ORIGINAL ARTICLE

Evaluation of Anthelmintic properties of Sesbania grandiflora Pers. (Kathurumurunga) against larvae of Toxocara canis and Haemonchus contortus – In Vitro Study

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ABSTRACT:

Sesbania grandiflora is a slender tree. It is a common garden plant in Sri Lanka, which grows well in mid and low country. In Sri Lanka, Sesbania grandiflora is used as a home remedy, in treatment of worm infections in humans. Toxocara canis is a helminth parasite infecting dogs and other canids. It also causes toxocariasis in humans. Haemonchus contortus is a nematode that infects goats and causes anaemia, marked reduction in growth and reproduction, and even death. Using this information, in vitro larvae migratory inhibition assay was carried out on Toxocara canis and Haemonchus contortus larve. This study revealed 98.1% and 94.3% larvae migration inhibition with larvae of Toxocara canis and Haemonchus contortus respectively. Least number of migrated larvae was observed in the positive control Levamisole and all the larvae were dead after migration. In decoction of S. grandiflora, all the migrated Toxocara larvae were dead and Haemonchus larvae were dead or in Grade 1 (inactive but occasional movement can be observed) condition. Inhibition of Toxocara larval migration and Haemonchus larval migration with decoction of Sesbania grandiflora and Levamisole are statically significant (p < 0.05). Since mean of LMI (larval migration inhibition) of Levamisole is greater than mean of LMI of Sesbania grandiflora with both larvae, Levamisole is more effective than Sesbania grandiflora. Based on these findings, the aqueous extract of leaves of Sesbania grandiflora is shows a statistically significant anthelmintic activity in in-vitro model.

Keywords: Anthelmintic properties, Haemonchus contortus, Kathurumurunga, Sesbania grandiflora, Toxocara canis

INTRODUCTION

Sesbania grandiflora (Family: Fabaceae; Sinhala name: Kathurumurunga; Sanskrit name: Agastya; Tamil name: Agathi) is a small erect, fast-growing, and sparsely branched tree that reaches 10 m in height. Toxocara canis, the round worm in dogs may produce Toxocariasis is humans.1,2 Haemonchus contortus is a parasite of goats which causes

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anemia. Resistances to anthelmintic drugs are increasing daily. Hence novel findings of anthelmintics have become a necessity. *Sesbania grandiflora* is used as a home remedy in treatment of worm infections and various other ailments. Therefore, authors decided to evaluate the anthelmintic effect of *Sesbania grandiflora* against larvae of *T. canis* and *H. contortus*.

**MATERIAL AND METHOD**

(a) Preparation of decoction of leaves of *Sesbania grandiflora* (*Kathurumurunga*)

Decoction was prepared using fresh leaves. 120 g (24 Kalan) of fresh leaves of *Sesbania grandiflora* are mixed with 1920 ml (8 Patha) of water and boiled down to one eighth of it, that is; 240 ml (1 Patha) to prepare decoctions.

(b) Anthelmintic properties against larvae of *Toxocara canis*

i. Collection of *Toxocara canis* eggs

Eggs of *Toxocara canis* were obtained from the faeces of young puppies and were embryonated for larval preparations as described by Rajapakse *et al.* (1992). Feces from the puppies were screened by direct smear method, before taking as samples for the research. The fecal samples were collected from all the positive animals. All positive samples were mixed in one liter of water containing 0.05% Teepol (Lankem Ceylon Ltd., Colombo) in a measuring cylinder and washed five times by sedimentation method to remove all fat and other fine materials.

Thereafter, the sediment was re-suspended in 500 ml of saturated salt solution and the suspension was centrifuged (at 1000g for 10 minutes) in order to separate the eggs. The surface layer of the supernatant solution containing the *T. canis* eggs was collected using a Pasteur pipette and washed with water through a filter of 100μm pore size in order to remove the coarse fibrous matter. The filtrate was then poured through a filter of pore size 50μm where *T. canis* eggs remained on the filter.

ii. Development of infective eggs of *Toxocara canis*

Freshly harvested eggs were stored in 0.1 N H₂SO₄ at a depth of 0.5 cm in Petri dishes (10 cm x 1.5 cm) in an incubator (Lindberg and May Pvt. Ltd., Australia) at 14.5 °C. At this temperature, the development of eggs was arrested without any substantial reduction of their viability. The eggs could be stored in this manner for 60 days. Whenever infective eggs were required, Petri dishes containing the required number of eggs were placed at room temperature (22°C-24°C). In the course of this second incubation, the culture was rocked gently once a day to ensure aeration. Eggs reach infective stage within 30-40 days. Thereafter eggs were washed twice by centrifugation at 150g for 15 minutes with 0.15 M Phosphate Buffered saline (PBS) (pH 7.2) to remove H₂SO₄ and the other organic matter and the eggs were recounted at 1:100 dilutions by the McMaster technique. Viability of the *T. canis* embryonated eggs was assessed by the light stimulation method before use as described by O’Lorcan *et al.* (1995).

iii. The storage and maintenance of larval cultures

*Toxocara* larvae to be used for experimental purposes will often have to be stored and this was done satisfactorily in a shallow layer of water. 40ml of a suspension containing not more than 3000 larvae per ml were placed in a tissue culture flask and kept in an incubator maintained at 10 °C. As the storage at low temperatures would induce inhibition of some population, care was taken not to use larvae while they were being conditioned. This means that the usage before 4 weeks of storage (larvae had been stored for 2 weeks to ensure a normal establishment rate) or after 16 weeks, was avoided.

iv. In vitro larval migration inhibition assay

The larvae migration inhibition (LMI) bioassay developed by Wagland *et al.* (1992) and modified by Rabel *et al.* (1994) was used to determine the effectiveness of extract of leaves of *Sesbania grandiflora* against infective larvae.

Decoction of *Sesbania grandiflora* (*Kathurumurunga*) was diluted by adding phosphate buffered saline (PBS). 1 ml of the solution was taken and diluted with 29 ml of PBS so as to obtain a transparent solution. Then the density was calculated (D=M/V) at room temperature (29°C). As the positive control levamisole 200 μg/ml was used, whereas phosphate buffered saline (PBS) was used as the negative control.

Then larval suspensions were added to wells (200 μl of suspension per well) and each well containing 800 μl of either controls (positive and negative) or plant extract, and were incubated at 37 °C in the wells of tissue culture plates. Three wells (replicate samples) were run for decoction and
for the controls. The process was repeated three times.

All the incubations were carried out in 24 well tissue culture plates overnight (18 hours), at 37 °C and pH 7.2. Following day solutions were transferred to sieves (20 μm mesh at one end) and left for 24 hours at room temperature for active larvae to migrate through the sieves, which were counted later.

On the next day, sieves were removed, Lugol’s iodine (0.1 ml) was added to the well and the number of larvae which had migrated was counted under the microscope. The viability and activity of the post migratory larvae with different plant remedies were observed and recorded as follows.

Grade 0 = Dead; No recovery after prolonged immersion in saline
Grade 1 = Inactive but occasional movement can be observed;
Grade 2 = Inactive but intermittent movement can be observed clearly;
Grade 3 = Slow moving;
Grade 4 = Active.

(c) Anthelmintic properties against larvae of Haemonchus contortus (in vitro)

i. Collection of the eggs of Haemonchus contortus

Fecal samples were collected directly from the rectum of goats in Kekirawa veterinary range, Sri Lanka. Fecal egg count was determined using the modified McMaster technique (Cringoli, 2011). 6 Faeces of high eggs per gram (EPG) of >5000 from each animal were collected for this study. All positive samples were then subjected to fecal culture for collection of infective larvae.

ii. Fecal culture and isolation of Haemonchus contortus larvae

Fecal cultures were prepared using faeces collected from infected goats. The faeces were broken up finely, using a large pestle and mortar, mixed with sterile dung or sawdust in 1:1 ratio, and dampened with distilled water until the mixture was moist and crumbly. Then the mixture was kept in wide-mouthed glass jars or enamel trays and incubated at room temperature for 10-14 days. The cultures were maintained by aerating the lower layers every day and, to prevent drying, by adding a few drops of water in order to maintain moisture. After 14 days, cultures were baermannized using wide-mouthed glass jars. The larvae were counted and assessed for viability and identification was carried out to the level of genus before being stored at 10 °C.

iii. The storage and maintenance of larval cultures

The storage and maintenance of larval cultures was carried as described under Toxocara canis

iv. In vitro larval migration inhibition assay

In vitro larval migration inhibition assay was carried out as described under Toxocara canis but infective larvae in unsheathed forms were used. The Haemonchus infective larvae that were subjected to test were in unsheathed forms. Sheathes were removed by incubating the larvae in sodium hypochlorite solution (0.025% available chlorine) for 10 minutes at room temperature, washing several times and concentrating to approximately 2500 larvae/ml PBS.

(d) Statistical analysis of larval migration inhibition of Toxocara canis and Haemonchus contortus

Larval migration inhibition of Toxocara canis and Haemonchus contortus were statistically analysed by using independent-sample t-test.

RESULTS

Results of the in vitro assay of Toxocara canis and Haemonchus contortus larval migration inhibition are given in Table 1 and Figure1. As shown in the table, decoction of Sesbania grandiflora was 98.1% effective in inhibiting Toxocara larval migration and 94.3% effective in inhibiting Haemonchus larval migration. Whereas larval migration inhibition of Toxocara canis and Haemonchus contortus with Levamisole were 99.7% and 96.6% respectively.

Inhibition of Toxocara larval migration and Haemonchus larval migration with decoction of Sesbania grandiflora and Levamisole are statically significant (p < 0.05). Since mean of LMI (larval migration inhibition) of Levamisole is greater than LMI of Sesbania grandiflora with both larvae, Levamisole is more effective than Sesbania grandiflora (Table2 and Figure2).

The viability of post-migratory larvae of Toxocara canis and Haemonchus contortus with controls and with decoction of Sesbania grandiflora are presented in Table 3 and Figure3. Maximum number of migrated larvae of Toxocara and
Haemonchus was observed in the negative control PBS. Least number of migrated larvae was observed in the positive control Levamisole and all the larvae were dead after migration. 1.2% of Toxocara and 1% of Haemonchus larvae were migrated in decoction of Sesbania grandiflora. All the migrated Toxocara larvae were dead and in Haemonchus larvae were dead or in Grade 1.

DISCUSSION

According to Ayurveda, Sesbania grandiflora possesses Tikta Rasa, Laghu and Ruksha Guna, Sheeta Veerya and Katu Vipaka. These properties lead reduction of Kaptha Dosha. Ayurveda describes three methods to treat Krimi Roga (worm infection). One of them is Prakrutti Vighata that it making the environment unfriendly for worms. S. grandiflora makes the surrounding unfriendly for worms by reducing of Kaptha Dosha in the environment. Ayurveda describes Anulomana (mild laxative) and Krimighna (wormicide) property of S. grandiflora. This acts against larvae of Toxocara canis and Haemonchus contortus as a vermifuge by paralyzing the muscles of larvae due to its Anulomana (mild laxative) property or kill them acting as a wormicide due to its Krimighna property. Jalalpure et al. (2006) had reported the anthelmintic effect against Pheritium pasthuma worm. Leaves of Sesbania grandiflora contains alkaloids, cardiac glycosides, flavanoids, saponins, steroids and tannins. It has been shown that plants with anthelmintic activities contain phytochemicals such as polyphenols, tannins, flavonoids, saponins which may act synergistically to kill worms. The anthelmintic activity of Sesbania grandiflora may be due to tannins, flavonoids, saponins.

CONCLUSION

It is concluded that Sesbania grandiflora (Kathurumurunga) is effective in inhibiting larval migration of Toxocara canis and Haemonchus contortus.

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REFERENCE


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### Table 1: Percentages of *in vitro* larval migratory inhibition of *Toxocara canis* and *Haemonchus contortus* infective larvae with decoction of *Sesbania grandiflora* (Kathurumurunga).

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Percentage (%) of larval migration inhibition (LMI)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td><em>Toxocara canis</em></td>
</tr>
<tr>
<td>Levamisole 200 µg / ml in PBS (Positive control)</td>
<td>99.7</td>
</tr>
<tr>
<td>Phosphate buffered saline (Negative control)</td>
<td>0</td>
</tr>
<tr>
<td><em>Sesbania grandiflora</em> (Kathurumurunga)</td>
<td>98.1</td>
</tr>
</tbody>
</table>

**Figure 1:** Percentages of *in vitro* larval migratory inhibition of *Toxocara canis* and *Haemonchus contortus* infective larvae with decoction of *Sesbania grandiflora* (Kathurumurunga)

### Table 2: Mean ± SD of percentages of *in vitro* larval migratory inhibition of *Toxocara canis* and *Haemonchus contortus* infective larvae with decoction of *Sesbania grandiflora* (Kathurumurunga)

<table>
<thead>
<tr>
<th>Larvae</th>
<th>Levamisole (<em>Positive control</em>) (Mean ± SD)</th>
<th>Phosphate buffered saline (<em>Negative control</em>) (Mean ± SD)</th>
<th><em>Sesbania grandiflora</em> (<em>Kathurumurunga</em>) (Mean ± SD)</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Toxocara canis</em></td>
<td>99.7 ± 0.6</td>
<td>0.00 ± 0.00</td>
<td>98.1 ± 0.6</td>
<td>1.85E-05</td>
</tr>
<tr>
<td><em>Haemonchus contortus</em></td>
<td>96.6 ± 1.21</td>
<td>0.00 ± 0.00</td>
<td>94.3 ± 0.8</td>
<td>0.00</td>
</tr>
</tbody>
</table>
**Figure 2**: Mean ± SD of percentages of *in vitro* larval migratory inhibition of *Toxocara canis* and *Haemonchus contortus* infective larvae with decoction of *Sesbania grandiflora* (*Kathurumurunga*).

**Table 3**: Viability of post-migratory larvae of *Toxocara canis* and *Haemonchus contortus* larvae with decoction of *Sesbania grandiflora* (*Kathurumurunga*).

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Percentage (%) of viability of <em>Toxocara canis</em> larvae</th>
<th>Percentage (%) of viability of <em>Haemonchus contortus</em> larvae</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Grade 0</td>
<td>Grade 1</td>
</tr>
<tr>
<td>Levamisole (Positive control)</td>
<td>55</td>
<td>0</td>
</tr>
<tr>
<td>Phosphate buffered saline (Negative control)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td><em>Sesbania grandiflora</em> (<em>Kathurumurunga</em>)</td>
<td>1.2</td>
<td>0</td>
</tr>
</tbody>
</table>

Grade 0 = Dead; No recovery after prolonged immersion in saline; Grade 1 = Inactive but occasional movement can be observed; Grade 2 = Inactive but intermittent movement can be observed clearly; Grade 3 = Slow moving; Grade 4 = Active.

**Figure 3**: Viability of post migratory larvae of *Toxocara canis* and *Haemonchus contortus* with decoction of *Sesbania grandiflora* (*Kathurumurunga*).