Snail control is one of the important tools to reduce the incident of fasciolosis. To attain this objective the present study undertaken to evaluate the molluscicidal potential of Terminalia arjuna bark and Tamarindus indica seed. The toxicity of both of plants were time and concentration-dependent. The toxicity of T. indica seed (12.00 mg/l) was more pronounced than that of T. arjuna bark (37.47 mg/l). Ethanol extract of both plants were more effective than other organic solvent extract. The 96h LC50 of column purified fraction of T. arjuna bark (3.12 mg/l) and T. indica seed (0.71 mg/l) was found to be highly effective against snail. Arjunolic acid and procynadine were isolated by column chromatography and characterized as active molluscicidal components in T. arjuna bark and T. indica seed respectively. Co-migration of pure arjunolic acid (Rf/0.80) and procynadine (Rf/0.77) with column purified extract of T. arjuna bark (Rf/0.80) and T. indica seed (Rf/0.77) demonstrate the same Rf value, confirm the presence of arjunolic acid and procynadine in their respective column purified fractions. The result of the present study clearly indicate that the T. arjuna and T. indica are the potential source of plant molluscicides.

Copyright©2017, Neelam Soni and Vinay Kumar Singh. This is an open access article distributed under the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

INTRODUCTION

Endemic fasciolosis is a serious parasitic disease affecting domestic ruminants as well as human population (Haridy et al., 2002, Ashrafi et al., 2016). The disease is closely linked to summer rainfall which favors fluke development and provides an optimum habitat for intermediate host, the snail. The two species most commonly associated as the causative agent of fasciolosis are Fasciola hepatica and Fasciola gigantica (Gebrie et al., 2015, Singh et al., 2015). Certain aquatic snail (Lymnaeidae and Planorbidae) are of great economic importance because they act as intermediate hosts of trematode (Kumar et al., 2014, Soni and Singh 2015). The economic losses due to the fasciolosis throughout the world are more than $3.0 billion and these losses are associated with mortality, morbidity and reduced growth rate, contamination of bulky liver, and increased susceptibility to secondary infection and expense of control measure and treatment (Ayaz et al., 2014, Gebrie et al., 2015). Treatment of Fasciola in mammalian host required multiple doses of antihelmintic drugs, which pose frequent side effect (Abdul-Samie et et al., 2010). Therefore the best method to control trematode infection is to control the population of vector snail by the use of molluscicide either synthetic or plant origin (Agarwal and Singh, 1988, Upadhyay et al., 2013, Quijano-Aviles et al., 2016). The high cost of synthetic molluscicides and their negative impact on environment as well as snail resistance to these compounds have given a line to study the plant molluscicides (Singh et al., 1996, Singh et al., 2014, Osman et al., 2014). Thus the use of bio-molluscicides has received increased interest, primarily because it could be an appropriate and inexpensive technology for snail control (Adadesanmi et al., 2007). The present study describes the molluscicidal activity of the plant of Terminalia arjuna (Family- Combretaceae) and Tamarindus indica (Family- Leguminaceae) against Lymnaea acuminata. Though both plants have great pharmacological significance in Indian Traditional medicine system (Amalraj and Gopi., 2017, Javed et al., 2016, Shaikh et al., 2017, Ribeiro et al., 2015).

MATERIALS AND METHODS

Animal collection

Adult L. acuminata lengths (2.6±0.30 cm) were collected locally from Ramghar Lake, located almost adjacent to D.D.U. Gorakhpur, University Campus India. Snails were acclimatized for 72 h in laboratory condition in dechlorinated tap water. Ten experimental animals were kept in glass aquaria containing 3 liter of dechlorinated tap water at 24±1°C. The pH of the water was 7.1-7.3 and dissolved oxygen, free carbon

*Corresponding author: Vinay Kumar Singh
Malacology laboratory, Department of Zoology, DDU Gorakhpur University Gorakhpur 273009 (U.P.), India.
dioxide and bicarbonate alkalinity were 6.5-7.3 mg/l, 6.2-6.5 mg/l and 102-106 mg/l, respectively.

Plants

The bark of *T. arjuna* commonly called as arjuna and seed of *T. indica* locally known as imli were collected from the Botanical garden of University campus and identified by Dr. S.K. Singh, Retired professor (plant taxonomist), Department of Botany D.D.U. Gorakhpur University, Gorakhpur, India.

Preparation of crude extract

The freshly collected stem bark of *T. arjuna* and seed of *T. indica* were kept in incubator at 45° for 24h. The dried parts of both plants were pulverized separately in electric grinder to obtained crude powder. The crude powder then sieved with the help of fine meshed cotton cloth to obtained fine crude powder, thus obtained were used for the toxicity experiment.

Organic solvent extract

Five gram of crude powder of both of plants parts were extracted separately with 100 ml each chloroform (99%), ether (98%), Acetone (99%), carbon tetra chloride (95.5%) and alcohol (95%) at the room temperature for 24h. Each extracts were subsequently evaporated under vacuum at room temp. The residues thus obtained were used for determination of molluscicidal activity. The bark powder of *T. arjuna* yielded 43 mg of chloroform extract, 90 mg of ether extract, 730 mg of acetone extract, 70 mg of carbon tetra chloride extract and 1008 mg of alcoholic extract. *T. indica* seed yielded 78 mg of chloroform extract, 93 mg of ether extract, 220 mg of acetone extract, 65 mg of carbon tetra chloride extract and 1110 mg of alcoholic extract.

Column Chromatography

50 ml ethanol extract of seed of *T. indica* extract subjected separately to silica gel (60-120) mesh Qualigens glass, Precious Electro Chemindus Industry Privet Limited, Mumbai, India) Chromatography through 95x45 cm column. Five milliliters fractions of elutents were eluted with 95% ethanol for each column preparation. Ethanol was evaporated under vacuum and the remaining solids obtained from all 5 ml elutents were used for the determination of molluscicidal activity.

Pure Compounds

Arjunolic acid (2,3,23-Trihydroxyolean-12-en-28-oic acid) and Procyanidine (cis,trans’-4,8’-Bi-(3,3’,4’,5,7-Pentahydroxyflavane) were purchased from sigma chemical Co. U.S.A.

![Arjunolic acid](image1)

![Procyanidine](image2)

**Arjunolic acid**

**Thin layer Chromatography**

Thin layer chromatography (TLC) was performed by the method of Jaiswal and Singh (2008) to identify active molluscicidal component present in the *T. arjuna* bark and *T. indica* seed. TLC was done on 20x20 cm precoated silica gel (Precious Electro Chemindus Industry Private Limited, Mumbai, India) using benzene/ethyl acetate (9:1,V:V) as the mobile phase. Co-migration of column purified fraction of plant along with their respective active component arjunolic acid and procyanadine was done for identification of molluscicidal component. TLC plate was developed by iodine.

Toxicity experiment

**Treatment protocol for concentration response relationship**

Toxicity experiments were performed by the method of Singh and Agarwal (1984). Ten experimental animals were kept in glass aquarium containing 3l of dechlorinated tap water. Snails were exposed continuously for 96h to different concentration of plant products separately. Six aquaria were set up for each concentration. The control animals were kept in the equal volume of water under similar condition without treatment. Mortality of snails was recorded at time interval of 24h up to 96h. The dead animals were removed immediately to avoid any contamination of aquarium water. The mortality of snail was established by concentration within the shell, no response to needle probe was taken as evidence of death. The LC values lower and upper confidence limits (LCL-UCL) slope value, t-ratio, g-value and heterogeneity factor were calculated by using Polo-Computer program software of Robertson et al (2007). The regression coefficient between exposure time and different value of LC50 was determined by the method of Sokal and Rohlf (1995).

**RESULTS**

The toxicity of crude bark powder of *T. arjuna* and seed powder of *T. indica* and their organic solvent extracts against *L. acuminata* were time and concentration dependent. There was a significant negative regression in between the exposure period and LC50 all treatments (P<0.05).

**Table 1. Concentration used for toxicity determination of different of preparation of *T. arjuna* bark and *T. indica* seed and their active component against *L. acuminata*.

<table>
<thead>
<tr>
<th>Material used</th>
<th>Test concentration (mg/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>T. arjuna</em> bark powder</td>
<td>50, 70, 90, 110</td>
</tr>
<tr>
<td>Ethanol extract</td>
<td>7, 9, 11, 13</td>
</tr>
<tr>
<td>Ether extract</td>
<td>9, 11, 13, 15</td>
</tr>
<tr>
<td>Acetone extract</td>
<td>9, 11, 13, 15</td>
</tr>
<tr>
<td>Chloroform extract</td>
<td>9, 11, 13, 15</td>
</tr>
<tr>
<td>Carbon tetra chloride extract</td>
<td>9, 11, 13, 15</td>
</tr>
<tr>
<td>Column purified</td>
<td>3, 5, 7, 9</td>
</tr>
<tr>
<td>Arjunolic acid</td>
<td>1, 3, 5, 7</td>
</tr>
<tr>
<td><em>T. indica</em> seed powder</td>
<td>10, 15, 20, 25</td>
</tr>
<tr>
<td>Ethanol extract</td>
<td>0.9, 1.5, 3.0, 5.0</td>
</tr>
<tr>
<td>Ether extract</td>
<td>1, 3, 5, 7</td>
</tr>
<tr>
<td>Acetone extract</td>
<td>1, 3, 5, 7</td>
</tr>
<tr>
<td>Chloroform extract</td>
<td>1, 3, 5, 7</td>
</tr>
<tr>
<td>Carbon tetra chloride extract</td>
<td>1, 3, 5, 7</td>
</tr>
<tr>
<td>Column purified</td>
<td>0.7, 0.9, 1.1, 1.3</td>
</tr>
<tr>
<td>Procyanidine</td>
<td>0.3, 0.5, 0.7, 0.9</td>
</tr>
</tbody>
</table>
DISCUSSION

The result of the present study clearly indicate that the plant of *T. arjuna* and *T. indica* are potent molluscicides. It has been shown that toxicity of crude or purified plant product is potent molluscicidal if, the LC50 is less than 100 mg/l (Hostettmann et al., 1987). In the present Study 96h LC50 of both plants are less than 100 mg/l. The higher toxicity of ethanol extract of *T. arjuna* bark powder and *T. indica* seed powder as compared to other organic solvent extract indicate that the molluscicidal component in the bark and seed are more soluble in ethanol extract. The phytochemical investigation has reported that the bark of *T. arjuna* has active component arjunolic acid (Verma et al., 2012). Experimental study of *T. arjuna* bark revealed that its bark shows significant anthelmintic activity (Yadav et al., 2013), antibacterial activity (Shinde et al., 2009, Jethinlalkhosh and Antony, 2013)) and analgesic activity (Biswas et al., 2011). The seed of *T. indica* found to have great pharmacological significance as it shows anti-inflammatory and analgesic activity (Nakhat et al., 2014), (Suralkar et al., 2012) antioxidant activity (Sandesh et al., 2014). Kalra et al (2011) noted peptic ulcer protective effect due to presence of
its polyphenolic compounds mainly procyanidin epicatechine and polymeric tannin. Their toxicities are time and concentration dependent, as evident from the negative regression between exposure period and LC50 value of the different treatment. The time dependent toxic effect of tested plants product may be due to uptake of active component by snail, which progressively increased in the body with an increased in exposure duration. It is also possible that the active compounds could change in to more toxic form in the aquarium water or in the snail body due to the action of various enzymes. In the toxicity study it was found that 96h LC50 of T. arjuna bark (57.47 mg/l) was higher than the T. indica seed (12.00 mg/l), indicate that the seed of T. indica are potentially more toxic molluscicides than T. arjuna bark. Co-migration of column extract of both plants and active component arjunolic acid and procyanidine on TLC plate was demonstrated, it indicate the same Rf value. It clears the presence of active component arjunolic acid procyanidine in bark of T. arjuna and seed of T. indica respectively. A compression of mollusccidal activity of column purified fraction of T. arjuna bark and T. indica seed with synthetic molluscicides clearly demonstrated that the former are more potent. The 96h LC50 of crude powder of T. arjuna bark (57.47 mg/l) and T. indica seed (12.00 mg/l) against L. acuminata was lower than the plant products has been reported earlier in our laboratory. The crude powder of Cinnamonum tamala leaf powder (830.90 mg/l) (Srivastava and Singh, 2005) Sapindus mukorossi fruit (119.57 mg/l), Terminalia chebula fruit (93.59 mg/l) (Upadhyay and Singh, 2011), Mimusops elengi bark (108.15mg/l), Bauhinia variegata leaf (238.17 mg/l) (Singh et al., 2015), Moringa oleifera leaf (22.52 mg/l) and Momordica charantia fruit (318.29 mg/l) (Upadhay et al., 2013). It is evident from steep slope value that small increase in concentration of different treatment caused marked mortality in snails. A t-ratio value is greater than 1.96 indicate that regression is significant. The value of heterogeneity factor is less than 1.0 denotes that in the replicate tests of random sample the concentration response line would fall within 95% confidence limits and thus model fits the data adequately. The index of significance of potency estimation value indicate that the value of mean are within the limits of all probability levels (90.95,99) as it is less than 0.5. (Robertson et al., 2007).

**Conclusion**

In the present study it can be concluded that the plants of T. arjuna and T. indica are the potent molluscicides. Both plants are native therefore easily available, economical, ecologically sound, and culturally more acceptable. For the proper
utilization of these plants products as molluscicides further extensive study are required to explore the mode of action of these components inside the snail body.

REFERENCES


********