Contents

A preliminary investigation of Vietnamese indigenous plants for anti-parasite activities in goats

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Summary

The anthelmintic effect of the Vietnamese indigenous plants Caliandra (*Calliandra spp.*), Xau Ho Do (*Mimosa pudica*), Keo giau (*Leucaena glauca)*, Xoan (*Melia azedarach*), Oi (*Psidium guyjava*), and Keo tai tuong (*Acacia farnesiana*) were examined *in vitro* using a larval migration inhibition (LMI) assay and in goats carrying a mixed nematode infection. Keo giau, Keo tai tuong and Caliandra inhibited greater than 80% of nematode larvae in the LMI assay. In goats, the plants Caliandra, and Xau ho do were able to reduce the worm egg counts by 74 and 75%, respectively, compared to the controls. The results indicated a potential of using these plants in ethnoveterinary management of parasitic diseases in goats.

Keywords: plants, anthelmintics, larvae, inhibition, goats

Introduction

Gastrointestinal parasites in ruminant animals such as cattle, sheep, and goats cause significant production losses to the farming communities through out the world. In Vietnam, the majority of livestock farmers are small holders, however, they contribute to approximately 25% to the total output of the agriculture sector. Parasitic diseases pose a significant threat to the farmers since the loss of animals will have a devastating effect on the income generating capacity. The current parasite control measures have relied heavily on anthelmintics. However, with the emergence of drug resistant parasites and the concern for the drug residues in meat, milk, and the environment, a cost-effective and sustainable alternative to these chemical drugs is therefore urgently required. Ethnoveterinary medicine has been used widely in developing countries as medicinal remedies. A review by Hammond et al. (1997) listed 23 plants that are routinely used in South East Asian countries as anthelmintics against gastro-intestinal parasites in ruminants, poultry, and pigs. Kokwaro (1993) described the use of 50 indigenous plants in Africa for the treatment of nematode and cestode parasites. The impact and possible therapeutic potential of these indigenous plants is great and are particularly appealing for livestock as these plants and/or their extracts may be readily incorporated into feed supplements such as pellets or feed blocks. In this paper, the effect of Vietnamese indigenous plants on nematodes *in vitro* and in goats is examined.

Materials and methods

The following aerial parts of the plants: Caliandra (*Calliandra spp.*), Xau ho do (*Mimosa pudica*), Keo giau (*Leucaena glauca)*, Xoan (*Melia azedarach*), Oi (*Psidium guyjava*), and Keo tai tuong (*Acacia farnesiana*) were collected from Bavi, Sontay, Vietnam. Plants were dried at room temperature and 20 g of the dried material was cut into small pieces and homogenised in 200 ml of distilled water in a blender. The homogenised material was filtered through two layers of gauze, centrifuged at 400g for 10 minutes, and stored at –20^{o}C before being used.

Faeces from goats naturally infected with nematode parasites were collected from the Goat and Rabbit Research Center in Bavi. The culturing of nematode larvae was as described by Anonymous (1971). The third stage larvae (L3), predominantly *Haemonchus contortus*, obtained

from the larval cultures were washed twice with Phosphate Buffered Saline (PBS) by centrifugation, counted, and adjusted to an appropriate volume with PBS. The L3 were used immediately in the Larval Migration Inhibition (LMI) assay.

Plant extracts were screened for their anti-parasite activity using the modified LMI assay (Wagland et al, 1992). The assay consisted of allowing the L3 migrate through glass tubing of approximately 30mm in length and 6mm in diameter with one end of the tube sealed with 20µm nylon mesh. Triplicates of 800 µl of either PBS (negative control), dilutions of plant extracts, or the positive control Levamisole hydrochloride (Sigma), were added to the tubes sitting in wells of 48 well plate. Two hundreds µl containing 300 nematode L3 were gently transferred down the side of the glass columns. The L3 were allowed to migrate through the mesh overnight at room temperature. The L3 recovered from the bottom of the 48 wells plate were counted using a microscope (Olympus). The % inhibition was calculated using the following equation:

$$\% \text{ Inhibition} = \frac{\text{Numbers of L3 in PBS wells} - \text{Numbers of L3 in treatment wells}}{\text{Numbers of L3 in PBS wells}}$$

Goats of the Saanen and Barbary breeds obtained from the Goat and Rabbit Research Center in Bavi were used in the evaluation of plants. They were approximately one year old and weighed between 20 and 30 kg. Two weeks before the trial, they were brought indoors and kept on raised wooden pens. Faecal samples were collected from all goats and subjected to nematode faecal egg count (FEC) using the McMaster's egg counting technique. Goats were allocated into groups using the criteria of group means FEC of within 10% of each other. Goats were kept indoors for the entire experiment. The goats were fed a staple diet composing of fresh leaves and grass. In addition, the groups were given free access to 2 kg/day of the tested fresh plant for 14 days. FEC was carried out on faecal samples collected from individual goats during the experiment.

One way analysis of variance was performed on untransformed data using the computer program Minitab (version 8.2).

Results and Discussion

The results of the LMI assay showed that Keo Giau, Keo Tai Tuong and Caliandra inhibited between 82, 78, and 88% of L3 respectively (Figure. 1). The plants Xoan, Oi and Xau ho do exhibited 70, 37, and 59% inhibition, respectively. The positive control Levamisole consistently inhibited greater than 90% when used at a concentration of 100ug/ml (Figure. 1).

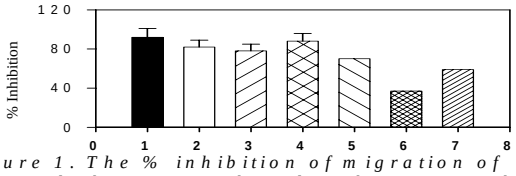

Figure 1. The % inhibition of migration of nematode larvae incubated with Levamisole (1), crude plant extracts (2: Keo giau; 3:Keo tai tuong; 4:Caliandra 5: Xoan; 6: Oi; 7: Xau ho do). Results (means + SD) are from 1-3 experiments.

The plants Keo giau, Caliandra, Xoan, and Xau ho do were evaluated in goats carrying a natural nematode infection. At the start of the experiment, the goats had a mean FEC burden of between 4000 to 5000 eggs per gram (EPG) of faeces. After 7 days of treatment, there were 63, 75, 10, and 65% reduction in the FEC in goats supplemented with Keo giau, Caliandra, Xoan, and Xau ho do, respectively, compared to the controls ($P < 0.05$ for Keo giau, Caliandra, Xau ho do only). After 14 days, there were 75, 92, 34, and 92% reduction in FEC compared to the controls ($P < 0.05$ for Keo giau, Caliandra, Xau ho do only). However, when the above plants were removed from feeding after 14 days, the reduction in FEC at day 18 from goats given Keo giau, Caliandra, Xoan, and Xau ho do were 35, 78, 60, 73%, respectively, compared to the controls ($P < 0.05$ for Caliandra, Xau ho do, Xoan only). At day 28, only goats that were fed with Caliandra, and Xau ho do had a FEC reduction of greater than 70% (74 and 75% respectively, $P < 0.05$) when compared to the

controls (Figure 2). Since, worm counts were not performed at the end of the experiment, the plants Caliandra and Xau ho do may either have an inhibitory effect on the parasite's eggs production or a deworming effect. The plant Xau ho do only inhibited 59% of the larvae in the LMI assay but it was able to reduce the egg worm burden by 75% at day 28 of the experiment. Similarly, Alawa et al. (2003) showed that the Nigerian medicinal plant *Annona senegalensis* inhibited significantly the hatching of eggs from *Haemonchus contortus in vitro*. It would be interesting to determine if the same plant has a deworming effect when tested *in vivo* in ruminants.

The results demonstrate the anti-parasite potential of the plants studied. However, further studies are needed to confirm the *in vivo* results and to identify the amount of plant needed to achieve the effect.

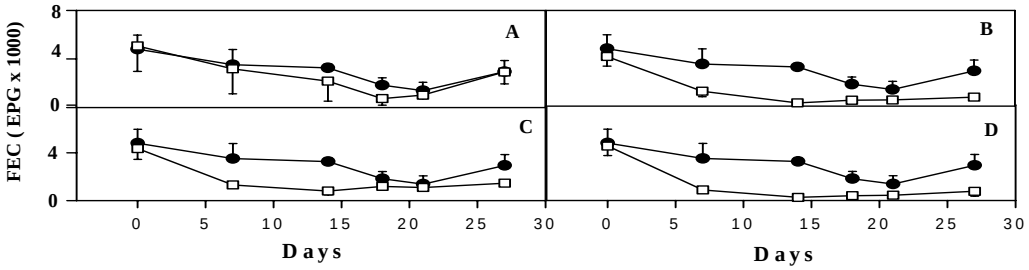

Figure 2. The anthelmintic effect the plants Xoan (panel A), Xau ho do (panel B), Keo giau (panel C), and Caliandra (panel D) on nematode parasites in goats. Control goats (●) are not fed with the above plants whereas treated goats (□) were fed with the respective plant. Results (means ± SD) from 4 goats per group.

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