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***In vivo* Efficacy of *Vernonia amygdalina* (Compositae) Against Natural Helminth Infection in Bunaji (*Bos indicus*) Calves**

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ABSTRACT

Fifteen Bunaji calves (*Bos indicus*) averaging 105±12.5 Kg liveweight and approximately nine months of age with natural helminth infection were distributed into three treatment groups of five animals each. Animals were either treated orally with aqueous extract of *Vernonia amygdalina* at a dose concentration of 1.1g/Kg body weight, a conventional anthelmintic or left untreated. *V. amygdalina* treatment produced 59.5% reduction in eggs per gram (EPG) of faeces which was significantly different ($P<0.001$) from the untreated control (-17.24%), whereas levamisole hydrochloride treatment produced 100% reduction in EPG. A total of six genera of helminths were recovered from the gastrointestinal tracts and liver of experimental animals. These were *Haemonchus contortus*, *Trichostrongylus* spp., *Bunostomum* spp., *Oesophagostomum* spp., *Fasciola* spp and *Dicrocoelium* spp. There was significant difference ($P<0.001$) in worm load between the different treatment groups. Except for *Haemonchus* spp, animals in the untreated group had significantly ($P<0.001$) higher worm load for all the genera of helminth recovered than those of the *V. amygdalina* treated group, indicating that *V. amygdalina* had no effect on *Haemonchus contortus*.

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INTRODUCTION

Helminthosis has long been recognized and still remains a problem responsible for losses in ruminant production in almost all regions of the world. Parasitic nematodes causing mortality, severe weight losses, low milk output and reproductive failure have been identified as the major cause of production losses of resource-poor livestock farmers in tropical Africa and South Eastern Asia (Alawa *et al.*, 2001; Alawa *et al.*, 2002; Bizimenyera *et al.*, 2008). The control of helminthosis has largely been limited to the use of chemotherapeutic agents, which have higher efficacy and higher safety Alawa *et al.* (2008). However, these chemotherapeutic agents are increasingly getting out of the reach of the resource-poor livestock farmers due to high costs. Anthelmintic resistance is now a serious problem in livestock management (Waller *et al.*, 1996). While research into new synthetic drug is continuous, the high cost will remain a problem and will

likely not make the drugs affordable to most farmers in Third World countries. Newer and more advanced methods of parasitic control such as the use of fungi and vaccines even if available may not be economically viable for these farmers. The search for alternative methods of endoparasite control by smallholder farmers has therefore been driven largely by economics. Pastoralists and other smallholder livestock owners have relied and continue to rely on locally available plants for treatment of their animals.

Vernonia amygdalina (Compositae), commonly referred to as bitter leaf, is native to Nigeria. This plant grows over a range of ecological zones in Africa. This plant is widely used across Africa for food and medicinal purposes. In traditional medicine, the roots and twigs are used for abdominal and other gastrointestinal problems in humans (Watt and Breyer-Brandwijk, 1962) while the decoction from the leaves are used as antimalarial in Guinea and as cough remedy in Ghana (Akinpelu, 1999;

Masaba, 2000). *Vernonia amygdalina* is also widely described by livestock farmers as a choice antihelmintic (Alawa *et al.*, 2008).

Improving ruminant production through control of helminthosis can contribute significantly towards solving the problem of marginal or resource-poor farmers, thereby helping to alleviate poverty and consequently enhancing livelihood (Sujon *et al.*, 2008). We therefore set out to validate farmer's response about the use of *V. amygdalina* as an anthelmintic in calves in an earlier report (Alawa *et al.*, 2008) following exactly the procedure described by the farmers.

MATERIALS AND METHODS

Preparation of plant extracts

Fresh leaves of *V. amygdalina* were obtained from farms around Zaria (Longitude 8° 31' 0" and Latitude 11° 59' 47"), Nigeria and identified by taxonomist of the Department of Biological Sciences, Ahmadu Bello University, Zaria, Nigeria. The voucher specimen No. 900675 is deposited in the herbarium of the Department of Biological Sciences, Ahmadu Bello University, Zaria, Nigeria. Fresh leaves (120g) were crushed, mixed with 100mL of water and sieved to obtain the drenching material for each animal. The method and dosage was based on farmers practice (Alawa *et al.*, 2008).

Experimental animals

Fifteen Bunaji calves averaging 105±12.5Kg liveweight and approximately nine months of age with natural helminth infection were distributed into three treatment groups of five animals each by blocking based on live weight and fecal egg count taken prior to commencement of trial. All animals were housed on concrete-floored pens and fed cured, cut and carry forage supplemented with concentrate 500g (cotton seed cakes and maize offal). Mineral lick and water was given *ad libitum*.

Treatment protocol/clinical trials

Animals in group 1 were treated with *V. amygdalina* 1.1g/Kg body weight, group 2 animals were left untreated (untreated control) and group 3 animals were treated with Levamisol hydrochloride. The plant extract was drenched with a stomach tube to group 1 animals once daily (morning) for six consecutive days, while group 3 animals were drenched with Levamisol hydrochloride once at the commencement of the trial.

Animals in all treated groups were closely observed for signs of toxicity or otherwise following treatment for 12 days. No adverse effects were physically observable throughout the duration of treatment.

Fecal egg count

Fecal samples were collected directly from the rectum of all animals on days 0, 2, 4, 6, 8, 10, and 12 and mixed with water thoroughly until a relatively liquid suspension was obtained. This was then washed through a 100-mesh (150µm pore size) sieve and then through another 400-mesh sieve (38µm pore size) with warm water. The material left on the sieve was back-washed and transferred into a 50mL tube (10mL fecal material/tube),

the tube was filled with water. Tubes were centrifuged for 5 minutes at 3000rpm, decanted, and the sediments transferred to another set of tubes. A 5 ml volume of magnesium sulphate (1.2 specific gravity) was added to each tube, and using two applicator sticks on vortex mixer, this was mixed until a suspension was achieved. The tubes were then filled with magnesium sulphate solution and centrifuged for 5min at 3000rpm, decanted, through a 400-mesh sieve, and the retained eggs were transferred into a 50mL tube. The number of eggs in the tube was determined using McMaster method (Sloss *et al.*, 1994).

Necropsy

Three animals per treatment group were slaughtered on day 12 post-treatment for total worm count. The gastrointestinal tract, liver and lungs were observed for presence of helminths. Also, the abomasal contents were collected and the walls of the abomasums washed with water. These washings and the contents of the large bowel were combined and washed through sieves of appropriate aperture for worm counts according to the procedure of MAFF (1986). The worms were identified based on their predilection site in the gastrointestinal tract and morphological characteristics such as size, presence or absence of sheath, length of buccal capsule, structure of tail, position of the genital primordium (Bowman, 2009). Percent reduction in fecal egg count was computed by the following formula:

$$\% \text{ Reduction} = \frac{\text{MeanEPG on day 0} - \text{meanEPG on day 12}}{\text{MeanEPG on day 0}} \times 100$$

Percent efficacy

Percent efficacy of *V. amygdalina* and Levamisol hydrochloride® were calculated as follow (Arundel, 1985).

$$\% \text{ Efficacy} = \frac{N - n}{N} \times 100, \text{ where}$$

N= Mean number of helminths in control (untreated) animals

n = Mean number of helminths in treated animals.

Statistical analysis

The daily egg count of each animal was transformed, by replacing the actual count x, by log₁₀ (x+1). The major effect of the transformation was to stabilize the variance and compensate for the zero counts (Snedecor and Cochran, 1969). The differences between means of the transformed egg counts for the different groups were examined vide analysis of variance with General linear model (GLM) of the SAS statistical package (SAS, 1987).

RESULTS

The mean eggs per gram of feces (EPG) of the animals of three groups pre- and post-treatment are presented in Table 1. There were significant differences (P<0.001) in the EPG between the treatments. At the end of the study, Untreated animals had significantly (P<0.001) higher overall mean egg counts than the *V. amygdalina*-treated animals. The *V. amygdalina* treated

calves had significantly ($P < 0.001$) higher egg counts than the Levamisol treated calves. The percent reduction in EPG is also presented on Table 1. *V. amygdalina* treatment produced 59.50% reduction in EPG which was significantly different ($P < 0.001$) from the untreated control group which showed 17.24% increase in EPG compared to the value before treatment. Levamisol hydrochloride treatment resulted in 100% reduction in EPG.

The mean worm load of the animals in the three groups is presented in Table 2. A total of six species of helminths were recovered from the gastrointestinal tracts of experimental animals. These were *Haemonchus spp*, *Trichostrongylus spp*, *Bunostomum spp*, *Oesophagostomum spp*, *Fasciola spp* and *Dicrocoelium spp*. There was significant difference ($P < 0.001$) in worm load between the different treatment groups. Except for *Haemonchus spp*, animals in the untreated group had significantly ($P < 0.001$) higher worm load for all the species of helminth recovered than the *V. amygdalina* treated group indicating that *V. amygdalina* had no effect on *Haemonchus spp*. No parasites were seen in the faces of calves treated with levamisol.

DISCUSSION

This trial concentrated on evaluating *V. amygdalina* for the treatment of natural helminth infection using the method of treatment described by livestock farmers in the area. The dosage and extraction method (water) were based on farmers practices. However, water extraction naturally set some limitations to the type and amount of compounds that can be extracted due to its polarity (Eloff, 1998).

The results of this trial demonstrate that *V. amygdalina* has some therapeutic activity against some nematodes (*Trichostrongylus axei*, and *Oesophagostomum spp*) and trematodes (*Fasciola* and *Dicrocoelium*) *V. amygdalina* efficacy against species like *Trichostrongylus colubriformis* and *Oesophagostomum* can be described as being poor, while it can be said to be very good against *Dicrocoelium* and *Fasciola spp*. This finding agrees with the report of Toyang *et al.* (1995) that *V. amygdalina* is

widely used as a broad spectrum anthelmintic by farmers in the Republic of Cameroun. The observed inactivity of *V. amygdalina* against *Haemonchus contortus* agrees with an earlier study where the plant was found to be ineffective against *Haemonchus contortus* in an *in vitro* egg hatch and fecal culture assay (Alawa *et al.*, 2003). It is also possible that the dose rate used in this study was as described by farmers and it did not undergo any prior dose determination studies and may not have provided sufficient knock-down effect.

The poor anthelmintic property of this plant extract against *Haemonchus* in this study is a disadvantage to its potential use as an anthelmintic, since Haemonchosis is a disease of economic importance in small ruminants. Several survey reports on gastrointestinal parasitism in Nigeria indicate a high incidence of *Haemonchus* infection (Onwuliri *et al.*, 1993; Nwosu *et al.*, 1996). However, confirmation of effectiveness of *V. amygdalina* against other nematodes observed in this work needs further studies.

According to Ketzis (1999), traditional healers and farmers often do not distinguish between different types of parasitic infections and usually assume that a particular treatment is effective against all parasites, while in reality such treatment may only be effective against selective species. Resource-poor farmers will continue to rely on herbal plants for animal health problems and so the search for potential plants as sources of newer and cheaper anthelmintics will continue.

The results of this study indicate that the crude extract of *V. amygdalina* has some anthelmintic activity against some nematodes. However, more studies such as dose dependent trials, purification and identification of the active principle should be carried out before a conclusion can be made as to the validity of this plant being a broad spectrum anthelmintic.

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Table 1: Mean fecal EPG of *V. Amygdalina* treated, untreated control and Levamisol treated Bunaji calves

Days post treatment	<i>V. amygdalina</i> treated (Group 1)	Untreated control (Group 2)	Levamisol treated (Group 3)
0	2828 ± 1121	1276 ± 802	296 ± 137
2	2320 ± 704	1780 ± 807	20 ± 15.5
4	2992 ± 980	1860 ± 1135	8.00 ± 8.00
6	1248 ± 373	1700 ± 990	8.00 ± 8.00
8	1332 ± 546	2004 ± 1011	84 ± 46.4
10	1204 ± 427	1824 ± 741	16.00 ± 9.80
12	1144 ± 512	1496 ± 668	0.00 ± 0.00
% Reduction in EPG	59.50%	-17.24* %	100%
Mean EPG	1463 ± 666b	1706 ± 879a	61.71 ± 32.1c

* There was an increase in egg count over day 0 values; EPG – Eggs per gram of faeces; A,b,c = value differ significantly from one another ($P < 0.001$).

Table 2: Mean worm load recovered from *V. amygdalina* treated, untreated control and levamisol hydrochloride treated Bunaji calves

Helminth	<i>V. amygdalina</i> treated (Group 1)	Untreated Control (Group 2)	Levamisol treated (Group 3)
<i>Haemonchus contortus</i>	1567 ± 145a	1267 ± 410a	0.00 ± 0.00
<i>Trichostrongylus axei</i>	300 ± 252b	1100 ± 361a	0.00 ± 0.00
<i>Oesophagostomum</i>	33.3 ± 233.3b	100 ± 57.7a	0.00 ± 0.00
<i>Bunostomum</i>	0.00 ± 0.00b	66.7 ± 66.7a	0.00 ± 0.00
<i>T. colubriformis</i>	600 ± 458b	1067 ± 481a	0.00 ± 0.00
<i>Dicrocoelium</i>	0.00 ± 0.00b	2 ± 21a	0.00 ± 0.00
<i>Fasciola</i>	0.00 ± 0.00b	3.00 ± 3.00a	0.00 ± 0.00

A, b = Values with different letters within a row differ significantly (P<0.001).

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