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MOLLUSCICIDAL EFFICACY OF MEDICINAL PLANT *SOLANUM SURATTENSE* AGAINST *FASCIOLA* VECTOR SNAIL, *LYMNAEA ACUMINATA*

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Abstract: Fasciolosis is one of the most serious food-borne parasitic diseases. These parasitic infections are caused by *Fasciola hepatica* and *F. gigantica* among cattle and human populations. The carrier of fascioliasis is a fresh water host snail, *Lymnaea acuminata*. The control of vector snails is a major tool in reducing the incidences of fasciolosis. Synthetic molluscicide causes adverse effects in the environment as well as on non target organisms. The present studies were designed for evaluation of molluscicidal efficacy of medicinal plant *Solanum surattense* against *L. acuminata*. The efficacy of *S. surattense* was concentration and time dependent. Toxicity experiment of dried leaf powder of *S. surattense* and their different organic extracts and column purified was continuously observed for 96h at different concentrations. Mortality was observed for 24, 48, 72 and 96h. Six aquariums were set up for each concentration. The control group animals were kept in the equal volume of water under similar conditions without treatment. Mortality of snails was recorded at intervals of 24h each up to 96h and lethal values were calculated. The 24h LC_{50} of dried leaf powder of *S. surattense* was 157.33mg/l and at 96h 150.26 mg/l. Among all the organic extracts, the ethanol extract of dried leaf powder of *S. surattense* was found more toxic. The present study revealed that the product of *S. surattense* has potent molluscicidal phytochemicals, which may be used as potent molluscicides for control of harmful snails.

Keywords: Fasciolosis, *Lymnaea acuminata*, Molluscicides, *Solanum surattense*.

INTRODUCTION

Fasciolosis is caused by *Fasciola hepatica* and *F. gigantica* (Mas-Coma *et al.,* 2007; Hacariz *et al.,* 2014). Infection of *Fasciola* has been reported in 81 countries in different parts of the world. It is a major worldwide zoonotic disease in ruminant's animals and human (Mas-Coma *et al.,* 2014; Cwiklinski *et al.,* 2016). It was estimated that between 2.4 million and 17 million people were infected around the world. However, these parasitic diseases in India are mainly caused by *F. gigantica* (Dalton, 1999). *Fasciola* is a trematode parasite belongs to the Platyhelminthes, which is a phylum of flatworms (Verma and Prakash, 2020). It has a complex lifecycle among intermediate host snails and definitive mammals hosted, including human (Carvedo and Cabad, 2020). They inhabit in the liver of cattle, sheep, goat, buffalo etc. and play an important role in growth, development and productivity of economically significant livestock (Kuchai *et al.,* 2011; Eshetu *et al.,* 2017; Kumar, 2021). In the northern part of Uttar Pradesh (India), fresh water snail, *Lymnaea acuminata* is an intermediate

host of *F. gigantica*, which is responsible for endemic fasciolosis (Singh and Agarwal, 1981; Kumar and Singh, 2006; Kumar *et al.,* 2011; Kumar *et al.,* 2012; Kumar *et al.,* 2013a and 2013b; Kumar and Singh, 2014; Kumar *et al.,* 2016; Kumar *et al.,* 2018; Kumar *et al.,* 2020). Some species of snails and slugs are also causing greater economical loss by damaging agricultural crops (Kumar, 2020). The control of snail population, thereby breaking the life cycle of *Fasciola* and reduce the incidence of zoonotic disease and economic loss are relevant (Kumar and Singh, 2006; Kumar *et al.,* 2009; Kumar *et al.,* 2018; Kumar, 2021). The control of snail population bellow threshold level by using molluscicides is well-recognized method for control of liver fluke infections. The use of synthetic molluscicides has been advocated that, it is not safer for environment (Agarwal and Singh, 1988). It should be effective for several life stages of the harmful snails, selective and harmless for non-target organism and safer for environment (Singh *et al.,* 1996).

Plant derived molluscicides are becoming alternative source of the synthetic molluscicides because they are more acceptable, cheaper and safer for non target aquatic organisms, as well as being potentially biodegradable and eco-friendly (Marston and Hostettmann, 1985; Kumar, 2021). *Solanum surattense* (Family Solanaceae) is herbaceous weed, which is widely distributed throughout the tropical and subtropical regions of the South East Asia (Khare, 2007). *Solanum surattense* is traditionally used for leprosy, cough, fever, dropsy, dysmenorrheal hypertension, cardiac disorder, epilepsy, asthma and depression (Singh *et al.,* 1979; Vaidyaratnam, 1994; Khan and Khan, 2019). Pharmacologically, it has been evaluated for analgesic, antibacterial, antidiabetic, antinociceptive, antioxidant, antifungal and larvicidal activities (Amirtharaj *et al.,* 2015; Ramar and Nandagopalam, 2011).

Different parts of *S. surattense* are used in the treatment of various diseases like asthma, fever, bronchitis, laxative, tuberculosis, kidney disorder, cough, constipation, tooth ache, sore throat, rheumatism and gonorrhea (Yadav *et al.,* 2014). It has antioxidant, antipyretic (Muthalik *et al.,* 2003), antiulcer, antimicrobial, anti-inflammatory and anthelmintic property (Pawar and Maheshwari, 2003). The aim of present study is to evaluate the molluscicidal efficacy of *S. surattense* against fresh water host snail *L. acuminata*.

MATERIALS AND METHODS

Collection of Snails

A number of adult snails, *L. acuminata* (2.610.32 cm in length) were collected from low lying submerged fields and ponds from Muhammadabad Gohna, Mau (U.P.) India. The snails were acclimatized for 72 hours in dechlorinated tap water at 27 ± 3^{0}C. The pH of water was 7.2-7.1 and dissolved oxygen, free carbon dioxide and bicarbonate alkalinity were 6.3-7.3 mg/l, 5.4-6.3 mg/l and 103.0-106.0 mg/l, respectively.

Plant and preparation of crude products

The fresh leaves of *Solanum surattense* collected from the college campus, was identified by Dr. A.K. Singh Department of Botany, S.G.N. Government P.G. College Muhammadabad Gohna, Mau (U.P.) India. All these leaves were washed by fresh water and dried in sun light 3 to 5 days and pulverized in the electric grinder for crude powder thus obtained, was then sieved with the help of fine mesh cloth. This fine crude powder was then used for toxicity experiments against snail, *L. acuminata*.

Extraction of organic solvent

Five gram crude leaf powder of *Solanum surattense* were extracted with 500 ml of 98% ether, 99.7% chloroform, 98% methanol, 98% acetone and 95% ethanol at room temperature for 24h. Each preparation was separately filtered through sterilized whatman No-1 filter paper and the filtered extracts where subsequently evaporated under vacuum (Jaiswal and Singh, 2008). The leaf powder of *S. surattense* yielded 235 mg ethanol, 215 mg chloroform, 250 mg ether and 270 mg acetone extracts. The residues, thus obtained, were used for the determination of molluscicidal activity.

Column purification

One hundred milliliters of ethanol extract fraction of dried leaf powder of *S. surattense* were subjected to silica gel (60-120 mesh, Qualigens

Glass, Precious Electrochemidus Private Limited, Bombay, India) chromatography through a 5 × 45 cm column. Five milliliter fractions eluted with ethanol (95%) were collected. Ethanol was evaporated under vacuum and the remaining solids obtained were used for the determination of molluscicidal activity of each fraction.

Determination of toxicity response

Toxicity of different organic extracts and column purified of *S. surattense* was performed by the method of Kumar and Singh (2006). Ten snails were kept in a glass aquarium containing 3 liter of dechlorinated tap water. These experimental animals were exposed continuously for 96h to different concentrations and preparation of *S. surattense* and mortality was observed for 24, 48, 72 and 96h. Six experimental aquariums were setup for each concentration. The control groups of snails were kept in the equal volume of water under similar laboratory conditions without treatment. The mortality of snails was recorded at interval of 24h each up to 96h. The mortality of snails was established by the contraction of body within the shell, no response to touch needle probe was taken as evidence of snail death. Lethal values (LC_{50}), slope values, t- ratio, 'g' value and heterogeneity factor were calculated using POLO computer programme (Robertson *et al.,* 2007).

RESULTS AND DISCUSSION

The dried leaf powder of *S. surattense*, different organic extract and column fractions against *L. acuminata* were concentration and time dependent. The 24h LC_{50} of dried leaf powder of *S. surattense* were 157.33mg/l and at 96h 150.26 mg/l (Table-1). Among all the organic extracts, the ethanolic extract of dried leaf powder of *S. surattense* was more toxic, after 24h, 48h, 72h and 96h exposure against *L. acuminata* the LC_{50} values were observed 147.53, 145.82, 143.82 and 140.82mg/l, respectively (Table-1). The 24h LC_{50} of the column purified fractions of dried leaf powder of *S. surattense* were 135.82 mg/l. The 96h LC_{50} of column purified fraction of dried leaf powder of *S. surattense* were 122.82 mg/l (Table-1). In all treatments column purified fraction was more effective.

The t- ratio was greater than 1.96 and the heterogeneity factor was less than 1.0. The g-value was less than 0.5 at all probability levels (90, 95 and 99) (Table-1). The slope values given in Table-1 were steep and the separate estimates of LC based on each of the six replicates were found to be within the 95% confidence limits of $Lc_{50.}$

The result clearly demonstrates that the dried leaf powder of *Solanum surattense* is potent source of molluscicides. The study of toxic efficacy revealed that molluscicidal components of *S. surattense* are soluble in different organic solvents and caused motility of snail, *L. acuminata*. The time and concentration dependent toxic efficacy of *S. surattense* and their different preparations may be either due to the uptake of the active moiety which progressively increases the amount of phytochemicals in snail body with increase in exposure period or it might be possible that the phytochemicals could change into more toxic forms in the snail body due to the action of various enzymes activities. The ethanolic extract of *S. surattense* was tested against *Plasmodium* which has significantly reduced their effect in infected mice (Garedaghi and Khaki, 2014). Higher toxicity of ethanol extract among other organic extracts indicates that molluscicidal phytochemicals are present in *S. surattense* and it's more soluble in ethanolic solvent.

The toxic efficacy of *S. surattense* is concentration and time dependent as the phytochemicals are dissolved in aquarium water and gradually diffuses in snail body and causes mortality with increases in exposure period. Suhas *et al.* (2009) reported that methanolic extract of *S. surattense* shows antibacterial activity against gram positive bacteria, *Streptococcus aureus* and *Bacillus subtilis* at 50, 75 and 100 μg/ml concentrations. Leaf extracts of *S. surattense* have larvicidal efficacy against *Culex quinquefasciatus* (Mahesh *et al.,* 2012). It's also having a number of alkaloids (Siddiqui and Faizi, 1983), sterols (Kusano *et al.,* 1973), saponine (Tupkari *et al.,* 1972), flavonoids and their glycosides (Debey and Gupta, 1936), tannins, gums (Sheeba, 2010). Several tannin bearing different families of plants have molluscicidal properties (Ayoub and Yankov,

Table: 1. Molluscicidal efficacy of dried leaf powder of *S. surattense* and their different organic extract, column purified against *L. acuminata* at different exposure periods.

Exposure periods	Values	Molluscicidal preparations (mg/l)						
		***S. surattense* dried leaf powder**	**Ether extract**	**Chloroform extract**	**Methanol extract**	**Acetone extract**	**Ethanol extract**	**Column purified**
24h	LC_{50}	157.33	150.82	151.68	149.59	151.13	149.53	135.82
	LCL	152.33	145.62	148.63	146.21	147.85	144.21	130.22
	UCL	160.98	155.82	152.86	154.36	156.34	150.83	138.64
	Slope Value	1.63±0.70	1.45±0.33	1.43±0.70	1.23±0.35	1.28±0.11	1.60±0.71	1.53±0.16
	t-ratio	3.61	5.33	3.83	2.66	3.29	3.77	2.61
	g-value	0.23	0.29	0.16	0.26	0.22	0.24	0.28
	Heterogeneity	0.25	0.17	0.21	0.31	0.27	0.20	0.22
48h	LC_{50}	155.62	149.75	150.11	148.69	151.61	145.82	130.62
	LCL	157.73	145.36	147.51	144.82	148.59	142.52	127.52
	UCL	158.92	153.22	154.86	153.89	154.63	150.35	133.86
	Slope Value	1.55±0.27	1.31±0.30	1.28±0.75	1.66±0.30	1.27±0.55	1.86±0.38	1.70±0.43
	t-ratio	2.29	3.13	4.81	3.54	3.20	4.56	4.41
	g-value	0.18	0.16	0.25	0.19	0.17	0.23	0.32
	Heterogeneity	0.18	0.26	0.15	0.28	0.45	0.15	0.31
72h	LC_{50}	153.33	147.55	148.69	146.42	149.85	143.82	125.85
	LCL	150.12	142.58	146.52	143.82	147.61	140.69	122.36
	UCL	156.32	152.38	153.66	149.32	154.73	147.82	128.73
	Slope Value	1.86±0.99	1.74±0.13	1.40±0.34	1.44±0.55	1.37±0.28	1.88±0.74	1.77±0.65
	t-ratio	2.77	3.11	4.82	3.53	2.66	3.91	2.27
	g-value	0.26	0.11	0.28	0.12	0.34	0.25	0.16
	Heterogeneity	0.36	0.34	0.29	0.28	0.23	0.31	0.19
96h	LC_{50}	150.26	146.89	145.63	144.82	147.99	140.82	122.82
	LCL	145.81	142.31	141.65	141.82	144.83	138.66	119.66
	UCL	156.96	151.80	150.99	149.39	152.62	145.72	125.86
	Slope Value	1.85±0.70	1.65±0.31	1.29±0.95	1.77±0.36	1.46±0.83	1.73±0.70	1.86±0.77
	t-ratio	3.51	4.88	2.71	3.56	2.95	4.56	3.85
	g-value	0.17	0.20	0.19	0.35	0.28	0.27	0.19
	Heterogeneity	0.34	0.16	0.32	0.28	0.21	0.34	0.25

Six batches of ten snails were exposed different concentration of the above molluscicides. Mortality was determined after every 24h. LCL- lower confidence limits UCL- upper confidence limits.

1986). Bahuguna *et al.* (2008) described that different leaf extract of *S. surattense* like petroleum ether, aqueous alcohol and chloroform for antiulcer activity areas like pH, total acidity, free acidity and ulcer. This plant is also used in treatment of insomnia, cold, worms (Mathur and Agrawal, 2011), laxative, enlargement of liver, aphrodisiac activities (Kiritikar and Basu, 2005; Gupta *et al.,* 2011), anti-nociceptive, molluscicidal and anti-fungal activity (Bhutani *et al.,* 2010). The anti-cancerous efficiency of *S. surattense* fruit extract might be attributed to the presence of flavanoids such as apiginene, querecitien, fisatin and luteolin, which known to be the potent inhibitors of cancer cell proliferation (Kumar and Pandey, 2014).

It is evident from result section that the steep slope values indicate that a small increase in the concentration of different treatment which caused mortality in snails (Table-1). A t-ratio value greater than 1.96 indicates that the regression is significant. Values of heterogeneity factor less than 1.0 denote that in the replicate

tests of random sample the concentration response lines would fall within the 95% confidence limits and thus the model fits the data adequately.

CONCLUSION

It can be concluded from the above study that the medicinal plant *S. surattense* can be used as potent molluscicide, as it is easily available and ecologically more acceptable for livestock keepers. These results, allied to the easy preparation of the extract, low cost of application, could make the aqueous extract of *S. surattense* an alternative molluscicidal efficient in the control of snails populations. Further studies are required to identify the actual phytochemicals constituents that are present in the crude extract of this plant which are responsible for molluscicidal activity.

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