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Research Article

Effect of *Jatropha curcas* Latex on L3 *Haemonchus contortus* Larval Motility

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ABSTRACT

The anthelmintic resistance has limited the control of gastrointestinal nematodes of small ruminants and thus has awakened interest in the study of plants extract as a source of anthelmintics. These experiments were carried out to evaluate the in vitro efficacy of Jatrophacurcas latex extract against Haemonchuscontortus larval motility. To evaluate the larvicidal activity, H.contortus L3 were incubated with the extracts with varying concentration of 5 mg/mL, 10 mg/mL, 15 mg/mL and 20 mg/mL at 27°C for 48, 72 and 96 hrs. The results were subjected to the Kruskal-Wallis test ($P<0.05$). The extracts showed dose-dependent larvicidal effects. These results suggest that *J.curcas* can be used to control gastrointestinal nematodes of small ruminants.

Keywords: Anthelmintics, *Jatropha curcas, Haemonchus contortus*, Larval motility and Larvicidal effects.

INTRODUCTION

Gastrointestinal parasitism was considered to be a multifaceted disease that causes declination of profitable livestock production especially among sheep and goats all over the world[1], including tropical and sub-tropical countries[2]. They infect all types of livestock but depending on various factors like severity, host range and life cycle. Nematodiases causes 28% of mortality in small stock and weight loss of about 3-8% in livestock, causing US$ 2 billion wastage per annum in many countries[3]. Among various intestinal parasites, *Haemonchus contortus* is one of the most significant and accountable parasite causing acute and sub-acute parasitic gastroenteritis of small ruminants[4]. In Malaysia predominantly Sabah, high-level stock losses from the farm were recorded for several years. As frequently accompanied by clinical signs indicating pathogenic levels of infections with the nematode parasite *H.contortus*[5]. The pathogenicity of these parasites is due to its obtained nutrition and shelter from animal host, which results in poor growth rate, reduced fertility, less immunity, damaged gastric function, high mortality and increased costs of management[1]. These parasites are controlled by the administration of synthetic anthelmintics. However, in many countries, the indiscriminate use of anthelmintic has resulted in the establishment of parasite resistance[6]. Evidence from various sources also indicated the severity of multiple anthelmintic resistances that reached extremity[7]. Therefore, the plant phytochemicals, or plant extracts, have become increasingly attractive as an alternative vector control agents. They show small or no harmful effects on non-target organisms and the environment[8].

Bioactive plants, an alternative for the control of gastrointestinal nematodes, are rich in secondary metabolites. Previous researches have emphasized the importance of certain plant extract as a source of anthelmintic. However, development of herbal substances in terms of controlling parasites may vary in their results, but are effective and compatible with human and animal life[9, 10]. *Jatropha curcas L.* (Physic nut) plant is one of that effective agent that have anthelmintic effect. *J.curcas* is commonly known as physic nut or purging nut and it belongs to Euphorbiaceae family. The plant is found in tropical regions of Africa, South America, South East Asia and India[11]. *J.curcas* is considered as a large shrub or a small perennial tree because of its 5 m height, but under several conditions their height can also reach up to 8 or 10 m[12]. The plant has soft wood with subtle grey bark and when cut the bark, produces white and milky latex[13]. The sap (latex) and crushed leaves of *J.curcas* have shown anti-parasitic activity. In addition to its use as anthelmintic, most plant parts are used for the treatment of various human and veterinary ailments. Phytochemical screening of *J.curcas* leaves and bark extract revealed the presence of alkaloids, flavonoids and steroids and these compounds are known to possess insecticidal and larvicidal properties[14]. Root and stem extract of *J.curcas* plant have been demonstrated to possess pesticidal and insecticidal effects[15]. Petroleum ether extracts of *J.curcas* has also been studied and analysed for positive larvicidal properties[16]. Even the latex of *J.curcas* plant contains antibacterial compounds against *Staphylococcus aureus*[17]. Up to our knowledge, there are no reports on *J.curcas* plant study against *H.contortus* parasites in Malaysia. Thus, the main purpose of this study is to determine the efficacy of

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Table 1: In vitro anthelmintic activity of *J.curcas* latex extract on L3 *H.contortus*.

Conc. (mg/mL) / Incubation time (hours)	Larval motility inhibition (%) *Jatropha curcas* (mg/mL)					Positive control	Negative control
	5	10	15	20	25		
48	0	0	4.76	17.39	50	0	0
72	5	22.73	23.81	50	55	21	0
96	50	58.82	57.14	66.67	70.59	50	0

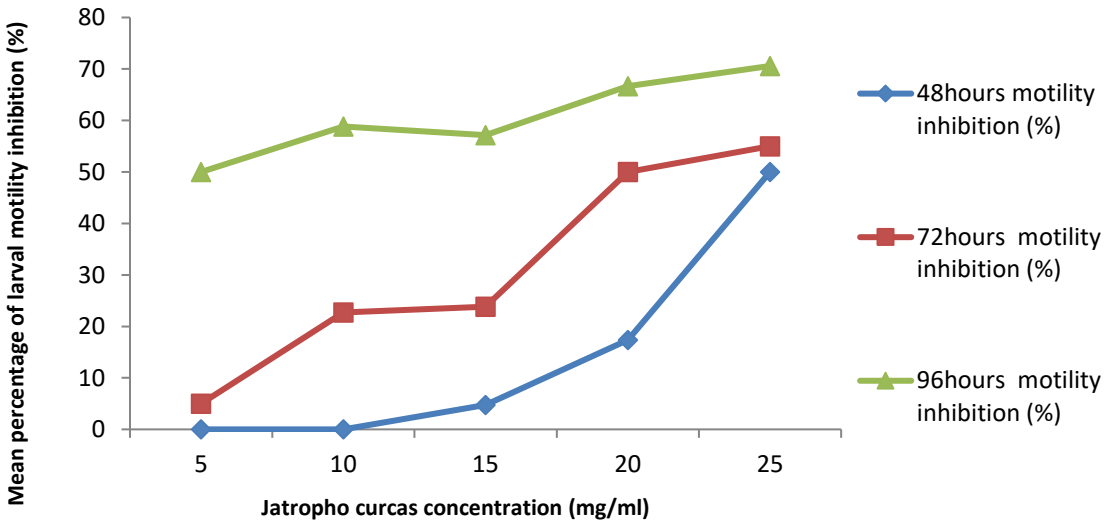

Figure 1: Mean percentage motility of larvae at different hours of exposure of nematode parasite *H.contortus* to increasing concentration of *J.curcas* latex extract.

J.curcas latex at different concentration which prevents the larval motility of L3, *H.contortus.*

MATERIALS AND METHODS

This research is aiming at evaluating the anthelmintic potential of *J.curcas* by incubating the L3 *H.contortus* with *J.curcas* latex at different concentrations.

Preparation of Jatrophacurcas latex

The latex was collected from the bark of the plant. The collected latex was centrifuged at 10,000 rpm for 10 mins to remove the unnecessary sedimentation. Then, the latex was dried by incubating them at 45-50°C for 2 days. The dried latex was weighed to estimate the yield.

Larval culture

From the rectum of naturally exposed goats, faecal sample were collected to make a positive samples for copro-culture which was needed for the procurement of third stage larvae (L3)[18]. After culture, third stage larvae (L3) were isolated and collected through Baermann's technique. The collected larvae were identified according to the morphological keys and stored at 4°C until next use.

Larval motility test

Samples of 50µL suspensions containing 20 L3 of *Haemonchus contortus* were distributed in 1.5mL Eppendorf tubes. Then, the latex extract of *Jatropha curcas* at concentration of 5mg/mL, 10mg/mL, 15mg/mL and 20mg/mL were added to all the tubes. Distilled water was used as negative controls. The samples were incubated at 27°C for 48, 72 and 96 hrs. The number of motile and non-motile larvae were counted, particularly focusing on the presence or absence of smooth sinusoidal movement, respectively. Results were expressed as % inhibition of larval motility as a representation of two independent experiments performed in triplicate[19].

Statistical analysis

The results were statistically evaluated by analysis of variance (ANOVA) and means were compared by Kruskal-wallis test at ($p<0.05$) level of confidence using SPSS 17.0 tool.

RESULTS AND DISCUSSION

Present study was done to analyze the efficiency of Jatropha curcas (J.curcas) latex extract against haemonchosis in goat. I*n vitro* anthelmintic activity of *J.curcas* latex extract to inhibit L3 of H.contortus larval motility was performed by experiment. The results showed that the latex extract of *Jatropha curcas* have anthelmintic properties against L3 *H.contortus.* Table 1 showed the larval motility inhibition (%) of *Jatropha curcas* bark extract at various concentration against L3 *H.contortus*.

From the Table 1, it was shown that the latex extract of *J.curcas* at concentration of 5mg/ml, 10mg/ml, 15mg/ml and 20mg/ml and 25mg/ml were used to assess the anthelmintic performance of the plant. Several investigators reported on the performance of medicinal plants extracts against the treatment of haemonchosis[20]. In vitro test to evaluate the inhibition of larval motility has been widely used in veterinary parasitology to the prospecting of novel anthelmintic agents[21]. The infective L3 larvae stage of the nematode species was chosen in this

research as they have high capacity to survive even in adverse conditions. Moreover, larvae stages are the feeding stages, and therefore they could even ingest or absorb medicinal components[22], thus make it more feasible in this research. The advantage of the assay is that compounds or materials to be tested are in direct contact with the infective life-cycle stage of the parasite. Most studies have been conducted on many other parasites with *J.curcas* plant extract. In this study, the efficacy was varied with the extract concentrations and compared with the negative control, distilled water and positive control, Albendazole. The larvae were incubated for 48, 72 and 96 hrs and the results were shown in Fig 1.

At 48 hours of incubation, the latex extract of *J.curcas* at concentration of 5mg/ml, 10mg/ml, 15mg/ml, 20mg/ml and 25mg/ml inhibited the motility of L3 by 0%, 0%, 4.76%, 17.39% and 66.67% respectively. At 72hours of incubation, the latex extract of *J.curcas* at concentration of 5mg/ml, 10mg/ml, 15mg/ml, 20mg/ml and 25mg/ml inhibited the motility of L3 by 5%, 22.73%, 23.81%, 50% and 55% respectively. Finally, at 96 hours, the motility of the L3 were observed to be much slower and the latex extract of *J.curcas* at concentration of 5mg/ml, 10mg/ml, 15mg/ml, 20mg/ml and 25mg/ml inhibited the motility of L3 by 50.00%, 58.82%, 57.14%, 66.67% and 70.59% respectively. The positive control and negative control had no significant effect on the larval motility inhibition. The larvae were moving actively as usual in both the controls. Unlike positive control albendazole, the latex extract of *J.curcas* at 15mg/ml, 20mg/ml and 25mg/ml had shown increasing reductions in larval motility of L3 *H.contortus* accordingly. Through observation, the movements of the larvae were also much slower and less active after incubated with *J.curcas* latex as compared to positive control. Based on the observation done, as the incubation time and concentration of J.curcas latex increases, the motility of the L3 larvae seem to be much slower. It was also observed that the larvae were started to prolong coiling themselves during this interval.

According to Siamba and Namasaka, (2008), a physical change manifested as coiling is associated with the larvae response to gradual external stress[23]. It was considered as a survival strategy aimed at conserving energy (lipid reserves) and protecting the parasite against molecular damage and ensuring survival long enough beyond the span of the stressful period. As compared to previous research, positive results were also found on L3 of *H.contortus* with the methanolic extracts of *A.indica* and *A.roburghiana*[24]. Molefe et al. (2012) have obtained positive result on L3 of gastrointestinal nematodes with *A.afra* and *Mentha longifilia* extracts[25]. Akter et al. (2014) also achieved the same positive results on L3 of H.contortus with medicinal plants named neem, tamak, korolla, halud, chatim, sharna lata and lazzabati[26]. The result of the plant extract showed dose-dependent larvicidal effects on *H.contortus*. Therefore, suggesting that *J.curcas* can be used to control gastrointestinal nematodes of small ruminant.

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CONFLICT OF INTEREST

None declared.

REFERENCES

1. Cala AC, Chagas ACS, Oliveira MCS, Matos AP, Borges LMF, Sousa LAD, Souza FA, Oliveira GP. Invitro anthelmintic effect of *Meliaazedarach* L. and *Trichiliaclasseni C.* against sheep gastrointestinal nematodes *ExpeParasitol.* 2012;10: 98-102.
2. Marley CL, Cook R, Barrett J, Keatinge R, Barrett J, Lampkin NH. The effect of birds foot trefoil (Lotus corniculatus) and chicory (Cichoriumintybus) on parasite intensities and performance of lambs naturally infected with Helminth parasites.Vet. Parasitol. 2003; 112: 147–155.
3. Eguale T, Tilahun G, Debella A, Feleke A, Makonnen E. In vitro and in vivo anthelminthic activity of crude extracts of *Coriandrumsativum* against *Haemonchus contortus. J. Ethnopharmacol*. 2007; 110: 428-33.
4. Fakae BB, Musongong GA, Chiejina SN, Behnke J, Gongeha L, Wakelin D. Variability in the resistance of the Nigerian West African Dwarf goat to abbreviated escalating trickle and challenge infections with *Haemonchuscontortus*. *Vet. Parasitol*. 2004. 122: 51–65.
5. Chandrawathani P, Yussof N, Waller PJ. Total anthelmintic failure to control nematode parasites of small ruminants on government breeding farms in Sabah, east Malaysia. *Veterinary Research Communications*. 2004; 28: 479-489.
6. Melo ACFL, Bevilaqua CML, Reis IF. Anthelmintic resistance to benzimidazole in gastrointestinal nematodes from small ruminants of semi-arid Brazilian northeast. *Ciencia Animal.* 2009;10:294-300.
7. Torres-Acosta JFJ, Mendoza-de-Gives P, AguilarCaballero AJ, Cuéllar-Ordaz JA. Anthelmintic resistance in sheep farms: update of the situation in the American continent. *Vet. Parasitol.* 2012; 189: 89–96.
8. Mathivanan T, Govindarajan K, Elumalai K, Ananthan A. Mosquito Larvicidal and Phytochemical Properties of *Ervantaniacoronaria* Stapf.(FamilyApocynaceae). *J.VectorBorneDis.* 2000; 44:178-180.
9. Shaalan EA, Canyon D, Younes MWF, Abdel Wahab H, Mansour AH. A review of botanical Phytochemicals with mosquitocidal potential. *Environ Int. 31*. 2005; 1149-1166.
10. Chaithong U, Choochote W, Kamsuk K, Jitpakdi A, Tippawangkoso, P,Chaiyasit D. Larvicidal effect of pepper plants on *Aedesaegypti (L.) (Diptera: Culicidae)*. *Journal of Vector Ecology*. 2006; 31: 138-44.
11. Schmook B, Seralta-Peraza L. J. curcas: distribution and uses in the Yucatan Peninsula of Mexico.In: Gu¨bitz G, Trabi M, Mittelbach M (ed) Biofuels and industrial products from Jatrophacurcas. DBV, Graz. (1997).

12. Divakara BN, Upadhyaya HD, Wani SP, Gowda CLL. Biology and genetic improvement of *Jatrophacurcas L* : a review. *Appl Energ.*2010; 87(3):732–742.
13. Horiuchi T, Fujiki H, Hirota M, Suttajit M, Suganuma M, Yoshioka A, Wongchai V, Hecer E, Sugimura T. Presence of tumor promoters in the seed oil of Jatrophacurcas L. from Thailand. *Jpn J Cancer Res.*1987. 78(3):223-226.
14. Ojha K, Pattabhiramaiah DM. Evaluation of Phytochemicals, Larvicidal Activity of JatrophaCurcasSeed Oil against AedesAegypti. *International Journal of Applied Research and Studies.* 2013; 2(12).
15. Aiyelaagbe OO, Adeyeni BA, Fatunsin OF, Arimah BD. In vitro antimicrobial activity and phytochemical analysis of *Jatrophacurcas* roots. *Int. J.Pharmacol.*2007; 3(1): 106-110.
16. Rahman M. In vitro and in vivo anthelmintic effects of some plants against gastrointestinal nematodes of goats. M.S. Thesis submitted to the Department of Parasitology, Bangladesh Agricultural University, Mymensingh. 2002.
17. Thomas R, Sah NK,Sharma PB. Therapeutic biology of *Jatropha curcas*: a mini review. *Current Pharmaceutical Biotechnology.*2008; 9(4): 315–324.
18. Zajac AM, Conboy CA. *Veterinary Clinical Parasitology*, 8th edition. Blackwell Publishers, UK. 2011.
19. Fereira LE, Castro PMN, Chagas ACS, Franca SC, Beleboni RO. In vitro anthelmintic activity of aqueous leaf extract of *Annona muricata L.(Annonaceae)* against *Haemonchus contortus from* sheep.*Experimental Parasitolog.* 2013.134: 327-332.
20. Rahuman AA, Venkatesan P. Larvicidal efficacy of five cucurbitaceous plant leaf extracts against mosquito species. *Parasitology Research*.2008; 103: 133–9.
21. Hounzangbe-Adote MS, Paolini V, Fouraste I, Moutairou K, Hoste H.In vitro effects of four tropical plants on three life-cycle stages of the parasitic nematode, Haemonchus contortus. *Res Vet Sci. 2005.*78:155-160.
22. Ferhat M, Muhammad Q, Zaheer-Ud-Din K., Nameen I, Shahid M, Muhammad L, Phool S. In vitro evaluation anthelmintic activity of essential oils from different parts Skimmalaureola (DC.) zucc.ex.walp., ver Nair. *Pakistan J Botany*.2011. 43:2915-18.
23. Siamba ND. Mechanisms of Hypobiosis in *Haemonchus Contortus* of Goats in Semi-Arid Lands (SAL) in Kenya. Thesis, submitted to the Department of Biological Sciences, MOI University. 2008.
24. Khan S, Afshan K, Mirza B, Miller JE, Manan A, Irum S, Rizvi SS, Qayyum M. Anthelminthic properties of extracts from Artemisia plants against nematodes. *Trop Biomed.* 2015. 32(2):257-68.
25. Molefe NI, Tsotetsi AM, Ashafa AOT, Thekisoe OMM. In vitro anthelmintic effects of *Artemisia afra* and *Mentha longifolia* against parasitic gastro-intestinal nematodes of livestock. *Bangladesh J Pharmacol*. 2012; 7:157-163.
26. Akter KN, Karmakar P, Das A, Anonna SN, Shoma SA, Sattar MM. Evaluation of antibacterial and anthelmintic activities with total phenolic contents of *Piper* betel leaves. *Avicenna Journal of Phytomedicine*. 2014. 4: 320–329.