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Laboratory evaluation of Molluscicidal and Cercaricidal Potential of *Artemisia annua* (Family: Asteraceae)

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Abstract

The study was aimed to assess the molluscicidal and cercaricidal potential of *Artemisia annua* leaves under laboratory conditions. The LC_{50} values of different crude extracts were tested for their molluscicidal activity against immature (Group I), young mature (Group II) and adult (group III) stages of *Indoplanorbis exustus* using Probit analysis. Snail size and different extracts of *Artemisia annua* strongly influenced the pattern of mortality and it was observed to be toxic to all the size/age classes of snails. Of the three extracts tested, carbon tetra chloride was most toxic against all the age classes (I, II, III) with the LC_{50} of 8.14ppm, 8.65ppm and 5.93ppm respectively. All the extracts also exhibited cercaricidal properties against two distome cercariae and the values were fairly below the molluscicidal range thus complementing a target specific approach towards control of trematodal infections.

Key words: *Artemisia annua*, molluscicide, cercaricide, *Indoplanorbis*, LC_{50}

Introduction

Schistosomiasis, a dreadful disease caused by parasitic trematode worms in humans as well as in animals is widespread in the world especially developing countries. It is considered second only to malaria as a major target disease of the World Health Organization [1]. Various species of fresh water snails act as intermediate host of schistosomiasis. The snails belonging to family Planorbidae are known to act as intermediate host of both human and animal schistosomiasis. The fresh water snail *Indoplanorbis exustus* (Mollusca: Gastropoda) is widely distributed in India and act as intermediate host of *Schistosoma nasale*, *Schistosoma spindale* and *Schistosoma indicum* [2], the causative agents of animal schistosomiasis and other trematodal diseases.

One of the possible approaches to eradicate or control this problem is to interrupt the life cycle of the parasitic trematodes by eliminating the snails, which are essential to their life cycle [1,3-5].

Past studies were initiated with the use of certain chemical and synthetic compounds against these vectors [6-9]. At present niclosamide (Bayluscide®, Bayer, Leverkusen, Germany) is the only registered molluscicide recommended by WHO against snail control programmes [1]. Since, the compound is also toxic to fish [7] and other compounds also contaminate aquatic environment [8,9], the use of natural products seems to be promising as they have a shorter environmental half life. The realization that plants could provide a cheap and readily available means of snail control has led to the screening of many plant species [10-17].

Artemisia annua Linn, a Chinese plant has been well established for its antimalarial potential [18,19] however, studies on its molluscicidal and cercaricidal activity are scarce. This has led us to make scientific investigations on the molluscicidal and cercaricidal potential of *Artemisia annua* leaves to prevent the transmission of trematodal diseases *via* the snail host, *Indoplanorbis exustus.*

Materials and Methods

Preparation of Extract

Artemisia annua, Chinese Plant a member of family Asteraceae is an annual herb famous for its antimalarial potential. The leaves of *Artemisia annua* were collected from the Botanical Garden of our Institute in the month of July. They were shade dried for four to five days and then kept in the oven at 35º C for 48 hours. The crushed leaves were subjected to soxhlet extraction and sequentially extracted with petroleum ether (60º -80º), carbon tetra chloride and methanol for 48 hours. Thereafter, the extracts were filtered to get rid of all particulate matter present in the extracts, distilled and evaporated till the crude extract was obtained.

Collection and Maintenance of snails

The planorbid snails, *Indoplanorbis exustus* of different sizes were collected from the irrigated rice fields of Dayalbagh, Agra (27°10′N, 78°′05′E) during July. Snails were hand captured and brought to the laboratory where they were maintained in the polypropylene aquaria containing tap water for acclimatization to laboratory conditions. They were fed with spinach *ad libitum* and water was changed thrice in a week. Dead animals were removed from the aquaria to avoid any contamination.

Bioassay for molluscicidal activity

Molluscicidal activity of different extracts of *Artemisia annua* was evaluated against *Indoplanorbis exustus*. Stock solutions were prepared by dissolving requisite amount of extract in minimum amount of acetone and made up with distilled water. Different dilutions were prepared from these stock solutions. Snails were divided into three groups on the basis of shell diameter, namely group I, group II, group III with shell diameter ranging from 0-5 , 6-10mm and 11-15 mm. 20 snails were tested for each dilution in 250 ml beakers with tap water. They were exposed for a period of 24 hours as the results after 48 hour exposure were mostly similar to those obtained with 24 hour period. Snails were not fed during exposure to the phytoextracts. The snails were considered dead when they remained completely motionless even after repeated tapping and had completely retracted into the shells. In case of doubt, the snails were transferred to clean tap water and observed for an hour. The dead snails were removed immediately from the beakers to avoid any contamination. The molluscicidal assays were carried out in triplicate while controls were run with three groups of each size class held separately in tap water. For cercaricidal studies, snails were screened for cercarial infections, if any. Freshly emerged cercariae were used for the study with twenty cercariae in each beaker and mortality was confirmed under microscope.

Morality patterns of the snails and cercariae

Bioassays were performed according to WHO procedures [1] at six concentrations to produce mortalities between 20-95% for calculating LC_{50}. In case, control mortality was observed from 5-20% it was corrected by applying Abbot's formula [20].

Results and Discussion

Water Quality

Experimental conditions of water were determined by the method of APHA [21]. Atmospheric and water temperature ranged from 32.5 to 34.5 ºC and 28.5 to 30.5º C respectively. pH of tap water was 7.3 to 7.5 while Dissolved oxygen content in ranged from 6.8 to 7.5 mg/L respectively.

Behavioral changes and poisoning symptoms

Exposure to aqueous extracts of leaves of *Artemisia annua* caused significant behavioural changes in the snails, *Indoplanorbis exustus*. In all experimental groups of snails, a consistent pattern of behavioural changes was noticed in the period before death. The initial 10 to 30 minutes was a period of hyperactivity during which sluggish snails moved rapidly in the test water followed by avoidance behaviour by emerging from the suspension (such emergent snails were regularly remerged). Prior to death, there was complete withdrawal of the body inside the shell. Extrusion of the whole head-foot region permanently outside the shell or lack of any movement of the body when touched with tip of the needle was considered to determine mortality of the snails. Sometimes, severe haemolysis preceded death of snails.

Dose-mortality response

The LC_{50} values of crude extracts of *Artemisia annua* leaves were tested for their molluscicidal activity against immature (group I), young mature (group II) and adult (group III) stages of *Indoplanorbis exustus*. No mortality was observed in the control groups over the course of the experiment. Snail size and different extracts of *Artemisia annua* leaves strongly influenced the pattern of mortality. These differences are summarized in table 1 in which 50% snail mortality are shown for different size groups with petroleum ether, carbon tetra chloride and methanolic extracts in tap and deionized water. The results clearly indicates that of the three extracts tested carbon tetra chloride extract was most effective in all the groups with least susceptibility in Group II and maximum susceptibility in Group III. Moreover, the aqueous suspension of various leaf extracts in deionized water gave higher mortality than tap water suspensions.

Table 1. Molluscicidal activity of various *Artemisia annua* extracts against different age classes of *Indoplanorbis exustus*

Extracts	Snail Groups	Regression Equation	LC_{50}±SE (ppm) at 24 hours exposure (Fiducial limits P <0.05)	Chi-Square (χ^2)
Petroleum Ether	I	-15.674+24.257x	7.12±0.09(6.95-7.28)	0.924
	II	-18.604+24.748x	8.99±0.11(8.79-9.20)	0.903
	III	-4.395+11.683x	6.37±0.16(6.05-6.70)	0.960
Carbon Tetrachloride	I	-9.476+15.896x	8.14±0.15(7.85-8.45)	0.933
	II	-15.769+22.164x	8.65±0.12(8.79-9.25)	0.942
	III	-2.932+10.258x	5.93±0.17(6.00-6.79)	0.919
Methanol	I	-16.667+17.552x	17.16±0.29(16.60-17.70)	0.924
	II	-19.599+19.420x	18.48±0.28(17.91-19.01)	0.940
	III	-14.19+15.006x	17.32±0.33(18.70-20.04)	0.936

Cercaricidal activity (following one hour exposure) of *Artemisia annua* extracts was evaluated against two cercariae from *Indoplanorbis exustus*. All the three extracts possess the cercaricidal potential and LC_{50} values are reported in table 2. Cercarial exposure to various aqueous extracts of *Artemisia annua* leaves caused significant behavioural changes that appeared within 5 to 10 minutes of exposure. The most prominent change induced by the phytoextracts was the metamorphosis of *Cotylophorum cotylophoron* cercariae into metacercariae, however light microscopic studies indicated that complete metamorphosis was arrested because of formation of defective cyst wall hence leading to cercarial mortality. However in the case of furcocercous cercariae i.e. *Cercariae indicae XVII* shedding of tail was observed which suppressed the motility of cercariae and hence preventing it from targeting its final host.

Table 2. Cercaricidal activity of various fractions of *Artemisia annua* against cercariae I and cercariae II after one hour exposure

Cercariae	Extracts	Regression Equation	LC_{50}±SE (ppm)	Chi-Square χ^2
Cercaria I (*Cotylophorum cotylophoron*)	Petroleum Ether	3.738+1.231x	1.06±0.22	0.911
	Carbon tetrachloride	3.529+1.396x	1.13±0.34	0.863
	Methanol	3.739+0.948x	2.14±0.17	0.947
Cercaria II (*Cercariae indicae XVII*)	Petroleum Ether	3.240+1.643x	1.18±0.21	0.919
	Carbon tetrachloride	2.711+1.898x	1.61±0.16	0.966
	Methanol	1.994+1.709x	5.73±0.46	0.856

The overall results from mortality patterns of two cercariae clearly indicates that petroleum ether extract of *A. annua* was most effective against both the cercariae. Carbon tetra chloride extract was found to be slightly less effective however; the methanolic fraction was least effective. Moreover, the concentrations needed to kill 50% cercariae were comparatively lower than those reported in literature [12,22].

The results of the present study clearly demonstrates that various leaf extracts of *Artemisia annua* are molluscicidal for all the three age/size classes of snails, with larger snails being more susceptible than the smaller ones. A similar size dependency of molluscicidal effect was also noted for a butanolic extract of *Phytolacca dodecandra* against *Biomphalaria glabrata* [13]. It has been reported that young snails are less susceptible than the adult stages [23-25]. The reason may be that the young mature stages of *Indoplanorbis exustus* are highly active and reproductive and hence more tolerant to the environmental stress of the aquatic medium. However, examples are also known, where smaller snails are less resistant than the larger ones [26].

A number of studies have been performed to control snails by using extracts from various plants [27,28]. The LC_{50} of *Nerium indicum* bark against *Lymnaea accuminata* was recorded to be 34.5 mg/L [29] whereas toxicity (LC_{50}) of *Azardirachta indica* against *Biomphalaria pfeifferi*, *Bulinus truncatus* and *Lymnaea natalensis* was recorded as 19.00, 10.96 and 15.13 mg/L respectively [30]. Molluscicidal activity of *Calendula micrantha officinalis* leaves was reported as 52.17 ppm [31] whereas, crude water extract of *Brassasia actinophylla* gave the LC_{50} of 23.73 ppm against *Indoplanorbis exustus* [15]. The LC values of latex of *Euphorbia pulcherima* of chloroform and carbon tetrachloride extracts have been reported which were decreased from 0.51 mg/L (24 h) to 0.24 mg/L (96 h) and 0.51 mg/L (24 h) to 0.21 mg/L (96 h) respectively, against *Lymnaea acuminata* and from 0.46 mg/L (24 h) to 0.11 mg/L (96 h) and 0.49 mg/L (24 h) to 0.17 mg/L (96 h) against *Indoplanorbis exustus* [32]. In the present study, LC_{50} of various extracts of *Artemisia annua* fall well below these values proving our experimental plant with potent molluscicide and cercaricidal properties.

Statistical analysis of the data on toxicity brings out several important points. The $\chi 2$ test for goodness of fit (hetrogeneity) demonstrated that the mortality counts were not found to be significantly hetergeneous and other variables e.g resistance etc. do not significantly affect the LC_{50} values, as these were found to lie within the 95% confidence limits.

The results of the present study using crude leaf extracts suggest that *Artemisia annua* has potential as a plant molluscicide and cercaricide. In order to confirm this potential, however, a great deal of additional information would be required. In particular, the toxicity of the leaf extracts towards snails as well as to other non-target organisms needs to be investigated as does the possible enhancement of toxicity using extraction techniques. For proper utilization of the plant as molluscicide and cercaricide, further studies are necessary to elucidate the isolation of the active component.

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