ABSTRACT

Indigenous system of herbal therapy is becoming an increasingly attractive approach to control parasitic infections, particularly in developing countries like India. Among parasites, helminths (Cestodes, Trematodes and Nematodes) and helminthic infections remain to be one of the major health problems affecting billions of people all over the world. They are the most common infectious agents of human beings of tropical countries contributing wide spread occurrence of undernourishment, anemia, eosinophilia and pneumonia. They are also responsible for considerable economic losses to the livestock industry of marginal farmers, particularly in developing countries. The poor hygienic conditions, limited availability of health care services, unsafe drinking water, poverties as well as favorable conditions of the tropics for the growth and development of helminths contributes enormously to the spread and propagation of helminthic infections. The use of commercial anthelmintic drugs of various types such as mebendazole, albendazole, praziquantel, levamisole etc. have been used over the last few decades as the most common method of controlling helminthiases all over the world. Although, a large number of effective and potential anthelmintic drugs have been developed, controlling of helminthiases still is one of the most challenging tasks because of the limited availability of commercial drugs to the rural areas and the resistance capacity developed by most of the helminths against those synthetic drugs.

Contribution of plants to fight against various diseases dates back several centuries, and has been documented by the ancient civilizations. The use and importance of plants and its botanicals for the same has never been neglected and a large number of plants are screened for their efficacy against different helminths. Several such studies
based on traditional medicinal knowledge were done throughout the globe as well as in Indian sub-continent to test the putative anthelmintic properties of different plants. *Alpinia nigra* belonging to the family Zingiberaceae is one such traditionally used medicinal plant, the shoot part along with a part of rhizome of which is used by the indigenous tribal people of Tripura, India, as vegetable; whereas the aqueous juice of shoot of the plant is consumed to get rid of intestinal helminth infection. Although earlier studies carried out on *A. nigra* have confirmed the anthelmintic potential of crude extract of the plant against giant intestinal fluke *Fasciola buski*, details of histomorphological and biochemical alterations in *F. buski* caused by the crude and its different fractions of the crude extract of the plant are not known. Therefore, in the present study detailed investigations was carried out involving crude extract of the plant and its different fractions responsible for anthelmintic properties and their possible mode of action on *F. buski*, a parasite of zoonotic importance in Northeast India.

It is observed that the shoot extracts of *A. nigra* possessed potential anthelmintic efficacy against the trematode parasite, *F. buski*. The ethanolic crude extract showed stronger anthelmintic property by paralyzing the parasites within 2.14 ± 0.48 h of incubation at a test concentration of 20 mg/ml PBS. Out of all the four solvent fractions of *A. nigra* crude extract used for treatment, ethyl acetate fraction showed better anthelmintic activity that paralyzed the parasites within 3.12 ± 0.42 h of incubation at similar concentration. Astragalin, the active compound of *A. nigra* also showed good anthelmintic property at its tested concentrations. The time taken for paralysis was 9.76 ± 1.05 h at its highest concentration of 0.10 mg/ml PBS, while at the same concentration the paralysis time for the reference drug praziquantel was 6.76 ± 1.44 h. The crude
extract and its different fractions of *A. nigra* were also found to cause significant changes in the quantity of trace elements. Ultrastructurally, *F. buski* exposed to different treatments viz., *A. nigra* crude extract, and its ethyl acetate fraction, astragalin and reference drug PZQ showed changes in the tegumental ultrastructure of the parasites. *A. nigra* crude extract treated fluke revealed shredded tegumental layer with large number of vacuole formation, swelling of basal lamina layer and release of internal tissue materials to the exterior. Similar extent of damages and distortions were observed in parasites exposed to ethyl acetate fraction and astragalin. Histochemical localizations of enzymes such as AcPase, AlkPase, ATPase, AchE, MDH and LDH in the tissue sections showed variations in their staining intensities between the control and treated flukes. Reductions in the staining intensities of almost all the enzymes were observed under study. Biochemical quantification of enzymes activities also revealed changes in their enzyme activities when the parasites were exposed to different treatments. The crude extract of *A. nigra* causes highest reduction in the activities of AchE, AcPase and ATPase whereas the ethyl acetate fraction of *A. nigra* crude extract and bioactive compound astragalin showed more or less similar extent of enzyme inhibition. The mechanism of enzyme activity of all the enzymes such as AcPase, AlkPase, ATPase, AchE, PK, PEPCK, MDH and LDH under study was found to be influenced by various physico-chemical properties such as pH, temperature, incubation time and substrate concentrations. Enzyme assay mixtures when incubated with different doses of treatments, alterations were observed in the kinetic parameters of all the enzymes. At its tested concentrations of 0.5 and 1.0 mg, the crude extract of *A. nigra* showed non-competitive inhibition in AcPase, ATPase and AchE activities, followed by mixed type of inhibition in LDH, PK and PEPCK and un-competitive type of enzyme inhibitions in
AlkPase and MDH activity. Ethyl acetate fraction of the crude extract and the bioactive compound, astragalin also causes a similar kind of alteration the kinetic parameters of all the enzymes studied.

Our preliminary \textit{in vitro} investigations on bioactivity of crude extract of \textit{Alpinia nigra} and its different fractions along with bioactive compound astragalin have revealed the potent anthelmintic property of the plant. However, in order to pinpoint the specific principal ingredient of the plant as an anthelmintic, it is suggestive that the ethyl acetate fraction would be a good choice to start with. Astragalin, an active component of the plant showed potential anthelmintic efficacy and therefore, there is a possibility of astragalin being the active principle of the plant responsible for anthelmintic activity. However, it is prerequisite to isolate and identify other active anthelmintic component(s) of ethyl acetate fraction of the plant, if any, to confirm the present finding and to study its exact mode of action.