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Full Length Research Paper

Study on the inhibition effects of some Vietnamese traditional medicinal plants on egg hatching and larval movement of goat *Haemonchus contortus*

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Six traditional herbs which are well-known in Vietnam for their effect on endoparasite treatment, including: *Achyranthes Bidentata Blume*, *Cassia Alata L*, *Embelia Ribes Burn*, *Ipomoea Hederacea Jacq*, *Leucaena Glauca Benth* and *Solanumtorvum Swartz* were collected to evaluate the inhibitory effect on the egg hatching and larval movement of goat *Haemonchus contortus*. The aqueous extracts was made and diluted to 5 %, 10 % and 20 %. All of the herbs showed inhibitory efficacies at different levels, therefore explains their traditional application as the anti-parasite plants. *Cassia Alata L* and *Embelia Ribes Burn* showed the best effect on the egg hatching, while *Achyranthes Bidentata Blume* showed the best effect on the larval movement. In all of 6 tested herbs and 3 tested concentrations, only *Ipomoea Hederacea Jacq* and *Leucaena Glauca Benth* extracts at 20 % had good inhibitory effect (more than 70%) on both of egg laying and larval movement. This result suggests that *Ipomoea Hederacea Jacq* and *Leucaena Glauca Benth* extracts at 20% have the most potential to apply in the *Haemonchus contortus* treatment. The follow-up *in vivo* experiments and clinical trials are still necessary to confirm the effect of those medicine plants and to widen their application.

Key words: Herbs, extracts, antiparasite, *Ipomoea Hederacea Jacq*, *Leucaena Glauca Benth*, goat, *Haemonchus contortus.*

INTRODUCTION

The appearance of synthetic drug resistance to human and animal pathogens has been increasing and become a global problem. Furthermore, side effects, expensive costs, handling hazards and concerns about residues on food and thread to human environments are also their remarkable drawbacks. Many researchers are now trying to search for new agents, in which medicine plants are listed as one of noticeable options. Medicine plants have long history of being used as traditional method and are usually considered as a lower cost and less dangerous side-effects than synthesis compounds (Bui Thi Tho et al., 2006).

Haemonchus contortus, commonly referred to as the barber pole worm, is a clinical problem for goats. Infested goats have lower growth rates, markedly reduced reproductive performance and have high rates of illness and death. *Haemonchus contortusis* is the most economically significant parasite of sheep and goats throughout much of the United States and the world (Rose Paddock, 2010). Anthelmintics (chemicals, drugs) are widely used to manage *Haemonchus contortus*, but their effectiveness has been significantly decreased be-

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cause of the emerging of althelmintic resistance (DeVaney JA, 1992; Joan Burke, 2005; Maria Lenira Leite, 2006).

In this study we focused on the *in vitro* inhibitory activity testing of 6 well-known antiparasitic medicine plants on the egg hatching and larval movement of goat *Haemonchus contortus*.

MATERIALS AND METHODS

The Parasite Egg collection

Fresh feces samples from the goats infected with *Haemonchus contortus* were collected immediately after the animal defecation, following the method described by Nguyen Thi Kim Lan et al. (Nguyen Thi Kim Lan et al., 2008). 100 g of feces were ground and mixed thoroughly with distilled water before filtering through the steel nets to remove the fibrous materials. The resulting suspension was left stand for 15 minutes before being centrifuged at 3000 revolutions per minute for 15 minutes. After the upper part was removed, the remained solution was pour into a special 500 ml bottle which contained saturated sugar solution.

The saturated sugar solution was added more to top up the bottle before being put and left stand for 1 hour horizontally, in which the convex side of the bottom was up. Because this convex side is specifically designed for the egg attachment, we collected the eggs by using the distilled water to wash this convex surface. The egg-containing water was then centrifuged at 1000 revolutions per minute for 10 minutes before removing the upper part. New water was added and the centrifuge was repeated in order to wash the eggs solution. The McMaster egg counting slide was used to count the number of the eggs, and then the concentration of the eggs was adjusted to 2000 eggs/ ml. This solution will be used for the experiment with herbal extracts and drugs.

The parasite larva collection

The parasite larva was collected immediately after the animal defecation, following the method described by Nguyen Thi Kim Lan et al., (Nguyen Thi Kim Lan et al., 2008).

600 g of feces was ground and put into the 500 ml bottle. The feces was then wetted thoroughly with water. The water was applied every 2 days to keep the feces wet. After 1 week, we collected the larva. We filled the bottle with water, used the petri dish to cover the bottle and then reversed the bottle (Photo 1). Pour the water to the petri dish until almost full, and left for 2 hours. The larva moved from the bottle to the water in the Petri dish, therefore we collected the larva by taking this water. The process was repeated for 3 times. The larva-containing water was kept in the beaker with the black nylon cover

Photo 1. Collect the larva from 1 week incubated goat feces

for 1 hour for the larva to precipitate, and the upper solution was removed. We counted the number of larva to adjust the concentration to 2000 larva/ ml to test with the herbal extracts and drugs.

Herb Extracts and Drugs

The leaves of the 6 herbs were dried in 50^{o}C for 24 hours before grinding into powder. 2.5 g of leaf powder was stirred with 10 ml of distilled water and left for 24 hs. We filtered the solution through the cheese cloth and then centrifuged at 3000 revolutions/ m before passing through number 2 qualitative paper. The resulted solution was adjusted to 10 ml and considered as the 25 % herbal extracts. The extracts were diluted to 5 %, 10% and 20 % to use in experiments.

4 common used anti-parasite drugs, including Levamisol (main compound: Levamisol, Central Veterinary joint stock company, Vietnam), Vimectin (main compound: Ivermectin, Hanvet joint stock company, Vietnam), Bivermectin (main compound: Ivermectin, Hanvet joint stock company, Vietnam), Ascarex D (main compound: Piperazine dihydrochloride, Hanvet joint stock company, Vietnam) were bought from the local veterinary pharmaceutical shops.

The Inhibition on Egg Hatching

50 µl of extracts at 5 %, 10% and 20 % was added into 96 well-plates. The negative control was made with 50 µl of phosphate-buffered saline (PBS) solution. The similar samples were also made with 50 µl of Levamisol, Vimectin, Bivermectin and Ascarex at 0.1 % to compare. After that, we added 100 µl of egg containing solution (at 2000 eggs/ ml). The plates were covered with the paraffin and then left for 16 hours. Finally, lugol's solution was added to all wells to kill the eggs. The numbers of eggs were counted, and the egg hatching inhibition of the extracts or drugs were calculated through the following formula:

$$\text{Inhibition percentage (\%)} = \frac{N(n) - N(e)}{N(n)} \times 100$$

N (n): the number of larvae in the negative control well
N (e): the number of larvae in the experimental well
The experiments were performed for 3 times. In our research, we separated the inhibition effect of the herbs on egg hatching into the 3 levels using the standard which is usually used in Vietnam to evaluate the anti-parasite effects of the herbs (Bui Thi Tho et al., 2006), including:
More than 70 %: good effect
From 50 to 70 %: intermediate effect
Less than 50%: weak effect

The Inhibition on Larval Movement

800 µl of different extract were added to each well of 48-well plate (Photo 2). The similar samples were made with 800 µl of Levamisol, Vimectin, Bivermectin and Ascarex at 0.1 % to compare. The negative control was made with 800 µl of PBS. The bottom of 8 mm diameter glass tubes were cut and covered with 25 µm pore-size nylon net. Each glass tube with covered nylon net were kept stand on a well of 48 well-plates by the rubber circles (Photo 3). Each well was added with 100 µl of the larvae – containing solution (2000 larvae /ml). After 16 hs, the glass tubes were removed and 1 drop of lugol's solution was added to each well in order to kill the larvae. The percentage of the larval movement inhibition of the extract will be calculated through the following formular:

$$\text{Inhibition percentage (\%)} = \frac{N(n) - N(e)}{N(n)} \times 100$$

N (n): the number of larvae in the negative control well
N (e): the number of larvae in the experimental well

Photo 2. The extracts and drugs were added on 48 well-plate

All of the experiments were performed for 3 times. In our research, we separated the inhibition effect of the herbs or drugs on larval movement into the 3 levels using the standard which is usually used in Vietnam to evaluate the anti-parasite effects of the herbs (Bui Thi Tho et al., 2006), including:
More than 70 %: good effect
From 50 to 70 %: intermediate effect
Less than 50%: weak or no effect

Photo 3. Eight mm glass tubes were kept stand on 48 well-plates by the rubber circles.

Statistic Analyse

Data was expressed as mean ± standard deviation (Mean ± SD). Data were analyzed using the Statcel software (Yanai Hisae, Laboratory of mathematics, Faculty of Science, Saitama University, 1998). The result was considered significant at probability value less than 0.05 ($p< 0.05$).

RESULTS

The Inhibition on Egg Hatching

The inhibition effects on goat *Haemonchus contortus* egg hatching of 6 herbals extracts and 4 commercial drugs

From Table 1, we see that *Cassia Alata L* and *Embelia Ribes Burn* showed the best effect on the egg hatching. At 5% concentration, those herbs had higher efficacy than the effect of Levamisol 1 %, which is the most effective drugs in the experiments. Second to *Cassia Alata L* and *Embelia Ribes Burn*, the good effect (more than 75 % of inhibition) was also observed in *Ipomoea Hederacea Jacq* at all of 3 concentrations and *Leucaena Glauca Benth* at the concentration of 20% (88.4 ± 0.5 % of inhibition). *Achyranthes Bidentata Blume* showed the intermediate effects at all of 3 tested concentrations, while only weak effect was observed on *Solanumtorvum Swartz*.

The Inhibitory on Larval Movement

From Table 2, we see that the best effect was observed in *Achyranthes Bidentata Blume*, with the good inhibition

Table 1. The inhibition effects on goat *Haemonchus contortus* egg hatching of 6 herbals extracts and 4 commercial drugs.

Herb	Concentration 20%	10 %	5 %
Achyranthes Bidentata Blume	58.7 ± 0.8 [d]	58.7 ± 0.8 [d]	53.2 ± 1.3 [d]
Cassia Alata L	**100.0 ± 0.0 [a]**	**100.0 ± 0.0 [a]**	**99.0 ± 0.4 [a]**
Embelia Ribes Burn	**99.8 ± 0.4 [a]**	**99.8 ± 0.4 [a]**	**99.0 ± 0.4 [a]**
Ipomoea Hederacea Jacq	90.0 ± 0.7 [a]	90.0 ± 0.7 [b]	87.8 ± 1.3 [b]
Leucaena Glauca Benth	88.4 ± 0.5 [c]	71.1 ± 0.8 [c]	66.7 ± 1.4 [c]
Solanumtorvum Swartz	13.3 ± 0.9 [e]	13.3 ± 0.9 [e]	7.7 ± 4.6 [e]
Drug	Concentration : 1 %		
Levamisol	93.8 ± 0.5 [A]		
Vimectin	92.5 ± 0.8 [A]		
Bivermectin	83.2 ± 1.4 [B]		
Ascarex D	81.5 ± 1.3 [B]		

Means with different superscripts (a, b, c, d, e) within each column are significantly different ($p < 0.05$) in the extract group by one-way ANOVA and *Post – hoc* Fisher's least significant difference test and the numbers with bold letters indicate the highest inhibition effect. Means with different superscripts (A, B) within each column are significantly different ($p < 0.05$) in the drug group by one-way ANOVA and *Post – hoc* Fisher's least significant difference test.

Table 2. The inhibition effects on goat *Haemonchus contortus* larval movement of 6 herbals extracts and 4 commercial drugs.

Herb	Concentration 20%	10 %	5 %
Achyranthes Bidentata Blume	**83.0 ± 1.1 [a]**	**76.6 ± 1.7 [b]**	**75.7 ± 1.2 [a]**
Cassia Alata L	64.0 ± 1.0 [c]	60.8 ± 1.0 [c]	48.3 ± 4.2 [c]
Embelia Ribes Burn	51.8 ± 0.6 [d]	30.7 ± 0.7 [e]	30.1 ± 0.3 [e]
Ipomoea Hederacea Jacq	73.3 ± 1.1 [b]	61.0 ± 0.9 [c]	43.8 ± 1.7 [d]
Leucaena Glauca Benth	76.8 ± 1.5 [b]	56.1 ± 0.6 [d]	28.6 ± 0.4 [e]
Solanumtorvum Swartz	84.5 ± 7.0 [a]	86.6 ± 1.4 [a]	57.1 ± 0.4 [b]
Drug	Concentration : 1 %		
Levamisol	86.9 ± 1.4 [A]		
Vimectin	19.7 ± 0.6 [B]		
Bivermectin	30.3 ± 0.3 [C]		
Ascarex D	49.2 ± 1.3 [D]		

Means with different superscripts (a, b, c, d, e) within each column are significantly different ($p < 0.05$) in the extract group by one-way ANOVA and *Post – hoc* Fisher's least significant difference test and the numbers with bold letters indicate the highest inhibition effect. Means with different superscripts (A, B) within each column are significantly different ($p < 0.05$) in the drug group by one-way ANOVA and *Post – hoc* Fisher's least significant difference test.

Table 3. Evaluation of 6 herbals extracts inhibition effect on egg hatching and larval movement of goat *Haemonchus contortus*.

Herb	Concentration					
	20%		10 %		5 %	
	Egg	Larval	Egg	Larval	Egg	Larval
Achyranthes Bidentata Blume	Inter	Good	Inter	Good	Inter	Good
Cassia Alata L	Good	Inter	Good	Inter	Good	Weak
Embelia Ribes Burn	Good	Inter	Good	Weak	Good	Weak
Ipomoea Hederacea Jacq	**Good**	**Good**	Good	Inter	Good	Weak
Leucaena Glauca Benth	**Good**	**Good**	Good	Inter	Inter	Weak
Solanumtorvum Swartz	Weak	Good	Weak	Good	Weak	Inter

Bold letters indicate the case of good inhibition effect on both eggs and larva.

on all of 3 tested concentrations. The good effect was also observed in *Solanumtorvum Swartz* at 20 % and 10 %, and in *Leucaena Glauca Benth* and *Ipomoea Hederacea Jacq* at 20%. Other herbs only showed the weak efficacy.

The Evaluation of Anti-Parasite Effect

From Table 3, we see that only the *Ipomoea Hederacea Jacq* and *Leucaena Glauca Benth* at 20 % had good inhibitory effect on both of egg laying and larval movement.

DISCUSSION

In our study, all of the extracts from 6 experimental herbs showed the inhibitory effect on egg hatching and larval movement of goat *Haemonchus contortus*. These results explain the traditional application of those herbs in the endoparasites treatment in Vietnam. Our results are in accordance with other studies, which also reported the parasiticide effects of *Achyranthes Bidentata Blume* (Do Tat Loi, 2003), *Cassia Alata L* (Maria Lenira Leite., 2006), *Embelia Ribes Burn* (Sajith Mohandas, 2013; Syed Asadulla et al., 2011), *Ipomoea Hederacea Jacq* (Do Tat Loi, 2003; Le Tran Duc, 1995), *Leucaena Glauca Benth* (Do Huy Bich, 1995; Do Tat Loi, 2003; Le Kha Ke, 1971) and *Solanumtorvum Swartz* (Do Tat Loi, 2003).

In our study, we see that only the *Ipomoea Hederacea Jacq* and *Leucaena Glauca Benth* at 20 % had good inhibitory effect on both of egg laying and larval movement. One herb or drug is considered as a good parasiticide therapy and can be applied for the treatment when it has good inhibition on both of egg laying and larval movement (Nguyen Thi Kim Lan et al., 2008). Therefore, we recommended that *Ipomoea Hederacea Jacq* and *Leucaena Glauca Benth* at the concentration of 20 % are the most potential candidates for the treatment of goat *Haemonchus contortus*. Our study suggests the possibility of applying herbs as a rich source of anti-parasite agents for the management of veterinary endoparasites, including the goat *Haemonchus contortus*, which is well-known for causing significantly economic lost in goat husbandary. However, our results are only tested inhibition effect on the egg hatching and larval movements, and follow-up research is important to determine the effect on the parasites and also to determine the active components in each extracts and confirm their mechanism.

CONCLUSION

The *Ipomoea Hederacea Jacq*, *Leucaena Glauca Benth* aqueous extracts at the concentration of 20 % have high potential to apply in the treatment of goat *Haemonchus contortus*

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