



ANTHELMINTIC ACTIVITY OF *Aristolochia indica* EXTRACTS AGAINST *Haemonchus contortus*

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Received - 04.05.2018

Accepted - 08.07.2018

Abstract

Haemonchus contortus is one of the most important parasites causing reduced production in livestock throughout the tropics and in most other countries. The present study has been undertaken to screen and evaluate a commonly used plant of Tamil Nadu namely, *Aristolochia indica* for its anthelmintic activity against *H. contortus* using *in vitro* test. Four different types of extracts viz aqueous, ethanol, chloroform and acetone were prepared and used for larval paralysis assay. The results revealed that all the extracts produced significant inhibition of the larval motility *in vitro*. At the end of one hour, the results showed that ethanolic extract (73.88 – 91.66%) was most effective, followed by aqueous (62.5–91.50%) and chloroform extract (42.42- 91.66%). The larval paralysis produced by all the extracts at the highest concentrations tested was below that of the positive controls, ivermectin (85-100%) and levamisole (90-100%). Results indicated that the ethanolic, aqueous and chloroform extracts of *A. indica* contained certain compounds active against *L₃* larva, potent enough to inhibit its normal motility. The larval paralytic action produced within a short duration of one hour also reflects that the active moieties were well

absorbed through the larval cuticle. LPA results of this study showed that the *L₃* paralytic activity was consistently above 90% in three of the *A. indica* extracts, except its acetone extract with a lower activity, which shows a strong potential for future use as anthelmintic for replacing synthetic compounds with resistance.

Key words: Anthelmintic activity, *Aristolochia indica* extracts, *Haemonchus contortus*, Larval paralysis assay.

Haemonchus contortus is one of the most important parasites causing reduced production in livestock throughout the tropics and in most other countries including India. Consequently, there is an urgent need to control infections caused by *H. contortus* in small ruminants. Over the past five decades, the control of this parasite has been achieved through the repeated use of currently available broad spectrum anthelmintics, which led to the emergence of nematode population resistant to these drugs (McKenna *et al.*, 1995). In addition to anthelmintic resistance (AR), the indiscriminate use of these anthelmintics increases the risk of chemical residues in food

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products (Muhammad *et al.*, 2004). Hence, novel approaches are required for nematode parasite control in small ruminants to address the problem of AR (Waller, 1997). Anthelmintic resistance in nematodes of sheep and goats has been reported in India (Yadav and Uppal *et al.*, 1992; Das and Singh, 2005). Gastrointestinal nematodes in sheep and goats are high during monsoon seasons in most of the agroclimatic zones in TamilNadu (Arunachalam, 2008) and during north east monsoon in certain zones (Jeyathilakan *et al.*, 2003).

Mali and Mehta (2008) also reported on the efficacy of alcoholic extract of stem bark of *P. granatum* in inhibiting hatching of *H. contortus* eggs. Tariq *et al.* (2009) evaluated the anthelmintic efficacy of aerial parts of *Artemisia absinthium* against GI nematodes of sheep which were comparable to albendazole. Keeping the above facts in view, the present study has been undertaken to evaluate a commonly used plant of TamilNadu namely, *Aristolochia indica* (family *Aristolochiaceae*) for its anthelmintic activity against *H. contortus* using *in vitro* test.

Materials and Methods

Aristolochia indica whole plant was collected washed and air dried in shade, ground in an electrical blender and was sieved (mesh size 20) to obtain fine powder. Four hundred grams of powder was macerated in one litre of water for 48 hours at room temperature to prepare the aqueous extract. Maceration was followed by double filtration using muslin cloth and Whatman no.1 filter paper, respectively. The filtrate obtained was dried by rotary evaporator. The same procedure was repeated to prepare the ethanol, acetone and chloroform extracts. The extracts were stored at - 20°C till further use. The four different extracts prepared from *A. indica* were subjected to qualitative and quantitative analysis for phytochemical constituents. The extracts were analysed using GC-MS in order to identify the active principles present.

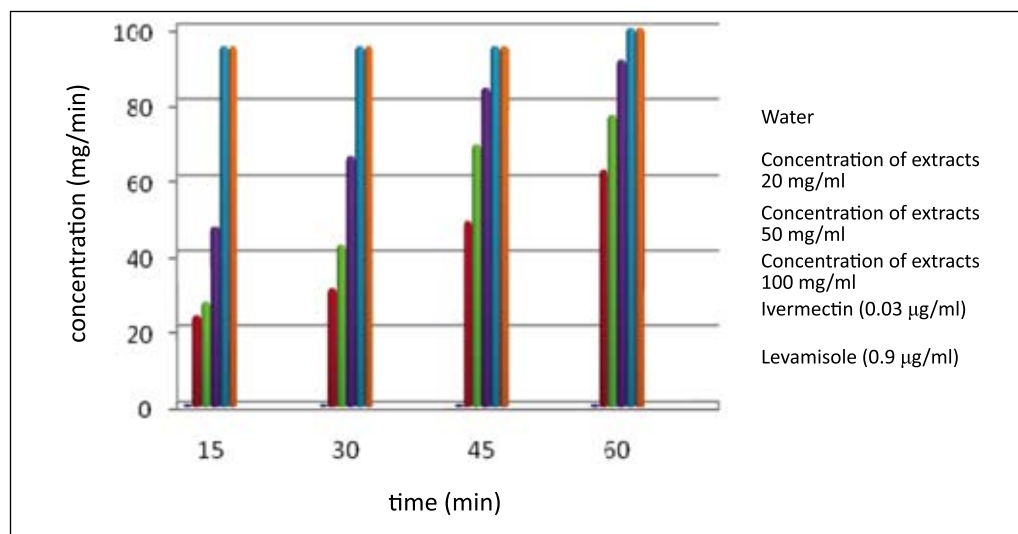
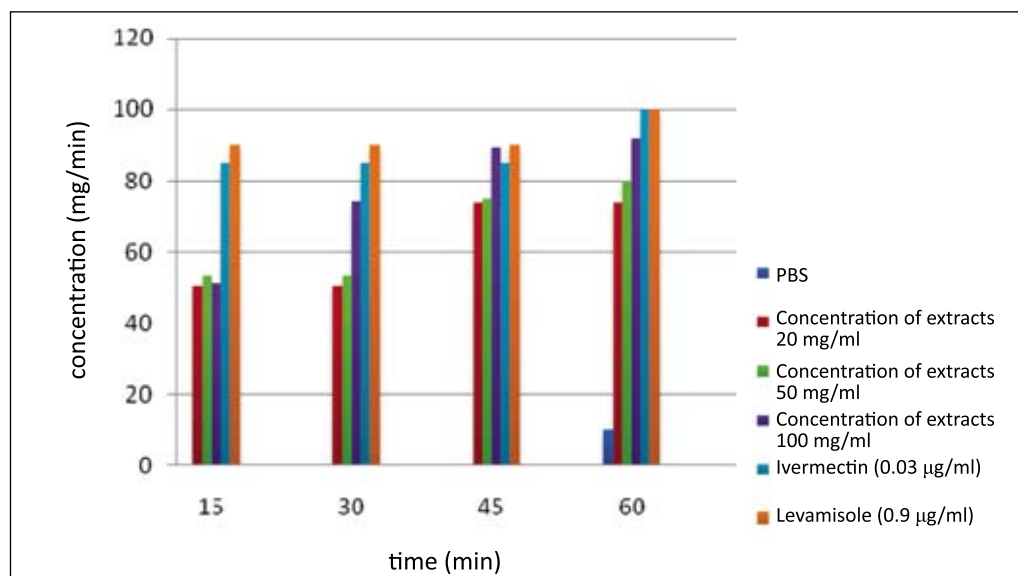
Larval Paralysis Assay (LPA): Larvae required for conducting LPA were raised through coprocultures as suggested by MAFF

(1971). LPA was conducted by the method described by Sutherland and Lee (1990). Third stage larvae of *H. contortus* harvested from coproculture were washed thoroughly with normal saline solution by repeated centrifugation. Approximately 25-30 L₃ larvae in 100µl were pipetted into the wells of a 24 multiwell plate (Corning®). Freshly prepared working solutions of ivermectin and levamisole, each 100µl at concentrations 0.03µg/ml and 0.9µg/ml, respectively were added to two wells as positive controls. Water or PBS was used as the negative control for aqueous and organic extracts respectively. Aqueous, ethanol, chloroform and acetone extracts of 100 µl each were added at concentrations of 20, 50 and 100 mg/ml. Normal saline solution, 100 µl was added to make up the volume to 300 µl. The counts of immobile and mobile larvae were taken at every 15 minutes interval for 60 minutes at room temperature. Three replicates were performed with the same extract concentrations and controls. The mean number of larvae paralyzed at each time interval was calculated and expressed in percentage.

Results and Discussion

The qualitative analysis for phytochemical components revealed that, tannin, phenol, flavonoid, terpenoid and quinine were present in all the extracts, whereas, saponin, coumarin and betacyanin had a varied distribution in the different extracts. Quantitative analysis revealed that total phenol content and flavonoids were higher in aqueous and ethanol extracts of *A. indica* compared to the other extracts.

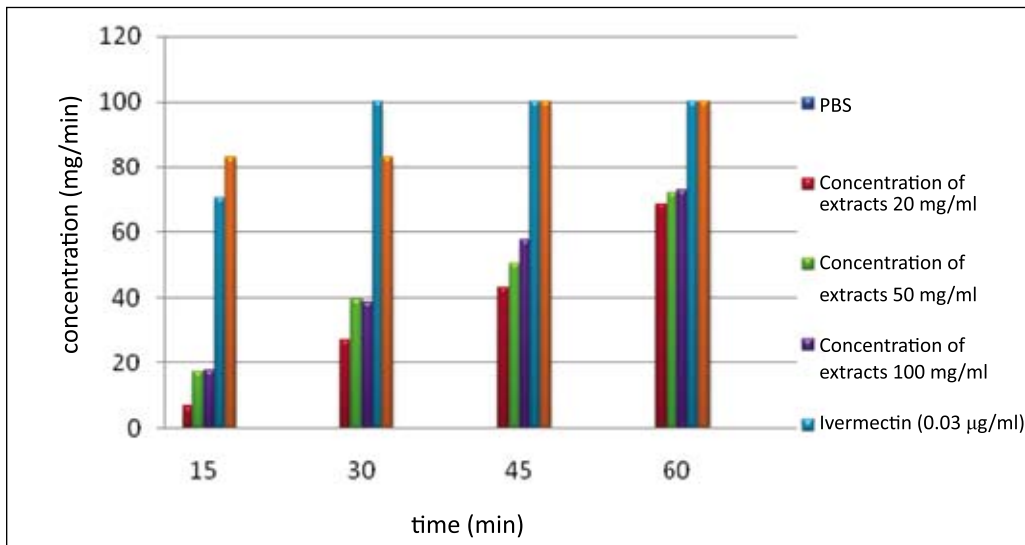
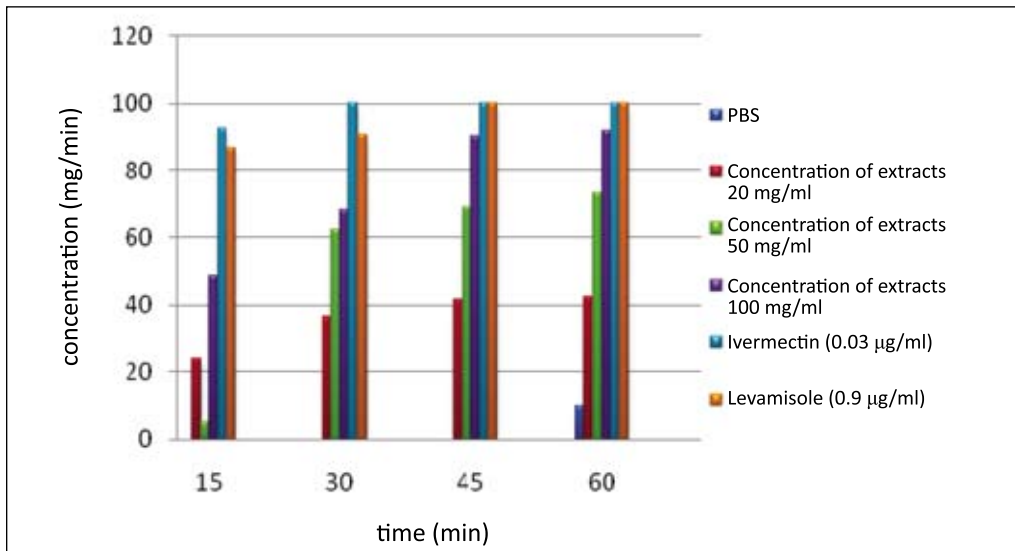
Results of LPA with aqueous extract of *A. indica* have been presented in figure-1. The results revealed that there was a significant ($P<0.01$) difference in the larval paralysis between the different concentrations of the extract. The effect of ivermectin and levamisole are significantly ($P<0.01$) higher than the aqueous extract at all the doses tested. While comparing the time dependent paralysis of *H. contortus* L₃ larvae, it was noted that the paralysis progressively increased with time for all the doses of aqueous extract tested. The percentage of paralysis attained for 20,

Figure-1: Percentage of larval paralysis caused by aqueous extract of *A. indica***Figure-2:** Percentage of larval paralysis caused by ethanol extract of *A. indica*

50 and 100 mg/ml concentrations at the end of one hour were 62.50, 77.00 and 91.50, respectively. Phytochemical analysis using GC-MS revealed that aqueous extract of *A. indica* contained diterpenoids and triterpenoids, *gamma* sitosterol, steroid and phenol (Table-1). The larval paralysis caused by the aqueous extract was similar to that produced by fresh juice of *Xanthium strumarium* leaves, which also comprised of similar constituents like sesquiterpene lactones (xanthinin, xanthumin, and xanthatin) α and β sitosterol and alkaloids

(Sharma *et al.*, 2003).

The percentage of larval paralysis caused by ethanol extract of *A. indica* has been summarised in figure-2. The results showed that with ethanolic extract, the doses 20 and 50 mg/ml caused similar responses in paralysis, whereas at 100 mg/ml dose the paralysis produced was significantly ($P < 0.01$) different from 20 and 50 mg/ml doses, except at 15th minute. Both the positive controls, ivermectin and levamisole, caused significant ($P < 0.01$)

Figure-3: Percentage of larval paralysis caused by acetone extract of *A. indica***Figure-4:** Percentage of larval paralysis caused by chloroform extract of *A. indica*

increase in the percentage of paralysis compared to the different doses of the ethanolic extract of *A. indica*. The significant ($P < 0.01$) difference in the percentage of larval paralysis with respect to time was observed only at the 45th minute unlike that of the aqueous extract. The maximum larval paralysis recorded with the ethanolic extract at the end of one hour of study was 73.88, 80.00 and 91.66% for the doses 20, 50 and 100 mg/ml, respectively. This finding was in agreement with the studies of Hounzangbe-Adote *et al.* (2005), who reported that alcohol extract of *Zanthoxylum*

zanthoxyloides caused larval paralysis in *H. contortus* also contained moderately good levels of tannins and/or flavonoids. The same study revealed that the alcoholic extracts of two other plants, *Newbouldia laevis* and *Carica papaya* found to be rich in flavonoids were also effective in causing paralysis of *H. contortus* larvae. Phytochemical analysis showed that the ethanolic extract was rich in tannins and different sesquiterpenoids. Tannins especially condensed tannins are polyphenolic compounds and several experiments have demonstrated their anthelmintic activity

Table- 1: Phytochemicals detected in the extracts of *A. indica* using GC-MS analysis

Extracts	Selected compounds identified in GC-MS	Class of compounds
Aqueous	Stigmast-5-en-3-ol (3, beta)	Sterol
	Olean-12-en-3-one	Triterpenoid
	Olean-12-en-3-one	Diterpenoid
	15,16-Dinorlabdane	Diterpenoid
	Methyl commate	Pentacyclic triterpenoid
Ethanol	Beta-linalool	Terpene alcohol
	Borneol	Monoterpenoid
	5-allyl-2-methoxyphenol	Simple phenol
	Beta-elemene	Sesquiterpenoid
	Guaia-3,9-diene	Sesquiterpenoid
	Dihydro alpha ionone	Terpenoid
	Tau-cadinol	Sesquiterpenoid
	Gama muurolen	Sesquiterpenoid
	Palustrol	Sesquiterpenoid
	Spathulenol	Sesquiterpenoid
	Ledol	Sesquiterpenoid
Acetone	Palustrol	Sesquiterpenoid
	Spathulenol	Sesquiterpenoid
	Ledol	Sesquiterpenoid
	Viridifloral	Sesquiterpenoid
	Gammasitosterol	Sterol
	Isospathulenol	Sesquiterpenoid
	Stigmast-5-en-3-ol (3, beta)	Sterol
	Humulane-1,6-dien-3-ol	Sesquiterpenoid
Chloroform	Tau- muurolol	Sesquiterpenoid

(Athanasiadou *et al.*, 2001; Paolini *et al.*, 2003). Overall, consistent results were found mostly with plant extracts that possessed the highest tannin content as indicated by the studies of Hoste *et al.* (2009) in which the anthelmintic properties of eight plants (chestnut, pine tree, heather, genista, brambles, oak tree, hazel bush and ash tree) extracts have been examined on the three main nematode species of small ruminants using larval migration inhibition assay (LMIA) and adult worm's motility inhibition assay (AMIA). Montellano *et al.* (2010) studied the effect of a tannin-rich plant *L. latisiliquum* on adult populations of *H. contortus* in sheep and suggested that a short term consumption of this legume could modulate directly the biology of adult *H. contortus* affecting the worm size and female fecundity. The ethanolic and dichloromethane extract of *Phytolacca icosandra*, which had high content of flavonoids, steroids, terpenoids and coumarins, produced inhibition of larval motility (Hernandez-Villegas

et al., 2011). The significant anthelmintic activity of aqueous and ethanolic extracts of *A. indica* against *H. contortus* using egg hatch assay (EHA) has been reported in our previous study (Mini *et al.*, 2013).

Larval paralysis caused by acetone extract of *A. indica* has been furnished in figure -3. During the 60th minute, all the doses (20, 50 and 100 mg/ml) produced same rate of paralysis, which did not differ significantly from each other (68.50, 72.00 and 73.00%, respectively). The standard drugs used brought about 100% paralysis of L₃ larvae, which was significantly ($P < 0.01$) higher than the extract tested at all doses.

Figure - 4 represents the percentage of larval paralysis caused by chloroform extract of *A. indica*. It was observed that after 15 minutes every dose produced paralysis which was significantly ($P < 0.01$) different from each other,

showing a dose dependent and time dependant increase in the percentage of larval paralysis. At the end of one hour, the maximum paralysis noted with the increasing doses was 42.42, 73.13 and 91.66%, respectively. The positive controls ivermectin and levamisole produced significantly ($P<0.01$) higher inhibition of larval movement. Phytochemical analysis revealed that the chloroform extract of *A. indica* contained more of tannins and sesquiterpenes. The findings of the present study are in accordance with those of Orduno *et al.* (2008). They showed that tannin rich extracts of *Havardia albicans* and quebracho were able to cause paralysis of *H. contortus* L₃ larva.

From the results of the present study it may be concluded that the different extracts of

A. indica produced inhibition of the *H. contortus* larval motility *in vitro*. The ethanolic extract was found to be most effective, followed by aqueous and chloroform extract. This indicated that the ethanolic, aqueous and chloroform extracts of *A. indica* contained certain compounds active against L₃ larva, potent enough to inhibit its normal motility. The larval paralysis produced within a short duration also reflects that the active principles were well absorbed through the larval cuticle enabling a very quick action. The larval paralysis produced by the different extracts of *A. indica* might be due to tannins, flavonoids, and terpenoids present in them as indicated through the quantitative phytochemical analysis. *A. indica* extracts showed a strong potential as anthelmintic however, *in vivo* studies are required to prove it.

Acknowledgement

Authors thank the Tamil Nadu Veterinary and Animal Sciences University, Chennai for providing the financial support and laboratory facilities to undertake this work.

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