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Full Length Research Paper

Anthelmintic activity of the crude aqueous leaf extracts of *Anogeissus leiocarpus* in sheep

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The safety and anthelmintic activity of the crude aqueous leaf extract of *Anogeissus leiocarpus* was investigated in sheep naturally infected with gastrointestinal nematodiasis using the feacal egg count reduction test and the controlled test. The results indicate that the extract had no significant (P>0.05) effect on the vital parameters such as temperature, respiratory and pulse rates nor did it produce any sign of toxicity in the treated animals. There were also no significant changes (P>0.05) in the body weight and haematological parameters of the albendazole or extract treated groups except the packed cell volume (PCV) of albendazole treated group and the white blood cells of the 400 mg/kg treated group and untreated group seven days post-treatment, which were significantly higher (P<0.05). In the feacal egg count reduction test, the extract produced dose-dependent reduction in the feacal egg count of the treated groups when compared to the untreated control. The result of the controlled test revealed that there was reduction in the number of worms recovered from the gastrointestinal tract of sheep treated with 400 mg/kg of the extract for three consecutively days than in the untreated control. It is, therefore, concluded that the crude aqueous leaf extract of *A. leiocarpus* could be tolerated by sheep and produced limited dose-dependent anthelmintic activity *in vivo*.

Key words: Anthelmintic, leaf, extracts*, Anogeissus leiocarpus,* sheep.

INTRODUCTION

Helminths are recognized as a major constraint to livestock production throughout the tropics and elsewhere (Adejimi and Harrison, 1997). The economic impact of parasitic gastroenteritis (PGE), which is caused by mixed infection with several species of stomach and intestinal round worms, as a production disease in ruminants lies not only in direct losses such as mortality associated with the clinical form of the disease but also indirect insidious losses as a result of weaknesses, loss of appetite, decreased feed efficiency, reduced weight gain and decreased productivity (Gibbs, 1986). Winrock International (1992) indicated that over $4 billion is lost in animal productivity as a result of animal diseases, with over half of this loss due to internal parasites such as helminthes. In an effort to reduce losses due to effects of helminth parasites on livestock industry, approximately $1.7 billion are spent annually worldwide in control measures (Lanusse and Princhard, 1993).

In Nigeria as in many tropical countries, control of helminthosis is mainly by periodic anthelmintic medication. However, as these are expensive and often unavailable to farmers in rural areas, livestock producers have continued to use indigenous plants as dewormers drawing upon centuries of knowledge of herbal medicines. Furthermore, some serious disadvantages of using manufactured drugs have become evident in the western world, such as resistance, food residues and environmental pollution (Dano and Bogh, 2003).

Anogeissus leiocarpus is a tree which is abundant in Nigeria and has found a wide use among Nigerian herba-

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lists (Ibrahim et al., 1997) and livestock farmers (Ibrahim, 1984; Onyeyili, 2000; Agaie, 2004). Aqueous extract of the root of the plant was reported to have anthelmintic activity against *Nippostrongylus braziliensis* in rats. Agaie (2004) also reported that the crude leaf extract of the plant prevented nematode egg hatch *in vitro*. The present study investigated the *in vivo* anthelmintic efficacy of *A. leiocarpus* crude leaf extract against clinical gastrointestinal nematodiasis in sheep.

MATERIALS AND METHODS

Plant material

The plant material was collected from Gidan Massalaci, Dange-Shuni local government of Sokoto State. The plant was identified and authenticated by Hajiya Rabi Ibrahim and Mal Mohammed Awwal of Department of Traditional Medicine and Botany, Usmanu Danfodiyo University, Sokoto respectively. The fresh material was collected, air dried, pounded and pulverized in a mortar, then sieved to a fine powder, which was kept in cellophane bags at 4^{o}C until used.

Extract preparation

100 g of the powdered leaf of the plant material was mixed with 2 l of distilled water in a 2 l beaker and boiled for 1.5 h. It was allowed to cool and then filtered through cheesecloth and a pack of cotton wool respectively. The filtrate was subsequently filtered using What man filter paper No.1 into a beaker. This was then concentrated by evaporation using a hot plate maintained at 60^{o}C and the extract stored in refrigerator until required. The extract yield was 10% (w/w).

Animals

Thirty sheep of both sexes of Ouda breed, weighing 15 – 39 kg and 6 months - 3 years old purchased from Sokoto Central Market were used. The animals were quarantined for two weeks and during the period they were screened and treated for haemoparasites, ectoparasites and bacterial infections using oxytetracycline L.A. (Vetinda Pharmaceuticals Limited, Gollapadu, India) and diazintol (Alfasan International, Holland).

Anthelmintic activity

The animals were divided into 6 groups of 5 animals each, placed on semi-intensive system of management and screened for helmintic (Stronglye) infection (MAFF, 1986) until a minimum parasite load (300 eggs per gram (epg) feaces for individual animal and 900 epg feaces was attained as group mean). The animals were then fed wheat bran and hay while water was provided *ad libtum*.

Animals in groups 1, 2 and 3 were given single graded doses (100, 200 and 400 mg/kg) of the extract orally while those in group 4 and 5 were given 10 mg/kg of albendazole and 0.5 ml/kg of physiological saline, respectively, through the same route. The animals in group 6 were treated with 400 mg/kg of the extract for 3 consecutive days. All the animals were assessed twice daily for signs of toxicity and general tolerability according to the method of Abdu et al. (2000). Physiological parameters such as temperature, respiratory and pulse rates were also taken before and 24 h following extract, saline or drug administration.

Faecal and blood samples were collected from all the animals on days 0 and 7 after extract, saline or drug administration from the rectum and jugular vein, respectively.

Packed cell volume (PCV), haemoglobin concentration (Hb), red blood count (RBC), white blood cell (WBC) and differential leucocytes counts were determined as described by Coles (1986) and body weight of the animals were also evaluated on day 0 and 7 using bathroom scale (Hana, Br. 9011). For parasite identification, salt floatation technique was used and parasite load was determined by MC Master technique (MAFF, 1986) while percentage egg reduction was calculated using the formula; ERC (%) = [(pre-post)/Pre] x 100 where pre is average pre-treatment (Epg) and post is average post-treatment (Epg).

Controlled test

8 days post extract, saline or drug administration, two animals from groups 5 and 6 were sacrificed and the gastrointestinal tract (GIT) examined for presence of worms as described by (MAFF, 1986). The percentage efficacy of the extract was then determined according to the method of Wood et al. (1995); % Efficacy = [(N-n)/N] x 100 where N is average number of worms found in controlled animals and n the numbers of worms in extract treated animals.

Statistical analysis

Test of significance between the mean parameters were performed using the t-test (Steel and Torrie, 1980).

RESULTS AND DISCUSSION

There were no signs of toxicity (Salivation, diarrhoea, skin reactions, etc) 24 h post extract administration in all the groups. Similarly the vital physiological parameters (temperature, respiratory and pulse rates) pre and 24 h post treatment showed no significant ($P>0.05$) changes (Table 1). There were also no significance changes ($P>0.05$) in the body weight of sheep treated with the extract or albendazole one week post treatment (Table 2). The extract produced dose-dependent reduction in the feacal egg counts of the treated groups. Treatment with 200 and 400 mg/kg produced 15.2 and 20.5% reduction in egg count while consecutive administration of 400 mg/kg for 3 days produced 39.5% reduction when compared to the untreated control. The group treated with 10 mg/kg of single dose albendazole showed 98.4% reducetion in FECR (Table 3).

Haematological parameters were not significantly ($P>0.05$) affected in both the treated and untreated groups except that there were significantly higher PCV in albendazole treated group 33.8 ± 3.0 7 days post treatment and 29.8 ± 4.57 at day O). The WBC counts in the sheep that received single dose 400 mg/kg and the untreated control were also significantly higher on day 7 when compared to day O (Tables 4 and 5). The result of the controlled test (Table 6) indicates that there was reduction in the number of worms recovered from the GIT of sheep that were treated with 400 mg/kg of the extract for 3 consecutive days when compared to the control. The overall efficacy of the treatment was 33% (Table 6).

Table 1. Vital clinical parameters of sheep treated with various doses of the leaf extract of *A. leiocarpus*

Extract treatment (mg/kg)	0 h			24 h		
	Temp (^oC)	Pulse rate	Resp. rate	Temp (^oC)	Pulse rate	Resp. rate
100	39.6±0.5	101±12	28±2	39.8±0.8	101±0	28±1
200	39.4±0.8	96±14	29±3	39.2±0.7	96±12	28±2
400 (Single dose)	39.4±0.8	97±13	28±2	39.4±0.8	97±13	28±2
400 (3 consecutive days	39.1±0.7	93±12	29±3	39.1±0.6	96±13	28±2
Control (untreated)	39.2±1.1	99±18	27±1	39.3±0.9	97±18	27±1
Albendazole (10 mg/kg)	39.3±0.5	99±18	27±1	39.1±0.4	97±18	27±1

Values are expressed as mean ± SD based on 5 observations.

Table 2. Mean body weight of sheep naturally infected with gastrointestinal nematodes and treated with graded doses of the leaf extract of *A. leiocarpus*

Extract/Drug treatment (mg/kg)	Body weight	
	Day 0	Day 7
Extract (100) (Single dose)	25.2	25.2
Extract (200) (Single dose)	23.8	23.6
Extract (400) (Single dose)	27.4	27.2
Extract (400) (3 consecutive days)	25.2	25.7
Untreated (Control)	30	28.4
Albendazole (10)	32.6	34.8

Table 3. The effect of leaf extract of *A. leiocarpus* on strongyle egg counts in naturally infected sheep.

Treatment (mg/kg)	Days post treatment				Egg Reduction (%)
	Day 0		Day 7		
	Mean e.p.g.	Range	Mean e.p.g	Range	
Extract (100) (Single dose)	900±666	300-900	1410±560	700-2000	-56.7
Extract (200) (Single dose)	1490±532	900-2100	1270±2950	800-1600	15.2
Extract (400) (Single dose)	244±1753	900-5400	1940±1126	300-3800	20.5
Extract (400) for 3 consecutive days)	1240±728	450-2000	760±370	200-1100	39.5
Untreated (Control)	1070±367	550-1500	3639±1961	1926-6400	-240
Albendazole (10)	1260±709	500-2400	20±45	0-100	98.4

Values for e.p.g are expressed as mean ± SD of 5 observations.

The results of the toxicity and tolerability test showed that the extract is safe and can be tolerated by sheep at doses used in this study. Nematodes have been reported to cause severe damages to the gastrointestinal tract GIT (Chiejina, 1987; Ikeme, 1997), the host therefore is forced to expend energy and protein repairing damages caused by parasites than for growth (Ketzis, 2000). This might be responsible for lack of significant changes in weight despite ant helmintic therapy.

Consecutive administration of the highest dose of the extract (400 mg/kg) produce only 40% FECR while the graded single doses were either not effective or did not produce substantial effect against strongylids worms. This suggests that the extract at doses used in this study is ineffective for curative anthelmintic therapy. Wood et al. (1995) reported that any anthelmintic product that reduces FEC by less than 80% during FECRT trial should be considered insufficiently active as a curative agent. However, a reduction of up to 40% could help reduce pasture contamination, which is an important aspect of helminth control in ruminant.

Although there were slight variation in the haematological parameters (PCV, HB, RBC) of all the groups between day O and 7, all the parameters were within the normal range reported by Gyang (1990). Significant changes observed in the PCV of albendazole treated group could

Table 4. Effects of leaf Extract of *A. leiocarpus* on the haematological values of sheep naturally infected with gastrointestinal nematodes.

Extract treatment (mg/kg)	PCV (%)		Hb (g/dl)		RBC (X10^9/ml)	
	Day 0	Day 7	Day 0	Day 7	Day 0	Day 7
100 (Single dose)	29±3.2	28.4±2.7	8.6±1.9	8.4±1.9	7.2±1.6	6.6±1.7
200 (Single dose)	28.8±5.9	29.5±6.0	10.6±1.8	10±1.2	8.1±1.2	8.3±0.5
400 (Single dose)	31±5.1	29.5±4.7	11.1±2.1	11±2.0	3.1±1.5	7.8±1.5
400 (3 consecutive days)	25±1.4	24.8±3.6	9.4±0.7	8.2±1.1	10.1±2.5	10.4±1
Control (untreated)	25.6±4.3	24±5.2	7.8±1.7	10±1.7	7.2±1.1	6.3±0.7
Albendazole	29.8±4.5	33.8±3.0*	8.8±1.2	11.7±1.8	7.7±1.6	8.1±2.1

* = P<0.05
Values are expressed as mean ± SD of 5 observations.

Table 5. The effects of leaf extract of *A. leiocarpus* on WBC and differential counts of sheep naturally infected with gastrointestinal nematodes.

Extract treatment (mg/kg)	WBC		Neutrophil		Lymphocytes		Eosinop	
	Day 0	Day 7	Day 0	Day 7	Day 0	Day 7	Day 0	Day 7
100 (Single dose)	13.2±3.0	14.4±2.6	46.6±8.2	46.4±11.5	45.6±8.7	44.8±14.0	3.6±2.1	4.2±2.2
200 (Single dose)	13.1±3.5	14.8±2.5	46±12.3	39±13.1	44.5±13.7	48.8±12.5	4.0±1.8	3.8±1.9
400 (Single dose)	11.7±2.1	17.1±1.6*	31.3±10.2	33.8±9.2	62.5±7.6	56.5±6.5	2.0±1.4	2.8±1.7
400 (3 consecutive days)	12.2±1.6	10.9±2.6	60.3±5.3	30.8±1.2	35.8±6.7	58.5±1.4	2.3±1.5	1.3±1.9
Control (Untreated)	13.3±1.8	17.5±2.0*	28±4.0	28±7.5	60.6±5.6	61.2±4.3	4.0±1.4	5.2±1.1
Albendazole (10 mg/kg)	12.0±2.3	10.8±1.7	40±3.6	47.6±1.8	52±3.9	48.2±10.8	2.6±1.5	1.6±1.9

* = P<0.05
Values are expressed as mean ± SD of 5 observations.

Table 6. Mean worm recovery from the gastrointestinal tract of sheep naturally infected with Strongyle and treated with water extract of *A. leiocarpus*.

GIT Segments	Parasite recovery		% Efficacy
	Treated	Control	
Abomasums	1020.3±251.7	1350±353.6	31.4
Small intestine	720±164.4	1075±213.7	33.0
Large intestine	781.8±213.6	1042.9±376.3	25.0
Total recovery	3141.8	4712.1	33.3

be attributed to reduction in blood loss due to parasite inhibition or clearance, which signifies recovery from helminthosis and improvement in health status (Lasisi et al., 2001; Onyeyili, 2001). The WBC and differential counts showed no significant changes except in the control (untreated group) and the group that was treated with single dose 400 mg/kg. Leucocytosis has been reported to be one of the haematological features of helminthosis in ruminants (Coles, 1986).

The overall efficacy of the controlled test was 33% for the 400 mg/kg treated group after 3 days of consecutive treatment. This is an indication that the extract is either not sufficiently active against adult parasites or the dose was inadequate. This is beside pharmacologic factors as earlier observed by Baggot and Mckeller (1994), Ladage et al. (1989) and Prichard (1985).

In conclusion, this study has shown that the aqueous crude leaf extract of *A. leiocarpus* could be tolerated by sheep and produced limited dose-dependent anthelmintic activity.

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