Full Length Research Paper

Nematicidal efficacy of methanol/methylene chloride extract of *Rauwolfia vomitoria* (Apocynaceae) on *Heligmosomoides bakeri* (Nematoda, Heligmosomatidae) parasite of the white mouse (*Mus musculus*)

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Parasitic diseases are the most common in tropical Africa where climatic and hygienic conditions favor their proliferation. Due to the limitations of synthetic therapy, there is a need to turn back to phytotherapy. Thus, the nematicidal efficacy of methanol/methylene chloride (v/v) extract of stem bark of *Rauwolfia vomitoria* (Apocynaceae) was tested on *Heligmosomoides bakeri*, a nematode parasite of *Mus musculus*. Seventy Swiss white mice of both sexes aged 5 to 6 weeks and weighing between 20 to 25 g were orally infected with a suspension containing about 100 to 110 one week old *H. bakeri* infective larvae (L₃). After the pre-patent period, infected animals were randomly divided into 7 groups of 10 animals each corresponding to the treatments. Thus, five doses of plant extracts [200, 400, 800, 1600, 3200 mg/kg body weight (bwt)] and a unique dose of 22 mg/kg bwt of albendazole were prepared. The nematicidal activity of the organic plant extracts compared to albendazole was assessed indirectly through faecal egg count reduction (FECR) and directly through total worm count reduction (TWC). Albendazole served as the positive control while 4% dimethylsulfoxide (DMSO) as placebo. Each animal received for 6 consecutive days a daily dose of 0.7 ml of the organic plant extract according to its weight and group. Albendazole was given as a single dose. *R. vomitoria* at doses ≥ 1600 mg/kg bwt was active on the adult *H. bakeri* and reduced significantly (P < 0.05) the FEC and TWC of the nematode. The dose of 3200 mg/kg BW showed a high nematicidal activity of 76. 21% FECR and 79.55% TWC, 7 days post-treatment. These results were similar to those obtained with albendazole, 88.67% and 85.60% respectively for FECR and TWC. These findings suggest that *R. vomitoria* is an efficient anthelminthic. However, further studies are required in order to establish its mechanism of action. In addition, toxicological studies should be carried out.

**Key words:** Methanol/methylene chloride extract, *Rauwolfia vomitoria*, Albendazole, *Heligmosomoides bakeri*, *Mus musculus*.

INTRODUCTION

Helminthiasis is among the most important disease in both humans and livestock, causing considerable
hardship and stunted growth. In 2002, the world health organization (WHO) estimated that parasitosis represented more than 40% of the total morbidity charge of diseases in the world (WHO, 2002). According to a 2004 report from the Cameroonian Public Health Secretary of State, 16.1 million inhabitants who live in Cameroon, more than 10 million are infected by helminthiasis (Essogo, 2004). Parasitic diseases kill more than 155,000 people annually with 97% deaths occurring in developing countries (WHO, 2002; Hoste et al., 2006) and about 20 billion people are infected (WHO, 2004). In the livestock sector, helminthiasis is among the most important animal diseases inflicting many economic losses such as low productivity and even mortality (Perry et al., 2002; Suleiman et al., 2005). To fight against helminthiasis, both humans and livestock farmers use synthetic drugs, which unfortunately present limitations such as high cost, irregular availability and side effects (Wabo et al., 2011b). The increasing resistance of parasites to chemotherapy (Cabaret et al., 2008) and the presence of residues in meat and milk (Gasbarre et al., 2001) have also been in concern in recent times. In this light, the search of alternative from nature can be justified (Paolini et al., 2003). Indigenous and herdsman had long recognized the importance of helminth parasites and have made several attempts in controlling them through herbs (Suleiman et al., 2005). Several studies have been carried out to scientifically validate medicinal plants used by traditionalist to treat helminths (Mpaone and Essomba, 2000; Wabo et al., 2005, 2009, 2011a, b). According to tradipractitioners, *Rauvolfia vomitoria* (Apocynaceae) has several therapeutic properties (ICRA, 2008). It is distributed all over the world especially in Asia and West and Central African countries. In Cameroon, especially in the “Bulu” and “Ewondo” speaking region, *R. vomitoria* is locally called “Medzanga Medzanga”. The plant has been used in lowering blood pressure (Amole et al., 2003) and as antimalarial remedy (Amole et al., 1993). It has also been reported to possess anagelsec (Amole et al., 2006), haematinic (Amole and Ogunjere, 2001), antipyretic (Amole and Onabajo, 1999) and anticonvulsant properties (Amole et al., 2009). However, no information has been reported on the anthelmintic properties of *R. vomitoria*. Major phytochemical constituents of this plant include alkaloids, glycosides, polyphenols and reducing sugars. The alkaloids have been reported to include reserpine, a well-known antihypertensive substance found in this plant (Gubar, 1993). The aim of this work was to validate the anthelmintic activity of *R. vomitoria* (Apocynaceae) in vivo, using *Heligmosoides bakeri*, since the in vitro tests have already been carried out and organic extract of *R. vomitoria* showed a high mortality rate on larvae than aqueous extract. The in vivo test was carried out to determine the fecal egg count (FEC) and total worm count (TWC) reductions on the adult of *H. bakeri*. *H. bakeri* is a standard experimental model used in testing anthelmintic molecules by pharmaceutical companies (Githiori et al., 2003a). It belongs to the superfamily Trichostrongiloidea as most of the nematodes of veterinary importance (Githiori et al., 2003a) and its biological cycle is easily maintained in the laboratory on white mouse (*Mus musculus*).

**MATERIALS AND METHODS**

**Plant**

The stem bark of *R. vomitoria* (Apocynaceae) used in this work was obtained from traders in Yaounde, Center Region and political capital of Cameroon. *R. vomitoria* is a small tree or shrub (5 to 15 m in height and 40 cm in diameter) found in tropical or subtropical regions in Africa. The leaves are oval with straight venation and clusters of tiny flowers (Akpanabiatu et al., 2008a). The plant was identified at the National Herbarium of Cameroon (NHC) with the voucher numbers 16887.

**Preparation of plant extracts**

Five hundred grams (500 g) of plant powder was mixed with 3 liters of methanol/methylene chloride (1/1; v/v) for 3 days in a 5 liter jar. The mixture was stirred at least twice a day using a spatula, after which the mixture was filtered using a sieve of 150 µ of diameter, cotton and whatman no. 2 filter paper. The filtrate was concentrated using a rotary evaporator heated at 82°C for about 12 h. The wet extract collected was distributed in 5 large aluminum plates (22 cm of diameter). These plates were carefully placed in an oven and heated at 45°C for 2 days. The dried extract obtained was stored in air tight containers for further use (Ciulei, 1982) in the Laboratory of Applied Biology and Ecology (LABEA) of the University of Dschang. The plant material was air-dried in the laboratory at room temperature. Two (2) g of extract was weighed and 0.8 ml of dimethyl sulfoxide (DMSO) added for better dilution of extract in water. The solution was completed to 20 ml with distilled water to obtain a final solution of concentration of 100 mg/ml. The quantity of the product received by each animal was determined using the following formula Vx = 0.032 bwt (where Vx is the volume of solution given to the animal, bwt, the body weight of the animal). This was administered to animals receiving the highest dose 3200 mg/kg bwt. For groups that received lower doses of 1600, 800, 400 and 200 mg kg⁻¹ bwt, the quantity of the highest dose was divided by 2 in each subsequent group. The treatment was repeated for 6 days.

**Preparation of the albendazole solution**

From a tablet of albendazole brought in a local pharmacy, fifty (50) milligrams were diluted with 0.8 ml of DMSO and distilled water was added to obtain the final volume of 50 ml, thus a solution of concentration of 1 mg/ml. A single dose of 22 mg kg⁻¹ bwt was administered using the following formula Vx = 0.022 bwt.

**Artificial infection of mice with filariform larvae L₃ of Heligmosomoides bakeri**

Seventy Swiss white mice aged 5 to 6 weeks and weighing 20 to 25
Evaluation of faecal egg count reduction (FECR)

After the pre-patent period (9 to 11 days), mice were isolated in individual cages to collect fecal pellets. The feces were homogenized in a mortar and a saturated salt solution added. After filtration through a sieve, the supernatant was used to fill 2 test tubes until the superior meniscus was formed. A cover slide was gently lowered and allowed for 5 min, after which cover slide was removed and placed on a slide. Eggs were counted at ×4 objective using a light microscope. As soon as the qualitative coproscopic analyses showed evidence of infection (presence of eggs in faeces of mice), animals were randomly allocated into 7 groups of 10 individuals each (a pilot study was carried out before establishing doses 200, 400, 800, 1600 and 3200 mg kg⁻¹ bwt). Each mouse was weighed prior to treatment and dosed based on the individual's body weight early in the morning each day for 6 consecutive days. The volume of tested product was slowly administered orally using a manual syringe of 1 ml capacity with a blunt ended needle. Groups 1 to 5 were treated orally with the stem bark extract of plants at different doses for 6 consecutive days, while group 6 was treated with a single dose of albendazole (22 mg kg⁻¹ bwt) as standard anthelmintic (positive control). Group 0 received 4% DMSO which served as negative control. FEC was taken for each mouse first thing in the morning before treatment on days 0, 3, 5 and 7 post treatments for each group. At the end of the treatment (day 7), the parasite burden (total worm count or TWC) was determined.

Evaluation of faecal egg count reduction (FECR) was determined by the following formula (Coles et al., 1992):

\[ \text{FECR} \% = 100 \times \frac{T}{C} \]

Where T and C are the geometric means of FEC in the treated and control groups, respectively.

Evaluation of total worm count reduction (TWCR)

Seven days post-treatment mice were euthanized using chloroform. The body cavity was opened to remove the small intestine which was placed in a large Petri dishes containing 20 to 30 ml of distilled water. This organ was opened longitudinally with a scissors and passed through the arms of a forceps. The exudates containing parasites was washed in the water and all worms recovered were counted under a dissecting microscope. The percentage of total worm count reduction (TWCR) was calculated using the following formula (Enriquez, 1993):

\[ \text{TWCR} \% = 100 \times \frac{\text{Total worm count in control} - \text{Total worm count in treated group}}{\text{Total worm count in control group}} \]

Statistical analysis

Statistical analysis of data was performed using two-way analysis of variance (ANOVA) followed by Waller-Duncan test for comparison between groups. Egg per gram of faeces (EPG) was analysed after its transformation into log (x + 1). The comparisons of means of fecal egg count (FEC) and total worm count (TWC) were done at p < 0.05. All analyses were performed using SPSS 19.0 program.

RESULTS AND DISCUSSION

Feecal egg count reduction (FECR)

Results on FECR are shown on Table 1. The negative control (4% DMSO) group showed increase in egg counts throughout the treatment period. This increase was highly significant (p < 0.05) when compared with groups that received the plant extract and albendazole. Albendazole showed significant reduction (p < 0.05) from day 3 post treatment, but this reduction was not significant after day three. A similar trend was observed with plant extract, where reduction observed on the third day after treatment was not significantly different (p > 0.05) when compared with days 5 and 7. The highest FECR rate was obtained with albendazole (88.67%), followed by 77.23% reduction with days 5 and 7 post treatment, which was not significantly different (p > 0.05) from day 3 post treatment. A similar trend was observed with plant extract, where reduction observed on the third day after treatment was not significantly different (p > 0.05) when compared with days 5 and 7. The highest FECR rate was obtained with albendazole (88.67%), followed by 77.23% reduction with plant extract at 3200 mg/kg bwt. FECR was dose dependent.

Effects of methanol/methylene chloride extract and albendazole on the parasitic intensity of the nematode/Total worm count reduction (TWCR)

For the TWCR, albendazole was the most effective (85.6%), while R. vomitoria produced 79.6% reduction in TWC at 3200 mg/kg bwt (Table 2). These results were not significantly (p > 0.05) different when compared statistically. The results showed that the TWC was dose dependent. The reduction in FEC and TWC observed in this study could be explained by the fact that when adult parasites come in contact with the product, their
Table 1. Mean variation ± standard deviation and the percentage of fecal egg count reduction (at day 3, 5 and 7) after treatment with 4% dimethylsulfoxide (DMSO), albendazole and different doses of methanol/methylene-chloride extract of *Rauvolfia vomitoria*.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dose (mg/kg bwt)</th>
<th>Period of treatment (days)</th>
<th>D5</th>
<th>D7</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>DO</td>
<td>D3</td>
<td></td>
</tr>
<tr>
<td>4% DMSO (negative control)</td>
<td>-</td>
<td>11000±8700</td>
<td>30570±8700 (0.0)</td>
<td>28550±2740 (0.0)</td>
</tr>
<tr>
<td>Albendazole (positive control)</td>
<td>22</td>
<td>14850±2980</td>
<td>2500±1500 (89.69±10.33)</td>
<td>3920±110 (83.81±13.35)</td>
</tr>
<tr>
<td></td>
<td>200</td>
<td>15500±2040</td>
<td>24600±7480 (34.18±3.43)</td>
<td>28900±6550 (33.19±3.79)</td>
</tr>
<tr>
<td><em>Rauvolfia vomitoria</em></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>400</td>
<td>5400±2420</td>
<td>13500±5050 (39.85±5.46)</td>
<td>14800±5670 (37.68±4.14)</td>
</tr>
<tr>
<td></td>
<td>800</td>
<td>8900±3320</td>
<td>11300±6000 (51.33±3.19)</td>
<td>18200±1470 (47.94±2.23)</td>
</tr>
<tr>
<td></td>
<td>1600</td>
<td>25200±6130</td>
<td>13400±2680 (60.22±5.16)</td>
<td>13200±3820 (60.24±5.13)</td>
</tr>
<tr>
<td></td>
<td>3200</td>
<td>14600±3040</td>
<td>7100±3470 (75.98±3.57)</td>
<td>6500±3550 (77.23±8.02)</td>
</tr>
</tbody>
</table>

a, b, c, d and e numbers with the same letter in the same column are not significantly different (p˃0.005). D: day; FECR: faecal egg count reduction; DMSO: Dimethilsulfoxide; ( ) : Percentage of fecal egg count reduction.

Table 2. Mean variation ± standard deviation of total worm count and the percentage of total worm count reduction (at day 7) after treatment with 4% dimethylsulfoxide, albendazole and different doses of methanol/methylene-chloride extract of *R. vomitoria*.

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Doses (mg.kgbwt⁻¹)</th>
<th>TWC</th>
<th>TWCR</th>
</tr>
</thead>
<tbody>
<tr>
<td>4% DMSO (negative control)</td>
<td>-</td>
<td>96±10</td>
<td>-</td>
</tr>
<tr>
<td>Albendazole</td>
<td>22</td>
<td>15.3±7</td>
<td>85.6^a</td>
</tr>
<tr>
<td></td>
<td>200</td>
<td>64±15</td>
<td>32.8^b</td>
</tr>
<tr>
<td></td>
<td>400</td>
<td>53.8±14</td>
<td>43.6^a,b,c</td>
</tr>
<tr>
<td><em>Rauvolfia vomitoria</em></td>
<td>800</td>
<td>49.8±9</td>
<td>59.4^c</td>
</tr>
<tr>
<td></td>
<td>1600</td>
<td>28.5±12</td>
<td>74.8^d</td>
</tr>
<tr>
<td></td>
<td>3200</td>
<td>23.5±13</td>
<td>79.6^a,e</td>
</tr>
</tbody>
</table>

a, b, c, d and e numbers with the same letter in the same column are not significantly different at p < 0.05. TWC: total worm count; TWCR: total worm count reduction; DMSO: dimethilsulfoxide; -: not determined.

metabolism is affected and some of the parasites die, thus leading to reduction in egg out-put.

Phytochemical screening of *R. vomitoria* revealed the presence of secondary metabolites such as polyphenolic compounds, tannins, alkaloids and flavonoids (Yondo et al., 2009). The stem barks of
plants are the site of storage of secondary metabolites responsible for the biological properties of medicinal plants extracts (Busindou, 1997). Polyphenolic compounds are known to inhibit parasite growth by causing apoptotic cell death, leading to the reduction of parasite and egg burdens (Inuoe, 1994; Rice, 1965; Adedapo et al., 2005). Tannins interfere with energy generation in helminth parasites by uncoupling oxidative phosphorylation (Martin, 1997), while alkaloids and flavonoids are good sources of chemicals responsible for the therapeutic activities of several medicinal plants (Debella, 2002). Flavonoids mode of action is linked to its unusual antioxidant pathway. Ribeiro et al. (1997) discussed that the lack of defense mechanisms against oxidative stress makes the parasites susceptible to drugs having an effect on the generation of reactive oxygen species. The increase in egg out-put after reduction may be due to the fact that parasites that did not die may show some level of resistance to the product, and start laying eggs.

Results of this study were consistent with that of Satrija et al. (1995) and Wabo et al. (2009) who tested the efficacy of Carica papaya and Canthium manii, respectively against this same parasite (H. bakeri).

Inhibition on egg production is an important aspect of the activity of some antinematodals as benzimidazoles and phenothiazines. Albendazole has a large broad spectrum on many parasites of medico-veterinary importance (Lagardère, 1995). Similar studies carried out by Onyeyili et al. (2001) shows a percentage of reduction of faecal count of 94.1% with this proprietary anthelmintic against mixed nematode species (Haemonchus contortus, Trichostrongylus colubriformis, Trichostrongylus axei, Oesophagostomum columbianum, Strongyloides papillosus and Trichuris ovis) at the dose 5 mg kg bwt of the sheep. This mouse host-parasite model used was suitable for a rapid evaluation of anthelmintic effect of R. vomitoria in a relatively short period. So, the reduction rate above 70% obtained puts this plant as a potential source of anthelmintic (Githiori et al., 2003b).

From the work that was aimed at assessing the anthelmintic activity of organic extract of R. vomitoria, we noticed that the methanol/methylene chloride extract of R. vomitoria has generated a high reduction rate of the ovary charge (76.21%) and parasitic load (79.55%) of the adults of H. bakeri. The effect of extract was dose dependent. Further research such as purification and identification of bioactive compounds and toxicological investigation are necessary.

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REFERENCES


