Paralytic effect of alcoholic extract of *Allium sativum* and *Piper longum* on liver amphistome, *Gigantocotyle explanatum*

T.U. Singh, D. Kumar, S.K. Tandan

**ABSTRACT**

**Objective:** To investigate the effects of alcoholic extract of *Allium sativum* and *Piper longum* on the muscular activity of a parasitic amphistome, *Gigantocotyle explanatum*.

**Materials and Methods:** Amphistomes were isometrically mounted to record the spontaneous muscular activity by using Chart 4 software program (Power Lab, AD Instruments, Australia) and to examine the effects of cumulative doses (100, 300, 1000, and 3000 µg/ml) of the plant extracts on the amplitude (g), frequency (per 10 min), and baseline tension (g) of the spontaneous muscular activity of the amphistome.

**Results:** Alcoholic extract of *A. sativum* produced significant reduction in the frequency and amplitude of contractile activity of the amphistome at 100 and 3000 µg/ml bath concentrations. Complete paralysis of the amphistome was observed after 15 min of addition of 3000 µg/ml concentration. Alcoholic extract of *P. longum* also caused paralysis following 15-20 min exposure of the amphistome to 3000 µg/ml concentration. In both the cases the amphistomes did not recover from paralysis following 2-3 washes.

**Conclusion:** The observations demonstrate the paralytic effect of alcoholic extract of *A. sativum* and *P. longum* on *G. explanatum*.

**KEY WORDS:** Alcoholic extract, *Allium sativum*, *Gigantocotyle explanatum*, *Piper longum*, spontaneous muscular activity

Parasitic infection is a major health problem throughout the world and is responsible for considerable economic losses to the livestock industry, particularly to poor livestock owners in developing countries. Other adverse effects of these parasites include loss of meat, wool, and egg production. Amongst helminths, infection caused by trematodes like amphistomes (*Gigantocotyle explanatum* and *Gastrothylax crumenifer*) and Fasciola sp. is more serious than that due to round worms. The prevalence of *F. gigantica* is very high in ruminants in the Indian subcontinent. These parasites cause hemorrhages and connective tissue proliferation at the site of attachment, vacuolar degeneration in the liver, and hyperplasia in the bile duct, thereby seriously affecting the health and productivity of infected animals.[1]

Chemotherapy is the only efficient and effective tool to cure and control the helminth infection, as efficacious vaccines against helminths have not been developed so far. Indiscriminate use of synthetic anthelmintics in domestic animals has resulted in the development of resistance in helminth parasites.[2-4] Further, residual toxicity, adverse reactions, high cost, and inaccessibility to the rural farmers are problems associated with these agents. Consequently, there is an urgent need to develop newer, selective, and eco-friendly agents to control helminth infections. Plant-based anthelmintics offer an alternative to overcome some of these problems and they can be both sustainable and environmentally acceptable. Unlike synthetic anthelmintics, plant-based anthelmintics with different modes of action could be of value in preventing the development of resistance.[5] Herbal drugs have been in use since ancient times for the treatment of a variety of acute and chronic parasitic diseases, both in human and in veterinary medicine.[6-7] The use of an extract of male fern (*Dryopteris felix mas*) against cestodes and trematodes and that of arecoline (from Areca catechu) against tapeworms of dogs and poultry has also been reported.[8]

In the search for plant-based anthelmintics, extracts of different medicinal plants have been tested for action against flatworms and roundworms in vitro and in vivo and have been found to possess anthelmintic activity. For example, an alcoholic extract of Mallotus philippinensis caused complete paralysis of *F. gigantica* in vitro,[9] garlic protected mice against Schistosoma infection, *A. sativum* has shown anthelmintic action in vitro against *Heterakis gallinae* and *Ascaridia galli*,[10] *Haemonchus contortus*,[11] a free-living nematode of Rhabditis sp., larvae of Nippostrongylus brasiliensis, and eggs of *Ascaris summ.*[12] In vivo *A. sativum* has demonstrated activity against strongyloids in donkeys.[13] *P. longum* has been reported to produce paralysis of *Ascaris lumbricoides*.[14] Moreover, extracts of the plants used in the present study, *A. sativum* and *P. longum*, have shown good hepatoprotective activity in rats.[15,16] Although *A. sativum* and...
P. longum have shown activity against roundworms, to the best of our knowledge, there is no study on the effects of these plants on G. explanatum. The present study evaluates the effects of alcoholic extract of A. sativum and P. longum on spontaneous muscular activity of G. explanatum.

Materials and Methods

Collection of parasites

Mature and healthy G. explanatum were collected from the bile ducts of freshly slaughtered buffaloes from the local abattoir in warm (38 ± 1°C) Hank’s balanced salt solution (HBSS) containing antibiotics (streptomycin sulphate - 6900 units @ 10mg/ml; and benzyl penicillin - 9900 units/liter). They were brought to the laboratory in an insulated container and were kept in the BOD incubator at 38°C ± 1°C until further use.

Preparation of alcoholic extract of the plant material

The plant materials (dried fruits of P. longum and bulbs of A. sativum) were pounded and then extracted with 70% ethanol under reflux. The alcoholic extract was concentrated under reduced pressure to a semisolid mass and made free from solvent.

Preparation of plant extract suspension

Suspensions (100 mg/ml) of the alcoholic extracts of A. sativum and P. longum were prepared in Tween-80 (final concentration 0.1%) and distilled water just before their use and further dilutions were prepared in HBSS solution.

Isometric mounting of G. explanatum and mechanical recording of the spontaneous muscular activity

Mature and active G. explanatum were mounted isometrically in HBSS solution at 38°C ± 1°C as per the method described for Gastrothylax crumenifer.[27] Briefly, the amphistomes were mounted with the help of two fine steel hooks. One hook was inserted 1-2 mm caudal to the anterior sucker and fixed to the tip of an aeration tube and another hook was inserted through the surface of the acetabulum and connected to the transducer (Power Lab, AD Instruments, Australia).

The isometrically mounted parasite was equilibrated without any tension for 30 min, following which 30 mg tension was applied and spontaneous muscular activity was recorded using Chart 4 software program (Power Lab, AD Instruments, Australia). Control recordings were made for 15 min before the addition of a drug. During the equilibration period, the bath fluid was changed once every 10 min. Three parameters, namely, frequency (total number of contractions in 10 min), amplitude (average of all peaks per 10 min or average tension) of spontaneous muscular contractions, and baseline tension (average of all minimum levels of contractions used for measuring amplitude) of the isometrically mounted G. explanatum were measured. Measurements were made for a period of 10 min immediately before the application of a dose of the extract or before a wash following application of a dose.

Effect of cumulative concentrations of plant extracts on spontaneous muscular activity of isometrically mounted G. explanatum

Cumulative doses (100, 300, 1000, and 3000 µg/ml) of the plant extract were added to the tissue bath with the isometrically mounted amphistome. Each dose was allowed to act for 15 min with concomitant recording of spontaneous muscular activity of the G. explanatum. The effects of various concentrations of plant extracts on frequency and amplitude of spontaneous muscular contractions and on the baseline tension of the isometrically mounted G. explanatum were recorded and compared with the control recording.

Statistical analysis

The results are presented as mean ± standard error of mean. To measure the level of significance, one way ANOVA with Tukey’s multiple comparison tests were applied.

Results

Recording of spontaneous muscular activity (SMA) of G. explanatum

The isometrically mounted G. explanatum exhibited SMA for several hours without significant change in amplitude, baseline tension, and frequency of the rhythmicity. The control (i.e., SMA recorded within 15 min of tension application) amplitude, baseline tension, and frequency of SMA were 0.31 ± 0.03 g (n = 6), 0.17 ± 0.02 g (n = 6), and 51.50 ± 3.52/10 min (n = 6), respectively. The amplitude (0.32 ± 0.02 g; n = 6), baseline tension (0.18 ± 0.02 g; n = 6), and frequency (51.83 ± 1.33/10 min; n = 6) of spontaneous contractions recorded after a period of 2 h, were not significantly different from those recorded within 15 min after applying the tension to G. explanatum [Figures 1A and B].

Effect of cumulative concentrations of alcoholic extract of A. sativum on SMA of G. explanatum

The amplitude of contractions of the amphistomes was significantly decreased to 0.20 ± 0.01 g (P < 0.05) and 0.10 ± 0.01 g, (P < 0.001) with 1000 and 3000 µg/ml concentrations of A. sativum extract, respectively, as compared to control (0.32 ± 0.03). The extract, at 1000 and 3000 µg/ml concentrations, also produced significant (P < 0.01 and P < 0.001, respectively) reduction in the frequency of the SMA of amphistome. The values of frequency of SMA of amphistome at 1000 and 3000 µg/ml concentration of the extract were 33.33 ± 2.73 and 24.00 ± 2.79, respectively. The alcoholic extract of A. sativum did not cause any significant change in the baseline tension up to a concentration of 1000 µg/ml. However, at 3000 µg/ml concentration, there was significant (P < 0.05) reduction in the baseline tension of the SMA of the amphistome. The extract produced complete paralysis of the fluke after 15-20 min of administration of 3000 µg/ml concentration [Figures 2A and B] and the SMA of the amphistome did not revive following 2 washes at 10 min intervals (data not shown). The recordings demonstrate a dose-dependent and progressive reduction in the SMA of the amphistomes from 100 to 3000 µg/ml concentration of the extract.

Effect of cumulative concentrations of alcoholic extract of P. longum on the SMA of G. explanatum

In isometrically mounted G. explanatum, the normal amplitude, baseline tension, and frequency of contractions recorded were 0.33 ± 0.02 g (n = 6), 0.17 ± 0.02 g (n = 6), and 52.83 ± 3.01/10 min (n = 6), respectively. The average
baseline tension was decreased significantly ($P < 0.01$) with 3000 µg/ml concentration of the extract of P. longum. The amplitude was reduced significantly to 0.19 ± 0.01 g ($P < 0.001$) and 0.11 ± 0.02 g ($P < 0.001$) at concentrations of 1000 µg/ml and at 3000 µg/ml, respectively, as compared to control. The frequency of SMA of the amphistome was significantly ($P < 0.001$) reduced at 1000 µg/ml and 3000 µg/ml concentrations of the extract [Figure 3A and B]. Paralysis of the amphistome ensued after 15-20 min of administration of 3000 µg/ml concentration of the alcoholic extract of P. longum. The paralyzed amphistome did not revive following 2-3 washes at 10 min intervals.
Discussion

The main finding from this investigation was that alcoholic extracts of A. sativum and P. longum produced complete paralysis of G. explanatum after 15 min of addition of 3000 µg/ml concentration of the extracts and the amphistomes did not recover from paralysis even after 2-3 washes.

Chemotherapeutic agents available for treatment of helminth infection act mainly through three different mechanisms, viz, by disruption of the neuromuscular physiology, by blocking the energy metabolism, and by disturbing the highly efficient reproductive system of the parasites. Several important anthelmintics cause paralysis of helmint parasites by disrupting one or the other aspect of their neuromuscular system.

The muscular activity of helminth parasites can be appreciated as SMA, which can be recorded mechanically with the help of a physiograph. The SMA can be quantified in terms of frequency, amplitude of rhythmic contractions, and baseline tension and these parameters can be measured before and following drug treatment and the values compared. The changes produced in the SMA of isometrically mounted amphistome by a drug, shows the involvement of the neuromuscular system on account of rapidity of action. Rapid and marked change in the SMA of an isometrically mounted parasite by a drug indicates that the neuromuscular system of the parasites has been affected. Thus, SMA of isometrically mounted parasite can be used to evaluate anthelmintic activity of new compounds in vitro. In the present study, the SMA of G. explanatum is grossly similar to that of G. crumenifer, S. mansoni, and F. hepatica.

We have observed in a separate study that alcoholic extracts of A. sativum and P. longum inhibited the gross (visually assessed) motility of G. explanatum at 4 h of incubation (under publication). In the present study both the extracts induced marked decrease in the amplitude and frequency of rhythmic contractions at 1000 µg/ml concentration and produced complete paralysis of the amphistome within 15 min recording in presence of their highest concentration (3000 µg/ml). It can be appreciated from the recording of the effect of alcoholic extract of A. sativum that the inhibitory effect was dose-dependent from 100 to 3000 µg/ml bath concentrations, although same could not be verified statistically. In case of P. longum also, a dose-dependent effect could be seen. Alcoholic extract of P. longum also demonstrated concentration-dependent inhibitory effect on the SMA of G. crumenifer from 1000 to 3000 µg/ml bath concentrations. Thus, the paralysis of G. explanatum is the effect of the alcoholic extracts of A. sativum and P. longum.

The chemotherapeutic value of both the extracts is also evident from an earlier study wherein P. longum (fruits), along with Azadirachta indica (bark), Butea frondosa (seeds), and Nigella sativa (seeds), produced broad spectrum anthelmintic action against roundworms (H. contortus and Oesophagostomum columbianum) and flukes (Paramphistomum cervi) in calves. In vitro, P. longum produced paralysis of A. lumbricoidea. Furthermore, A. sativum has also been shown to possess anthelmintic action in vitro against H. gallinae and A. galli. H. contortus, etc., and in vivo against strongylodes in donkeys. The present observations provide evidence for the paralytic effect of alcoholic extracts A. sativum and P. longum on amphistomes.

In conclusion, the observations demonstrate a paralytic effect of alcoholic extract of A. sativum and P. longum on G. explanatum by progressive reduction in the SMA, which may be associated with their inhibitory effect on the neuromuscular system of the amphistome.

Acknowledgment

The authors are thankful to the Director, Indian Veterinary Research Institute, Izatnagar (U.P.), for providing the necessary facilities to complete this work.
References


