administration of the drug and extracts resulted in a remarkable improvement in the haematology of sheep 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 [20-22].

Reduction of worm load by the powder and aqueous extracts of *C.papaya* in this study is a positive and welcome development in our local helminths struggle, because the fruits and seeds of *C.papaya* 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 powder and aqueous 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.

In conclusion, the trials conducted so far have proved that the seeds of *Carica papaya* has reasonable pharmacotherapeutic properties against intestinal nematodes of sheep. It can therefore be recommended that further research be embarked on by the willing veterinary pharmaceutical industries to determine the toxicity level, in-depth study of mechanism of action, determination of lethal dose, therapeutic level and economic viability of the *Carica papaya* seeds as a de-worming agent in sheep and other ruminants before adoption for commercial use and production.

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