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In vivo EFFECTS OF FOUR MEDICINAL PLANTS ON NEMATODES OF GOAT

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AUTHORS' CONTRIBUTIONS

This work was carried out in collaboration between all authors. Authors AOA and IBO designed the study, wrote the protocol and interpreted the data. Authors AOA and IBO anchored the field study, gathered the initial data and performed preliminary data analysis. Author AOA managed the literature searches and produced the initial draft. All authors read and approved the final manuscript.

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ABSTRACT

Medicinal plants are very cheap and accessible to rural people for treating ruminant's diseases. The aim of this study was to investigate *in vivo* effects of four medicinal plants (*Vernonia amgydalina, Ocimum grattisimum, Nicotiana tabacum and Talinum triangulare*) on nematodes of goat. The ethanolic extracts of these medicinal plants were made and the dose used for the goats include 1 and 2 g/kg for *Vernonia amgydalina*, 1 and 2g/kg for *Ocimum grattisimum*, 0.5 and 1 g/kg for *Nicotiana tabacum* and 0.5 and 1 g/kg for *Talinum triangulare.* The goats were infected with L3 stage larval of nematodes of *Trichostrongyle sp, Haemonchus contortus, Bunostomum and Strongloides sp* and after the build up of worm after some days, they were treated using *in vivo* assay through oral administration of the plants extracts. The faecal samples were collected 3, 7 and 11 days and examined quantitatively through McMaster Counting technique for the number of parasites. The results showed that only 2g/kg of *Vernonia amgydalina* and 1g/kg of *Talinum triangulare* showed decreased of 91.6% and and 69% in the mean egg per gram (epg) output of the faecal sample. But other medicinal plants did not show any decrease in epg. This indicates that *Vernonia amgydalina* and *Talinum triangulare* could be used as antihelmintic plants to treat goat diseases thus helping to improve the health status of the goat.

Keywords: *Vernonia (Asterales); Ocimum (Lamiales); Nicotiana (Solanales); Talinum (Caryophyllales);* nematode; antihelmintic.

1. INTRODUCTION

Parasites such as nematodes had been found to cause great economic loss in ruminants through reduction in the production of milk and meat from ruminants. These parasites have been shown to have antihelmintic resistance to synthetic drugs, as a result another method of remedy has being by the use of medicinal plants that have medicinal value.

Medicinal plants such as *Vernonia amgydalina, Ocimum grattisimum, Nicotiana tabacum and Talinum triangulare* had been reported to have medicinal uses to treat ruminant diseases [1]. These medicinal plants have been shown to contain chemical constituents such as alkaloid, Tannin, Saponin and Flavonoid [2].

In vivo antihelmintic activity of *Vernonia amygdalina* on nematode in goat in Uganda has been studied [3].

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Also In vitro and *in vivo* antihelmintic activity of *Nicotiana tabacum* and *Ocimum gratisimum* on nematode of ruminants had been studied [4,5,6,7,8].

In vivo method has been found to have greater importance in examining some important problems such as antihelmintic resistance. It's importance can be employed in this study.

The aim of this study was to use *in vivo* method to investigate antihelmintic activity of four medicinal plants.

2. MATERIALS AND METHODS

2.1 Collection of Plants

Plant materials (*Vernonia amygdalina, Ocimum grattisimum, Nicotiana tabacum and Talinum triangulare*) were collected from around the farmer's house (Mr Akinyemi) that grows and sell the plants in Ado-Ekiti and dried for 2 months. They were authenticated by Mr Omotayo in the Department of Plant Science at Ekiti State University Ado-Ekiti. The Voucher number for the medicinal plants include: *Nicotiana tabacum*. L (Solanaceae)- UHAE. 2015/30, for *Ocimum gratissimum* L. – UHAE. 2015/31, for *Talinum triangulare.* Wild – UHAE.2015/32 and *Vernonia amygdalina*. Del-UHAE.2015/33 and they were preserved in Department of Plant Science at Ekiti State University Ado-Ekiti. The part of the plants used was leaf.

2.2 Extraction

The method of extraction used by [9] was used for this extraction. The leaves of the medicinal plants (*Vernonia amygdalina, Ocimum grattisimum, Nicotiana tabacum and Talinum triangulare*) after air dried for 2 months were grinded separately into powder using a blender. And they were put inside a separate labelled container. 500 g of *V. amygdalina* was soaked in 2.5 L of ethanol, 400 g *of O. grattisimum* was soaked in 2 L of ethanol, 250g of *T. Triangulare* was soaked in 1.250 L of ethanol and 500 g of *N. tabacum* was soaked in 2.5 L of ethanol. The ratio of weight of the plants to ethanol was 1:5 i.e 100 g of plant material: 500 ml of ethanol. They were soaked for 72 hrs. After which it was filtered using muscline cloth. The filtrate was left to stand for 11 days for the ethanol to evaporate to remain the extract of the plants.

2.3 Culturing and Harvesting

Culturing and harvesting method used was similar to the method used by [10]. The faecal samples were collected from free range goats around Ado-Ekiti. They were now mixed with saw dust to allow aeration of the faecal sample and put inside 250 ml beaker, covered with paper foil and kept in the dark cupboard for 7 days.

2.4 Harvesting of Feacal Sample

After 7 days the faecal samples were put inside muscline cloth tied and use stick to hold it across it inside a beaker. Warm water was poured on it, to allow the parasite to flow from the faecal sample inside the cloth to the water and left for 45mins, to allow migration of L3 stage larval from the faecal sample into the water. After 45mins, the tied cloth was removed and the water containing the parasite was decanted to collect the parasite at the bottom of the beaker. And it was now observed under microscope to see the parasite third stage larva (L3 stage larval) present. 4000 larvae in 10 ml, 400 larvae in 1 ml and 40 larvae in 1 drop.

2.5 Animal Infection

Twenty seven small African goat (3-4 months old) of weight 3-6.4 kg of both 23 male and 4 female were purchased from local market at Mimiko Market in Akure. They were infected on the 10^{th} day of purchased with 10 ml of L3 larval solution containing about 4000 larvae and left for 7 days before the faecal sample was collected from the floored ground. Each collection was put in a container labelled with the number of the animal. The qualitative analysis of the samples was done through floatation method to determine the type of eggs and quantitative analysis was done to determine the quantity of eggs through McMater Counting Technique.

2.6 Floatation Methods

The method used by [3] for floatation method and McMaster counting technique was employed in this study. About 3 g of faeces collected from the animals were weighed in a 50 ml beaker and 30 ml of water was added. They were mixed thoroughly using a homogeniser at 2,000 rpm for 20 seconds. 57 ml of sugar solution was added. The faeces mixture was sieved through a tea strainer into sample bottle capped with a rubber bung and inverted ten times to mix. Pasteur pipette was used to quickly fill both sides of a McMaster counting Chamber. It was allowed to stand for 3 minute to allow the eggs to rise to the surface. The eggs contained at both sides within the ruled area were counted. The eggs counted were multiplied by 100 to get eggs per gram of the faecal sample.

2.7 *In vivo* Assay

The *in vivo* assay used was similar to the one used by [11]. Before the start of the experiment, the animals were checked and examined whether they have any disease. The animals were treated with 1 tablet of Albendazole and checked for the number of parasite present after 7 days. After which the animal were infected with third stage larva (L3 larval) solution of 10 ml containing 4000 larvae and left for 11 days to examine the faecal sample for the level of infection.

The animals were divided randomly into five groups each containing three animals and assigned different treatments as described below:

> Group 1 served as a negative control and received no treatment, Group 2 received single dose of *Vernonia amydalina* extract 2g/kg and 1g/kg, Group 3 received single dose of *Ocimum grattisimum* extract 2 g/kg and 1g/kg, Group 4 received single dose of *Talinum triangulare* extract 1g/kg and 0.5 g/kg while group 5 received single dose of *Nicotiana tabacum* 1 g/kg and 0.5 g/kg respectively.

Faecal samples from each animal were collected in the morning, starting from day 0 pre treatment and at days 3, 7 and 11 post treatments, the samples were evaluated for the type of worms by floatation method and quantified using McMaster technique.

> % reduction= C-T × 100/C
> C= Control epg at day 7 and day 11
> T= Treated epg at day 7 and 11days

2.8 Statistical Analysis

For *in vivo* assay of nematode T-test was used and siginificant at $P<0.05$ significant level.

3. RESULTS

3.1 *In vivo* Effects of Four Medicinal Plants on Goats Infected with Nematode

During *in vivo* study, the mean egg per gram (epg) output of goats treated with *Vernonia amygdalina, Ocimum grattisimum, Talinum triangulare and Nicotiana tabacum* ethanolic extract and untreated control were presented in Table 1. The mean epg trends of control group steadily rose from day-zero to throughout the experimental period. The percentage mean epg output reduction of 91.6% and 69% respectively by day 7 and day 11 for goat treated with 2 g/kg *Vernonia amgydalina* and 1 g/kg *Talinum triangulare*. Other plants did not show any reduction. *Vernonia amgydalina* treated animals at 2 g/kg at day 7 showed statistically significance difference in the egg per gram output when compared to negative control at $P<0.05$ significance level. *Talinum triangulare* treated animals at 1 g/kg showed statistically significance difference when compared to negative control by day 11 at $P<0.05$ significance level. Other animals treated with *Ocimum grattisimum and Nicotiana tabacum* did not showed significant difference rather than increase and death by day 12 at $P<0.05$ significance level (Table 1). It took *Talinum triangulare* (11 days) longer time to cause reduction compared to *Vernonia amgydalina* (7 days). The animals treated with *Nicotiana tabacum* were found to ran unconscious and died earlier. But other animals treated with other plants did not show any abnormal behavioural effect. Lower concentration for *Vernonia amgydalina and Talinum triangulare* were found to be non effective in the animals.

4. DISCUSSION

4.1 *In vivo* Effects of Four Medicinal Plants on Goats Infected with Nematode

In vivo study showed that *Vernonia amygdalina and Talinum triangulare* have antihelmintic effect on L3 stage larval of nematode, which was supported by the study of [12,13,14[discovered antihelmintic effect of *Vernonia amydalina in vivo*. The reduction in egg production on 7^{th} day could be due to death of parasites, while later picked up of epg in 11^{th} day, may be due to the maturation of the young stages of the parasites. This implies that the extracts were effective in mature parasites but not in young ones. In the absence of intervention with the plant extract the epg would have risen to show trends as the control group and possibly causing death. A repeat of the experiment after every 7 days would probably continue to control the build up of the worms, if the dosage was more than once. The antihelmintic effects shown by *Vernonia amygdalina* and *Talinum triangulare* could be due the presence of tannin in these plants [2,15,16] which have antihelmintic effect [17].

Lack antihelmintic effect of *Ocimum gratisimum and Nicotiana tabacum m*ight be due to the type of extract (ethanolic extract) used, which might not be effective *in vivo* but in vitro. As the studies from [5] used methanolic extract as opposed to the ethanolic extract used in this study for *Nicotiana tabacum.* Also [8] used essential oil for antihelmintic effects of *Ocimum grattisimum* in contrast to ethnaolic extract used in this study. The unconcious effects of *Nicotiana tabacum* on the treated animals could be due to the effects that it normally has on the brain and it's being

Table 1. Mean antihelminthic effect of 4 plants *in vivo*

Dose/plant	Day 0	Day 3	Day 7	Day 11
2g/kg Va	798±3.0	695±4.0	86.6±3.0*	900±0.0
1g/kg Va	293±8.0	600±0.0	4000±0.0	4648±3.0
2g/kg Og	100±0.0	693±8.0	1598±3.0	1700±0.0
1g/kg Og	100±0.0	693±8.0	Dead	Dead
1g/kg Tt	98±3.0	798±3.0	3100±0.0	283±0.7*
0.5g/kg Tt	98±3.0	695±4.0	800±0.0	995±4.0
1g/kg Nt	150±5.0	695±4.0	Dead	Dead
0.5g/kg Nt	100±0.0	698±3.0	Dead	Dead
Negative control	798±3.0	995±4.0	1198±3.0	1300±0.0

*N.B. * Significance difference from negative control at P<0.05 using t-test*

used as the stimulants. *Nicotiana tabacum* is a highly toxic plant due to the presence of nicotine alkaloid content. That affects the central nervous system and heart. This makes the animal treated with *Nicotiana tabacum* to ran unconscious and died earlier than other animals. But other animals treated with other plants did not show any abnormal behaviour because they did not contain high alkaloid which is very toxic that can make the other animals to behave abnormal. If high concentration of *Vernonia amgydalina and Talinum triangulare* are being used there is probability that there will be better effects on the animals. *Vernonia amygdalina and Talinum triangulare* would have cured the infection totally if the dosage used was more than once.

5. CONCLUSION

In conclusion, *Vernonia amgydalina and Talinum triangulare* could be recommended in the daily diet of ruminants, so as to daily expel worms and hence improve health status of the ruminants.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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