In Vivo Trial of *Combretum molle* Stem Bark Crude Extract as Anthelmintic in Sheep

DOI:10.36108/jvbs/8102.10.0140

Simon M.K.¹,², Michael I.A.², *Mohammed B.R.¹*, and Agbede R.I.S¹

¹Department of Parasitology and Entomology, Faculty of Veterinary Medicine, University of Abuja.

²Department of Infection and Host Defense, Graduate School of Medicine, University of Chiba, 8-1 Inohana, Chuo-ku, Chiba 260-8670, Japan.

³Department of Animal Production Technology, Federal College of Wildlife Management Technology, New Bussa, Niger State

**ABSTRACT**

Gastrointestinal nematodes remain a major constrain to productivity of livestock including sheep. Ethno veterinary practices using a wide variety of indigenous plants have been employed for the effective control of these parasites. However, little is known in the application *Combretum molle* bark in the treatment of Haemonchosis. This study was therefore conducted to determine anthelmintic efficacy of crude extract of *Combretum molle* against experimental Haemonchus contortus infection in sheep in vivo. Eighteen (18) helminth free lambs were randomly divided into three groups (A, B and C) of six animals each and were artificially infected with 10,000 larvae of *Haemonchus contortus*. The lambs were treated orally 7 days post infection. Here, Group A was treated with crude extract of *C. molle* at 200 mg /kg, B with Albendazole at 200 mg /kg and C with water at 5 ml/kg. Anthelmintic activity was assessed by comparing the number of eggs recovered from the treated lambs. Results revealed that the mean fecal eggs output per gram for groups A, B and C after treatments were 716.67, 96.67 and 12080 respectively. The depolarization produced by *C. molle* was significant (p< 0.05). The results of this study supported the ancient uses of some of the tested plants in the treatment of intestinal helminthoses. Hence, the active crude of *C. molle* could be a potential source of anthelmintic agent against *H. contortus* of sheep. Further pharmacological and toxicological studies are required to establish their use in the sheep production industry.

**Keywords**: Albendazole, *Combretum molle*, *Haemonchus contortus*, in vivo, Sheep

*C*Corresponding email: balarabemohammed161@yahoo.co.uk
Tel. +234(0)803 855 7168

*This Paper was accepted on 17th November, 2017 and published 24th April, 2018*
INTRODUCTION
In Nigeria, sheep are important sources of meat (mutton), milk and other by-products such as hide for domestic use and export [1]. However, these are threatened by gastrointestinal nematode infection, often considered a major impediment to small ruminant production [2]. Generally, parasitic helminths are major causes of substantial losses in small ruminant production resulting in significant economic losses in many developing countries including Nigeria [3]. Most notably, *Haemonchus contortus*, a blood sucking abomasal parasitic nematode causes a disease known as Haemonchosis in ruminants and is characterised by anaemia, anorexia, loss of condition, and eventual death of the host animal [4]. The disease is often established in the tropical and subtropical regions of the world [5]. The prevailing ecological conditions including humidity, temperature and rainfall in these regions predispose the growth and development of these parasites [6, 7]. Currently, Haemonchosis control strategies in small ruminants rely heavily on the repeated use of conventional anthelmintic chemotherapy [8]. The extensive and indiscriminate usage of these anthelmintics has emanated in the development of resistance [9].

In view of the aforementioned problems, the exploration of an effective anthelmintic from a reasonably inexpensive and available plant derived raw materials becomes highly apparent [10]. Previous studies revealed that plant derived anthelmintics can adequately curtail the intensity of parasite infestation in sheep and are auspicious alternatives to orthodox anthelmintics [11, 12, 13]. This therefore suggests that there is a tremendous prospect of exploring composite with anthelmintic efficacy from the indigenous plant materials. This study therefore was conducted to evaluate the anthelmintic efficacy of the stem bark of *Combretum molle* against Haemonchosis of sheep.

MATERIALS AND METHODS
Collection and preparation of plant samples
Fresh stem bark of *Combretum molle* was collected for 4 months within the premises of the Federal College of Wild Life Technology, New Bussa, Niger State and identified as described by [14]. The stem bark (Figures 1 & 2) collected was air dried and pulverized into powder using mortar and pestle (laboratory ceramic).

Combretum molle as an Anthelminthic in Sheep

Figure 2: An image showing the stem bark of Combretum molle (close range) in its natural habitat. Adapted from http://pza.sanbi.org/sites/default/files/images/plants/9948/combremolle4.jpg.

A stock solution of the crude extract was prepared by dissolving 500 g of the powdered plant material in 3 liters of water, allowed to stand overnight and filtered by maceration. The solution was concentrated by allowing it to stand for 3 hours. The supernatant was discarded and the concentrated layer dried into a solid by evaporation on a water bath at 60°C.

Study population
Eighteen (18) apparently healthy adult Yankasa Sheep of both sexes with an average age of 3-5 months were purchased at Wawa Market. The animals were housed in a well-ventilated, clean roofed building. All the animals were well fed and given water ad libitum. They were dewormed using Albendazole prior to experimental infestation with larvae of Haemonchus contortus.

Helminth parasites
Adult female Haemonchus contortus were harvested from the abomasal contents of the sheep slaughtered at the New Bussa abattoir and identified in Helminthology Laboratory of the Department of Parasitology and Entomology, Ahmadu Bello University, Zaria. The adult worms were crushed in culture material (vermiculite) to liberate the eggs and thereafter cultured in a bottle jar. The jar was left standing at room temperature in an incubator for 7 days. Larvae were harvested using the modified Baermann's techniques.

The parasite was crushed in mortar with pestle and about 60 ml water was added to the crushed worms and filtered in a 100 mesh sieve (100 X 150 µm). The assay was done using the modified McMaster method as described by [15]. The filtrate was placed in three 20 ml centrifuge tubes and centrifuged at 2000 g for 15 min. The supernatant was decanted and the volume of the sediment was adjusted to 20 ml in a graduated test tube. Using a hypodermic needle, 0.3 ml of the sediment was placed on a McMaster slide (Weber Scientific International, England®) and the eggs were counted under light microscope. The number of eggs per ml was estimated.

Experimental design
The sampled (18) sheep were randomly divided into groups A, B and C of 6 animals each. A total of 10,000 Haemonchus contortus L3 was used to infect each animal orally. The animals were screened daily for faecal egg output for one week from the day of infection.
Seven days post infection, Sheep in Group A was treated with crude extract of *Combretum molle* at 200 mg/kg. Group B was treated with Albendazole at 200 mg/kg whilst group C received water at 5 ml/kg as described by [16]. Twenty four (24) hours post treatment; faecal samples were collected from each animal daily for one week for faecal egg count using the modified McMaster method as described by [15].

**Data analysis**

Means of data obtained from the experimental treatment were analyzed using the software package for graph pad prism (version 4.0…2003). Statistical significance for the anthelminthic effect of crude extract and Albendazole was assessed by ANOVA. Subsequently, Borferroni’s multiple comparison tests was used with P value of < 0.05 considered as significant.

**RESULTS**

The mean faecal eggs output per gram for groups A, B and C before treatment are 9166.67, 9250 and 8950 respectively. The parasitism produced by the parasite in both groups was not significantly different (p<0.05) (Table I).

<table>
<thead>
<tr>
<th>S/N0.</th>
<th>GROUP A</th>
<th>GROUP B</th>
<th>GROUP C</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>9800</td>
<td>10000</td>
<td>10000</td>
</tr>
<tr>
<td>2</td>
<td>7500</td>
<td>9800</td>
<td>9800</td>
</tr>
<tr>
<td>3</td>
<td>10000</td>
<td>7500</td>
<td>9000</td>
</tr>
<tr>
<td>4</td>
<td>8700</td>
<td>10000</td>
<td>7600</td>
</tr>
<tr>
<td>5</td>
<td>10000</td>
<td>8700</td>
<td>8200</td>
</tr>
<tr>
<td>6</td>
<td>9000</td>
<td>9500</td>
<td>9100</td>
</tr>
<tr>
<td>TOTAL</td>
<td>55000</td>
<td>55500</td>
<td>53700</td>
</tr>
</tbody>
</table>

 Mean with letter *a* shows no significant difference between their means at p<0.05 as determined by one-way ANOVA.

The mean faecal eggs output per gram for groups A, B and C after treatments are 716.67, 96.67 and 12080 respectively. The deparasitization produced by *C. molle* and Albendazole were significant (p< 0.001) when compared to that produced by the control (water). There is however a significant difference (p< 0.005) in the deparasitization produced by *C. molle* and Albendazole (Table II).
Table II: Faecal Egg Output Per Gram after Treatment

<table>
<thead>
<tr>
<th>S/N</th>
<th>GROUP A</th>
<th>GROUP B</th>
<th>GROUP C</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>C. molle extract 200mg/kg</td>
<td>Albendazole 200mg/kg</td>
<td>Control H₂O 5ml/kg</td>
</tr>
<tr>
<td>1</td>
<td>500</td>
<td>20</td>
<td>12000</td>
</tr>
<tr>
<td>2</td>
<td>900</td>
<td>100</td>
<td>11000</td>
</tr>
<tr>
<td>3</td>
<td>600</td>
<td>40</td>
<td>14000</td>
</tr>
<tr>
<td>4</td>
<td>300</td>
<td>80</td>
<td>13000</td>
</tr>
<tr>
<td>5</td>
<td>500</td>
<td>300</td>
<td>10000</td>
</tr>
<tr>
<td>6</td>
<td>1500</td>
<td>40</td>
<td>12500</td>
</tr>
<tr>
<td><strong>TOTAL</strong></td>
<td><strong>4300</strong></td>
<td><strong>580</strong></td>
<td><strong>72500</strong></td>
</tr>
<tr>
<td><strong>MEAN ± SE</strong></td>
<td><strong>716.67 ± 160.55</strong></td>
<td><strong>96.67±42</strong></td>
<td><strong>12080± 583.3</strong></td>
</tr>
</tbody>
</table>

Mean with * within the column are significantly different at p<0.001, while those with the letter a shows significant differences between their means at p<0.05 as determined by Borferroni’s multiple comparison test.

The deparasitization produced by C. molle and Albendazole were significant (p< 0.001) when compared to that produced by the control (water). There is however a significant difference (p< 0.005) in the deparasitization produced by C. molle and Albendazole.

DISCUSSION

Parasitic diseases including gastrointestinal nematodes (GIN) remain a major constraint to sheep productivity in Nigeria [17]. In recent times, ethno veterinary practices, relating to the use of medicinal plants for the treatment of various ailments continue to play a major role in the control of helminthic diseases [18]. In this study, C. molle is demonstrated to be effective against experimental H. contortus infection in sheep at a non-toxic dose of 200 mg/kg [16]. The active principles in C. molle have significant anthelmintic efficacy. However, the efficacies of C. molle were comparable to that of Albendazole (a conventional anthelmintic) at a dose rate of 200 mg /kg. The anthelmintic activity of this plant might have been influenced by yet to be identified factors. For instance, the sheep might have generated a strong T. cell dependent immune response that brought about expulsion of the worms from the intestine [19]. It can be suggested that C. molle and some medicinal plants could serve as reliable therapeutic agents against helminthoses in traditional veterinary medical practices [20]. A number of these plants grow
singly in the wild, making the task of locating them more tedious at times. The ineffectiveness of some of these plants and some of the dosages could be attributed to a persistent re-infestation as pastures were highly contaminated with helminth eggs. Another factor could be because Albendazole had been properly refined than *C. molle* extracted from stem bark. It is likely that the thick walls of the cells from the stem bark reduced compounds from plant materials and this also is largely dependent on the solvent or medium and the method of extraction employed [21]. Extraction with water limit the amount and types of compounds extracted due to polarity [22]. However, hot water extracts from the plant materials are methods routinely used by farmers in Africa and represent a common means of preparing this plant for the treatment of animals [23].

The findings from this study suggest that *Combretum molle* appears to possess some anthelminthic properties that may support the use of this plant by local farmers in traditional animal health care. However, further pharmacological and toxicological researches are to establish its effectiveness against gastrointestinal nematodes of sheep in the nearest future.

**ACKNOWLEDGEMENT**

The authors wish to thank all the technical staff of the Department of Veterinary Parasitology and Entomology, Ahmadu Bello University, Zaria for their kind assistance and support.

**REFERENCES**


