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Asian Journal of Animal and Veterinary Advances

Academic
Journals Inc.

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OPEN ACCESS Asian Journal of Animal and Veterinary Advances

ISSN 1683-9919
DOI: 10.3923/ajava.2018.30.34

Research Article
Efficacy of *Potentilla fulgens* Root Powder and Their Different Organic Extract Against Fresh Water Vector Snail *Lymnaea acuminata*

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Abstract

Background and Objective: Fasciolosis is an endemic disease that causes severe economic conditions and affecting cattle populations even the human. The control of snail population is major tool in reducing the incidences of fasciolosis. The present study was designed for studying the effect of dried root powder of *Potentilla fulgens* (*P. fulgens*) use as molluscicides against vector snail *Lymnaea acuminata* (*L. acuminata*). **Materials and Methods:** Toxicity experiment of different organic extracts and column purified of *P. fulgens* was continuously observed for 96 h at different concentration. Mortality was observed for 24, 48, 72 and 96 h. Six aquariums were setup 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 interval of 24 h each up to 96 h by using POLO computer programme. **Results:** The dried root powder of *P. fulgens* at 96 h LC_{50} against *L. acuminata* was 133.62 mg L^{-1}. Among different organic extracts, ethanol extract was more toxic than other organic extract. The ethanol extract of *P. fulgens* was more toxic (24h LC_{50}-108.65 mg L^{-1}) against *L. acuminata.* The 96 h LC_{50} of column purified fraction of dried root powder of *P. fulgens* was 28.69 mg L^{-1}. **Conclusion:** The present study showed that the product of *P. fulgens* has potent molluscicidal activity. The product of *P. fulgens* may be used as potent molluscicides.

Key words: Molluscicides, *Potentilla fulgens*, *Lymnaea acuminata*, *Fasciola gigantica*, fasciolosis

Received: August 11, 2017 Accepted: October 02, 2017 Published: December 15, 2017

Citation: Pradeep Kumar, Kumari Sunita and D. K. Singh, 2018. Efficacy of *Potentilla fulgens* root powder and their different organic extract against fresh water vector snail *Lymnaea acuminata*. Asian J. Anim. Vet. Adv., 13: 30-34.

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Fasciolosis is a parasitic serious disease which caused by *Fasciola hepatica* and *F. gigantica*[1-2]. *F. hepatica* and *F. gigantica* is a major worldwide parasitic disease of domestic ruminants animals and human[3-4]. These parasitic diseases in India are mainly caused by *F. gigantica* in animal[5]. They live in the liver of cattle, sheep, goats and buffaloes, which have a significant importance on growth rate, developments and productivity of ruminants and therefore, are considered economically significant[6-7]. The fluke *F. hepatica* is widely distributed in temperate zones, whereas *F. gigantica* is typically found in tropical zones around the world[8,9]. Snail *Lymnaea acuminata* is the intermediate host for the liver fluke *F. gigantica*, which is responsible for endemic fasciolosis in the Northern part of Utter Pradesh, India[10-18]. An effective control of fasciolosis includes strategic use of anthelmintic drugs and control of intermediate host snail to reduce the incidence of fasciolosis. The population of vector snail *L. acuminata*, there by breaking the life cycle of fluke reduces the incidence of fasciolosis[12,19]. The control of vector snail population by using molluscicides is well-recognized method for the control of fasciolosis. However, it has been advocated that the use of synthetic molluscicides is not environmentally safe[11].

Alternatively plant origin molluscicides are becoming increasingly popular because they are cheaper, more acceptable and safer than their synthetic molluscicides, as well as being potentially eco-friendly and biodegradable[12,20]. *Potentilla fulgens* (Family: *Rosaceae*) is commonly called Himalayan Cinquefoil in English, Bajradanti in Hindi[21]. *P. fulgens* is common medicinal plants which are found in Northeast India and used in Ayurvedic, Unani, Siddha, Chinese and Tibetan systems of medicine due to high content of polyphenols in their aerial and underground parts[22-25]. The pharmacological studies reported that *P. fulgens* possesses hypoglycemic, anti-hyperglycemic, antitumor, anti-hyperlipidemic, antioxidant, antiulcerogenic and antinflammatory properties, Kaul *et al.*[26], thus supporting its ethnotherapeutic use. The present study to evaluate the molluscicidal activity of *P. fulgens* dried root powder, different organic extracts and column purified against vector snail *L. acuminata.*

MATERIALS AND METHODS

Experimental animals: Adult *L. acuminata* (2.60±0.30 cm in length) were collected from low lying submerged field of Maheshra lakes in 2017, Gorakhpur (U.P.) India. The snails were acclimatized for 72 h in dechlorinated tap water at 26±2°C. The pH of water was 7.1-7.2 and dissolved oxygen, free carbon dioxide and bicarbonate alkalinity were 6.4-7.2, 5.1-6.2 and 102.0-105.0 mg L^{-1}, respectively.

Plants: The fresh dried root of *Potentilla fulgens* were procured from local market in Gorakhpur, (UP) India.

Preparation of crude plant products: Dried root of *P. fulgens* were pulverized separately in the electric grinder and the crude powders thus obtained, were then sieved with the help of fine mesh cloth. This fine powder was then used separately for toxicity experiments against vector snail *L. acuminata*.

Organic solvent extracts: Two gram dried roots powder *P. fulgens* were extracted with 200 mL of 98% ether, 99.7% chloroform, 98% methanol, 98% acetone and 95% ethanol at room temperature for 24 h. Each preparation was filtered separately through sterilized Whatman No-1 filter paper and the filtered extracts where subsequently evaporated under vacuum[27]. The residues, thus obtained, were used for the determination of molluscicidal activity. The root powder of *P. fulgens* yielded 250 mg ethanol, 320 mg chloroform, 360 mg ether and 410 mg acetone extracts.

Column purification: One hundred milliliters of ethanol extract fraction of dried root powder of *P. fulgens* 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.

Toxicity for concentration-response relationship: Toxicity experiment of different organic extracts and column purified of *P. fulgens* was performed by the method of Kumar and Singh[12]. Ten experimental animals were kept in a glass aquarium containing 3 L of dechlorinated tap water. Snails were exposed continuously for 96 h to different concentrations and preparation of *P. fulgens* and mortality was observed for 24, 48, 72 and 96 h. Six aquariums were setup for each concentration. The control animals were kept in the equal volume of water under similar conditions without treatment. Mortality of snails was recorded at interval of 24 h each up to 96 h. The mortality of snails was established by the

contraction of body within the shell, no response to needle probe was taken as evidence of snail death. The mortality data were observed after every 24 h up to 96 h.

Statistical analysis: The Lethal values (LC_{50}), lower and upper confidence limits (LCL and UCL), slope values, t- ratio, 'g' value and heterogeneity factor were calculated using POLO computer programme[28]. The regression coefficient applied between exposure time and different values of LC_{50} was determined by the method of Sokal and Rohlf [29].

RESULTS

Molluscicidal activity of dried root powder of *P. fulgens* and their different fractions of organic extract against *L. acuminata* were time and concentration dependent. The LC_{50} of dried root powder of *P. fulgens* at 24 h were 166.76 mg L^{-1} and at 96h 133.62 mg L^{-1} (Table 1). Among all the organic solvent extract fractions, the ethanol extract of dried root powder of *P. fulgens* were more toxic (Table 1) . The LC_{50} of ethanol extract of dried root powder of *P. fulgens* at 24 h against *L. acuminata* were 108.65 mg L^{-1}. The column purified fractions of all the organic solvent extract fractions were highly toxic. The LC_{50} of the column purified fractions of dried root powder of *P. fulgens* at 24 h were 48.63 mg L^{-1}. The 96 h LC_{50} of column purified fraction of dried root powder of *P. fulgens* were 28.69 mg L^{-1} (Table 1).

The slope values given in Table 1 were steep and the separate estimates of LC based on each of the 6 replicates were found to be within the 95% confidence limits of LC_{50}. 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). There was significant negative regression ($p<0.05$) between the exposure time and LC_{50} of the treatments (Table 1).

DISCUSSION

The results of the present study clearly demonstrated that the dried root powder of *P. fulgens* is potent source of molluscicides. Toxicity study revealed that toxic components of *P. fulgens* are soluble in water and caused motility of snail *L. acuminata*. Their toxic effects are time as well as concentration dependant as evident from negative regression between exposure time and LC_{50} of different treatments. The time dependent toxic effect of *P. fulgens* plant products may

Table 1: Toxicity of *P. fulgens* their different organic extract and column purified against *L. acuminata* at different time exposure

Exposure period	Molluscicides	LC_{50}	LCL	UCL	Slope value	t-ratio	g-value	Heterogeneity
24 h	*P. fulgens* (DRP)	166.76	150.85	196.32	1.63±0.76	3.60	0.27	0.22
	Ether extract	135.62	128.39	140.69	1.62±0.26	3.26	0.26	0.25
	Chloroform extract	138.96	124.31	140.76	1.54±0.78	4.11	0.18	0.27
	Methanol extract	140.12	135.72	145.26	1.44±0.28	4.59	0.25	0.20
	Acetone extract	120.75	117.19	124.29	1.32±0.88	4.15	0.36	0.21
	Ethanol extract	108.65	102.71	113.76	1.48±0.32	4.59	0.13	0.19
	Column purified	48.63	47.61	55.63	1.58±0.28	4.23	0.22	0.18
48 h	*P. fulgens* (DRP)	158.29	155.42	162.72	1.96±0.33	3.66	0.21	0.22
	Ether extract	132.08	128.72	136.78	1.75±0.31	4.04	0.26	0.20
	Chloroform extract	134.73	130.44	138.82	1.42±0.38	4.57	0.19	0.25
	Methanol extract	135.62	130.48	139.46	1.18±0.38	3.15	0.18	0.29
	Acetone extract	116.58	113.26	119.73	1.66±0.52	4.81	0.24	0.26
	Ethanol extract	102.09	98.69	105.72	1.72±0.48	3.06	0.18	0.22
	Column purified	39.66	35.78	43.62	1.28±0.30	4.54	0.21	0.25
72 h	*P. fulgens* (DRP)	145.30	141.80	149.28	1.61±0.38	3.50	0.20	0.28
	Ether extract	129.75	125.70	133.55	1.28±0.26	4.16	0.18	0.30
	Chloroform extract	128.19	124.48	134.48	1.51±0.63	3.58	0.20	0.26
	Methanol extract	129.55	126.49	131.75	1.67±0.43	4.82	0.26	0.30
	Acetone extract	112.30	108.66	114.63	1.73±0.63	3.18	0.20	0.25
	Ethanol extract	97.58	94.43	100.63	1.89±0.33	4.59	0.24	0.28
	Column purified	34.53	31.78	37.93	1.72±0.69	4.52	0.21	0.19
96 h	*P. fulgens* (DRP)	133.62	129.66	138.87	1.84±0.38	3.85	0.18	0.33
	Ether extract	125.67	122.63	128.49	1.96±0.28	4.21	0.20	0.21
	Chloroform extract	122.44	119.76	125.96	1.78±0.33	3.58	0.25	0.35
	Methanol extract	120.36	117.26	124.83	1.51±0.75	4.54	0.16	0.33
	Acetone extract	98.63	94.60	102.58	1.84±0.38	4.06	0.25	0.20
	Ethanol extract	87.63	81.45	92.63	1.58±0.25	3.75	0.20	0.22
	Column purified	28.69	24.48	31.49	1.69±0.29	4.32	0.19	0.24

Six batches of ten *L. acuminata* were exposed different concentration of the above molluscicides. Mortality was determined after every 24 h, LCL: Lower confidence limits, UCL: Upper confidence limits, DRP: Dried root powder

be either due to the uptake of the active moiety which progressively increases the amount of active component in the snail body with increase in exposure duration or it might be possible that the active compound could change into more toxic forms in the aquarium water or in the snail body due to the action of various enzymes. Higher toxicity of ethanol extract among other organic extracts indicates that molluscicidal components present in *P. fulgens* plant are more soluble in ethanol.

The toxicity of *P. fulgens* plant products is time-dependent. It may be due to the uptake of the active moiety which progressively increases in snail body with increase in exposure period. Laloo *et al.*[30], reported the ethanolic root extract of *P. fulgens* preventing gastric ulcers in rats due to antihistamine and H^+K^+-ATPase inhibitory activities. It may be possible that the different active component of *P. fulgens* in snail body could change the different enzyme activity. Ray *et al.*[31], has been reported that the alcoholic extract of dried root powder of *P. fulgens* reduced significantly vital tegumental enzyme activity of acid phosphatase, alkaline phosphatase and adenosine triphosphatase (ATPase) in cestodes parasite *Raillietina echinobothrida* and trematodes *Gastrothylax crumenifer*, respectively. The acid phosphatase (ACP) is a lysosomal enzyme Aruna *et al.*[32], which plays an important role in catabolism, pathological necrosis, autolysis and phagocytosis[33]. The enzyme alkaline phosphatase plays a critical role in protein synthesis[34] shell formations, Timmermans[35] other secretary activities[36] and transport of metabolites[37] in gastropods. *P. fulgens* root extract is rich in polyphenolic components[30] with the maximum quantity of phenolic tannins. Jaitak *et al.*[38] reported the root extract of *P. fulgens* contain high amount of tannin and flavonoid. Several tannin bearing different families of plants have molluscicidal properties[39].

It is evident from the steep slope values indicate that a small increase in the concentration of different treatment in Table 1 caused mortality in snails. A t-ratio value greater than 1.96 indicated 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. The index of significance of the potency estimating values indicates that the value of the mean are within the limit at all probability level (90, 95 and 99) since it is less than 0.5.

CONCLUSION

It can be concluded from the study that the molluscicidal activity of *P. fulgens* can be used as potent molluscicide as it is easily and ecologically more acceptable by livestock keepers. For proper utilization of this plant products as molluscicides further studied are however, necessary to elucidate the mode of action of active molluscicidal components in snail body.

SIGNIFICANCE STATEMENTS

The present study concluded that the dried root powder of *Potentilla fulgens* may be used as potent molluscicides. These plant parts have great potentiality as molluscicides. For proper utilization of these plant products as molluscicides further studies are however, necessary to elucidate the mechanism and mode of action in the snail body.

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