In vivo evaluation of antihelmintic potential of *Napoleonaea vogelii* Hook & Planch (Lecythidaceae): A traditionally used nematicidal medicinal plant in West Africa

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**ABSTRACT**

The need for research into alternative therapeutic agents for the control of helminth infections is of great interest for animal health. Medicinal plants play a considerably role in the discovery of new molecules. In this study, *in vivo* antihelmintic potential of *Napoleonaea vogelii* leaves was evaluated against gastro-intestinal nematodes (GINs) in sheep. An 80 mg/kg single dose oral suspension of leaves ethanolic extract was administered to 3-6 months lambs aged naturally infected with GINs. Biological (bodyweight, diarrhea index), hematological (Famacha, hematocrit) and parasitological (fecal eggs count reduction, reduction of adult worms) parameters were determined. The results showed that the ethanolic extract generated a weight gain (5.8%) in treated sheep and showed moderate action on diarrhoea (diarrhoeal index = 67.3%) but had weak effect on hematocrit as compared with the control (Untreated) group. *N. vogelii* was highly effective against adult *Oesophagostomum columbianum* (94.4%) and *Cooperia curticei* (100%), but exhibited a marginally lower activity against *Gaigeria pachyscelis* (61.5%) and *Trichostrongylus axei* (42.2%). These results indicate that *N. vogelii* exhibited some potential and can be used as natural antiparasitics for the control of GINs in small ruminants.

**Key words**: *Napoleonaea vogelii*, in vivo efficacy, gastro-intestinal nematodes, small ruminants, West Africa.

**INTRODUCTION**

Parasites of livestock cause diseases of socio-economic importance throughout the tropics. The current financial and agriculture losses caused by parasites have a substantial impact on farm profitability worldwide, mainly in West African poor resource countries (Roeder et al., 2013). In Sub-Saharan countries, gastrointestinal nematodes infections (GINs) are responsible for heavy direct and indirect losses in livestock (Waller, 1987), due to severe harm and death to domestic animals (Kaminsky et al., 2013). As in developed countries (Epe and Kaminsky, 2013), in many West African countries treatment of helminth infections remains the mainstay of worm control in grazing animals and also is based on imported manufactured anthelmintics beside medicinal plants. However, cost, illiteracy and unfamiliarity with imported antihelmintics,
resulting in incorrect usage in rural areas may cause development of resistance favored by genetic features of many parasitic helminths of veterinary importance. Anthelmintic resistance poses a large threat to welfare of farm animals (Shabaly, 2013). The constraints in the use of conventional medicines in West Africa underline the advantages of medicinal plants as an alternative treatment. Livestock producers in rural continue to use those plants as dewormers, drawing upon centuries of knowledge.

Plant based medicines used to treat gastrointestinal infections in earlier times have been credited with having specific anthelmintic activity (Mehlhorn et al., 2011). Most of the West African medicinal plants screening for action against helminths have been shown to possess high in vitro potential. However, according to Klimpel et al. (2011), although in vitro testing of plant extracts for antiparasitic efficacy provides good results, it does not always guarantee in vivo test systems. Therefore, suitable in vivo animal models provide a more accurate reflection of the effectiveness of plant extracts.

*Napoleonaea vogelii* Hook & Planch (Lecythidaceae) is a perennial shrub which is native of upper Guinean forest. The geographical distribution of this plant species extends from Sierra Leone to Nigeria, covering all the coasta
d

MATERIALS AND METHODS

Selection of the studied plant and preparation of crude extract

The leaves of *N. vogelii* were collected in July 2010 in Azagué (South-Eastern Côte d’Ivoire), latitudes 5°35’ and 6°15’ N and longitude 3°55’ and 4°40’ W. Leaves were cleaned, dried under air-conditioning room (18 °C) and grounded to obtain powder. Crude extract was prepared from 100 g of powder in 100 ml of ethanol 90%, under mechanical stirring (3 × 24 h). The ethanol was evaporated in a rotary evaporator (rotavapor) at 40°C, frozen and lyophilized.

Animals and parasites

Thirteen ewe-lambs of West African Dwarf sheep “Djallonké,” from 6 to 12 months aged, were bought at the market of Port-Bouët (Abidjan). These sheep were naturally infected by GIWs from their local origin. They were reared and fed under same conditions.

The animals were selected using recommendations of the World Association for the Advancement of Veterinary Parasitology (WAAVP) and the Veterinary International Cooperation on Harmonization (VICH) (Powers et al., 1982; Coles et al., 2006).

Sheep were distributed in three groups of four sheep for groups I and II and five sheep for group III according to the mean of Fecal Eggs Count Reduction (FECR) observed a week before treatment. Mac-Master method modified by Gordon and Whitlock (1939) was used for FECR. Solution of chloride Sodium (NaCl, D = 1.19) was used for parasites eggs floating.

For in vivo assays, extract (80 mg/kg) and fenbendazole (7.5 mg/kg) were suspended in a solution composed of Tween 80 (%5) and water (95%). Groups I and II (positive control) were treated at single oral dose of 80 and 7.5 mg/kg, respectively with the ethanolic extract of *N. vogelii* leaves and fenbendazole. Group III (negative control) received distilled water.

Evaluation of in vivo anthelmintic efficacy

The efficacy of *N. vogelii* leaves was evaluated using fecal egg reduction and adult worm reduction. Biological and parasitological parameters such as weight gain, diarrheal index, rate of anemia and fecal egg count reduction (FECR) test were recorded each three days during the three weeks (Soro et al., 2013), and the necropsy was followed after this period (Coles et al., 2006).

Bodyweight gain

Animals were weighted using anybody weighs balance and weight gain was expressed using the formula below:

\[
\text{Bodyweight gain (BWg)} = \frac{(\text{BW} \times \text{BWg}) - \text{BW}}{\text{BW}} \times 100
\]

\[\text{BWg = bodyweight before treatment; BW = bodyweight after treatment}\]

Diarrheal index

Diarrheal index was based on observation of the back-train stain of animals. Feces consistency was scaled (I1 = liquid feces; I2 = soft feces; I3 = normal feces) using visual charter of Cabaret (2004). The percentage of appearance of each values of the scale was calculated for each studied group of sheep.

Hematocrit

Blood was collected from jugular vein in heparinized tube
(Improvacuter ®) and centrifuged at 4000 rpm during five minutes in a centrifugal machine of hematocrit (J.P. Selecta ®). The values of hematocrit were read with a reading grid (Murray et al., 1977) and expressed in percentage of hematocrit (% H) using the following formula below:

$$\% H = \left(\frac{Hx - Ho}{Ho}\right) \times 100$$

Ho = Hematocrit before treatment; Hx = Hematocrit after treatment

**Fecal egg count reduction test**

During 28 days (one week pretreatment and three weeks post-treatment), fresh feces were collected from the rectum of sheep. The eggs and coccidia oocysts were counted using Mac-Master method. The efficacy of the extract was determined on the basis of percent reduction in the fecal eggs count according to the formula of Coles et al. (1992):

$$\text{FECR} = \left(1 - \frac{Mx}{Mxo}\right) \times 100$$

Mx = means of fecal eggs count after treatment; Mxo = means of fecal egg count before treatment

**Counting of adult worms during necropsy**

After sacrificing animals by throat cutting, the digestive tract was removed and abomasum, small intestine and large intestine were separated by binding at the two ends and different junctions between organs (Graber and Perrotin, 1983; Kaufmann, 1996). The delimited compartments were opened longitudinally. Contents were collected under tape water in a bucket and passed through a sieve (200 μm). The residues were spilled in another bucket and three liters of water were added. The suspension was homogenized; 200 ml were taken for a systematic identification and counting of parasites with optical microscope (M × 100). The corresponding number of adult worms counted in three liters was arithmetically calculated. The percent efficacy (%E) of treatment was expressed using the formula below:

$$\% E = \left(\frac{Mc - Mx}{Mx}\right) \times 100$$

Mc = mean of adults worms in group III (control negative); Mx = mean of adults worms in treated groups (I or II).

According to Reinecke (1973, 1980) and Powers et al. (1982), efficacy of extract was classified as high (% E > 90 %); moderate (% E = 80–90 %); low (% E = 60–80 %), and ineffective (% E < 60 %).

**Statistical analysis**

Comparison of means between the three groups of sheep tested was performed with variance analysis (one-way ANOVA) using SPSS 20. When ANOVA showed significant difference between the three groups tested, the complementary test of the multiple comparisons of means (Turkey test) was applied to determine the level of relationship between groups (Westlake, 1971). The test was significant if P < 0.05. The correlation between quantitative parameters such as strongles, hematocrit and bodyweight was determined at P < 0.05.

**RESULTS**

**Effects on biological parameters**

**Bodyweight gain**

A weight gain of 5.85 ± 1.88% was recorded three weeks post-treatment in treated group with *N. vogelii*. The dewormed sheep in group I had a higher average weight gain than those treated with fenbendazole (-0.3 ± 1.3% -0.3 ± 1.2%) which in turn always exceeded the untreated group (- 4.6 ± 1.5%). Statistically, *N. vogelii* was the most effective (Table 1). However, there was no significant difference between group II (fenbendazole) and group III (negative control).

**Hematocrit**

There was a decrease of hematocrit of -1.0 ± 1.2% and - 6.2 ± 1.5% in group I (*N. vogelii*) and group III (negative control) respectively. In group II (fenbendazole), an increase of hematocrit (5.7 ± 1.3%) was recorded three weeks post-treatment (Table 1). Statistically, there was a significantly difference between the plant extract and fenbendazole that was the most effective.

**Diarrheal index**

After administration of *N. vogelii* leaves extract, a stop of diarrhea was observed in animals during the first week, with 100% of diarrheal index. However, at the end of the experiment, only 67.5% of treated animals with this extract gave normal feces. With fenbendazole, 58% of feces were normal (I3) and 42% soft (I2) at three weeks after treatment. In group III, 53% of normal feces (I3) and 47% of soft feces (I2) were recorded at the end of the assays (Figure 1).

**Effect on fecal egg excretion**

The extract of *N. vogelii* leaves was ineffective on fecal egg reduction of all studied gastrointestinal parasites, with %E
Table 1: Effect of *Napoleona vogelii* leaves extract on hematocrit and bodyweight in naturally infected sheep.

<table>
<thead>
<tr>
<th>Hematocrit (%)</th>
<th>Treatments</th>
<th>Before treatment</th>
<th>Post-treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Week 0</td>
<td>Week 1</td>
</tr>
<tr>
<td>Group I: N. vogelii leaves ethanolic extract (Essay)</td>
<td>Mean ± SEM</td>
<td>NA</td>
<td>-1.9 ± 5.0</td>
</tr>
<tr>
<td>Group II: Fenbendazole (positive control)</td>
<td>Mean ± SEM</td>
<td>NA</td>
<td>-2.3 ± 4.5</td>
</tr>
<tr>
<td>Group III: Untreated (negative control)</td>
<td>Mean ± SEM</td>
<td>NA</td>
<td>-5.0 ± 0.9</td>
</tr>
<tr>
<td>&lt;sup&gt;P&lt;/sup&gt;</td>
<td></td>
<td>0.43</td>
<td>0.002</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Bodyweight gain (%)</th>
<th>Treatments</th>
<th>Before treatment</th>
<th>Post-treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Week 0</td>
<td>Week 1</td>
</tr>
<tr>
<td>Group I: <em>N. vogelii</em></td>
<td>Mean ± SEM</td>
<td>NA</td>
<td>2.3 ± 1.4&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Group II: Fenbendazole (positive control)</td>
<td>Mean ± SEM</td>
<td>NA</td>
<td>-0.3 ± 1.3&lt;sup&gt;ab&lt;/sup&gt;</td>
</tr>
<tr>
<td>Group III: Untreated (negative control)</td>
<td>Mean ± SEM</td>
<td>NA</td>
<td>-3.3 ± 1.6&lt;sup&gt;ab&lt;/sup&gt;</td>
</tr>
<tr>
<td>&lt;sup&gt;P&lt;/sup&gt;</td>
<td></td>
<td>0.061</td>
<td>&lt;0.000</td>
</tr>
</tbody>
</table>

Values with the same superscript letter are not significantly different (P < 0.05 or P < 0.001).
SEM: standard error of mean, NA: not applicable.

Figure 1: Effect of *Napoleona vogelii* leaves extract on diarrheal index in natural infected sheep.

**Index 1:** liquid feces, **Index 2:** soft feces, **Index 3:** normal feces.
Table 2: Effect of *Napoleona vogelii* leaves on fecal eggs and oocysts excretion against gastrointestinal nematodes in naturally infected sheep.

<table>
<thead>
<tr>
<th>Internal parasites</th>
<th>Treatments</th>
<th>Before treatment</th>
<th>Post-treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Strongles</td>
<td></td>
<td>Week 0</td>
<td>Week 1</td>
</tr>
<tr>
<td>Group I: <em>N. vogelii</em> ethanolic extract (Essay)</td>
<td>Mean ± SEM</td>
<td>4123</td>
<td>1954 ± 137(^b)</td>
</tr>
<tr>
<td>FECR (%)</td>
<td>NA</td>
<td>52.6</td>
<td>0.9</td>
</tr>
<tr>
<td>Group II: Fenbendazole (positive control)</td>
<td>Mean ± SEM</td>
<td>4073</td>
<td>17 ± 8(^a)</td>
</tr>
<tr>
<td>FECR (%)</td>
<td>NA</td>
<td>99.6</td>
<td>99.6</td>
</tr>
<tr>
<td>Group III: Untreated (negative control)</td>
<td>Mean ± SEM</td>
<td>4118</td>
<td>3430 ± 270(^c)</td>
</tr>
<tr>
<td>FECR (%)</td>
<td>NA</td>
<td>16.7</td>
<td>-21.9</td>
</tr>
<tr>
<td><em>P</em> value</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
</tr>
</tbody>
</table>

Values with the same superscript letter are not significantly different (*P* < 0.05 or *P* < 0.001).

FECR: fecal egg count reduction, SEM: standard error of mean, NA: not applicable, BW: bodyweight.

Table 3: *In vivo* efficacy of *Napoleona vogelii* leaves extract against adult gastrointestinal nematodes in naturally infected sheep.

| Internal parasites | Treatments | Group III: Untreated | Group I: *N. vogelii* | Group II: Fenbendazole | *P*
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Haemonchus contortus</td>
<td>Mean ± SEM</td>
<td>478 ± 42(^b)</td>
<td>400 ± 41(^b)</td>
<td>0 ± 0(^a)</td>
<td>0</td>
</tr>
<tr>
<td>PR (%)</td>
<td>NA</td>
<td>16.3</td>
<td>100</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Trichostrongylus colubriformis</td>
<td>Mean ± SEM</td>
<td>592 ± 42(^b)</td>
<td>506 ± 66(^b)</td>
<td>26 ± 11(^a)</td>
<td>0</td>
</tr>
<tr>
<td>PR (%)</td>
<td>NA</td>
<td>14.3</td>
<td>95.6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Trichostrongylus axei</td>
<td>Mean ± SEM</td>
<td>45 ± 8(^b)</td>
<td>26 ± 7(^ab)</td>
<td>7 ± 4(^a)</td>
<td>0.009</td>
</tr>
<tr>
<td>PR (%)</td>
<td>NA</td>
<td>42.2</td>
<td>83.3</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
of 52.6 to 0.5%. Fenbendazole exhibited a percentage of egg reduction of 97.5%. Statistically, the fecal egg reduction was more important with fenbendazole than plant extract. However, as compared with the negative control group, the plant extract showed some positive effect (Table 2). The plant extract reduced the number of coccidia at 16.2% while fenbendazole was totally ineffective.

**Effect against adult worms**

The necropsy revealed the presence of helminths in sheep digestive tract, such as *Haemonchus contortus* and *Trichostrongylus axei* (in abomasum), *Trichostrongylus colubriformis*, *Strongyloëdes papillosus*, *Gaigeria pachyscelis* and *Cooperia curticei* (in small intestine), *Oesophagostomum columbianum* and *Trichurus globulosa* (in large intestine) and *Tænia* sp. (in large and small intestines).

*N. vogelii* leaves extract showed high efficacy against *O. columbianum* (94.4%) and *C. curticei* (100%). This extract’s efficacy was 61.5% against *G. pachyscelis* and 42.2% on *T. axei*. A marginally lower effect was observed against *H. contortus* (16.3%), *T. colubriformis* (14.3%) and *T. globulosa* (5.6%). No efficacy was recorded against *S. papillosus*. Fenbendazole was effective against all the studied parasites, except against *O. columbianum* (% E = 16.6%). Statistically, *N. vogelii* was more active than fenbendazole against *O. columbianum*, but the two compounds showed similar antihelmintic activities against *C. curticei* (Table 3).

**DISCUSSION**

The *in vivo* antihelmintic activity of *N. vogelii* was evaluated in naturally infected sheep in order to access its efficacy against GINs. This plant showed beneficial effects on certain biological parameters such as weight gain with an increase of 5.8% and diarrheal index of 67.5% as compared with the control groups. According to Höglund et al. (2013), such results indicate that the parasite challenge in the negative control group was sufficiently high to result in production loss. The effect on diarrhoea is of interest because diarrhoea is one of the main signs of GINs that could cause rapid death in animals. The decrease of diarrheal index from 100% in the first week to 67.5% in the third week can be explained by the administration of a single oral dose. The plant extract was highly effective against *C. curticei* (100%) and *O. columbianum* (94.4%). *O. columbianum* is the second prevalent nematode in small ruminants in Côte d’Ivoire (Komoin-Oka et al., 1999; Achi et al., 2003). This nodular worm is a considerably pathogenic parasite in sheep, goats and cattle (Mehlhorn, 2007). In addition,
oesophagostomosis are common zoonosis found in small ruminants, cattle and primates including humans (Krief et al., 2008; Guillot et al., 2011). In West Africa, they are limited only to Ghana and Togo at the moment (Guillot et al., 2011). *N. vogeli* may also have interest in controlling this parasitic infection in human.

*C. curtici* is a common parasite of the small and/or large intestine that has relatively low pathogenicity. But this helminth contributes to parasitic gastroenteritis in grazing small ruminants (Roebert et al., 2013) with main signs such as anorexia and loss of bodyweight (Mehlhorn, 2011). So, efficient treatments with the extract of *N. vogeli* against these parasites are of interest in reducing the negative effects of these symptoms.

Disappointingly, *N. vogeli* leaves was ineffective, *in vivo*, against *H. contortus* (%E = 16.6), despite the high *in vitro* activity reported against this helminth (Diehl et al., 2004). This finding is in full agreement with the state of Klimpel et al. (2011) that good results obtained *in vitro* does not always guarantee *in vivo* efficacy. Moderate or low activity was observed against the remaining helminths found in the studied sheep. Higher or multiples doses may probably result in promising activity against gastrointestinal nematodes. However, to the best of our knowledge, the *in vivo* anthelmintic activity of *N. vogeli* is reported for the first time in this study.

**Conclusion**

The *in vivo* anthelmintic evaluation showed that *N. vogeli* could have interest in the control of GINs infections in small ruminants. The extract has generated bodyweight gain and was effective against diarrhoea in naturally infected sheep with GINs. A high efficacy was observed against *O. columbianum* and *C. curtici* in the current study, but very weak activity against *H. contortus*. However, *N. vogeli* could be candidate for development of anthelmintic drug for the control of GINs in small ruminants with reduced spectrum. Further studies are needed with administration of high or multiples doses of this plant extract.

**ACKNOWLEDGMENTS**

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