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Original Research Article

ETHANOLIC COLUMN EXTRACT OF *Potentilla fulgens* AND THEIR EFFECTS ON BIOCHEMICAL CHANGES IN LIVER FLUKE VECTOR SNAIL *Lymnaea acuminata*

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ABSTRACT

Parasitic trematodes, *Fasciola hepatica*, and *F. gigantica* are causes fascioliasis in both livestock and humans. The snail *Lymnaea acuminata* is an intermediate host of the *Fasciola*. One of the major preventive steps against fascioliasis in the control of the vector snail *L. acuminata*. Effect of sub-lethal concentration of (20% and 60% of 96h LC₅₀) column purified fraction of *Potentilla fulgens* on free amino acid, protein, and nucleic acid (DNA, RNA) levels in nervous tissue of host snail *L. acuminata* were studied. Alteration in the levels of free amino acid, protein, and nucleic acid in nervous tissue was time and concentration-dependent. Withdrawal experiment demonstrates that there was a significant recovery in free amino acid, protein, DNA, and RNA levels in nervous tissues of snail *L. acuminata* were withdrawn from 7 days which treated with 20 and 60% of 96h LC₅₀ for 96h.

KEYWORDS: *Potentilla fulgens*; *Lymnaea acuminata*; Amino Acid; Protein; Nucleic Acid; Fascioliasis

Liver fluke (*Fasciola hepatica* and *F. gigantica*) causes liver cirrhosis and it is endemic worldwide diseases which caused severe economic loss and affecting a large cattle population and human (Al-Malki and Degheidy, 2013). The freshwater host snail *Lymnaea acuminata* is an intermediate host of liver fluke which causes fascioliasis among cattle in the eastern part of India (Singh and Agarwal, 1981). The effect of liver fluke can be controlled by the reduction of snail population due to which break the life cycle of fluke (Kumar and Singh, 2006, Singh *et al.*, 2008, Kumar *et al.*, 2009, Kumar *et al.*, 2011, Kumar *et al.*, 2014, Kumar *et al.*, 2016, Kumar *et al.*, 2018a, Kumar, 2021). Phytochemicals of the plant-derived would be an effective tool for selective killing of the intermediate host snail with a minimal adverse effect on the other non-target animal and environment. The dried root powder of medicinal plant *Potentilla fulgens* and their different organic extracts, column purified fractions are potent molluscicides against snail *Lymnaea acuminata* (Kumar *et al.*, 2018a), and *Indoplanorbis exustus* (Kumar *et al.*, 2018b). Their different preparations are also inhibiting the fecundity, hatchability, and survival of the young snail *I. exustus* (Kumar, 2021). The present study aims is to evaluate the sub-lethal molluscicides of ethanolic column purified fraction of medicinal plant *Potentilla fulgens* on the endogenous level of free amino acid, protein, and nucleic acid (DNA, RNA) in the nervous tissue of the snail *L. acuminata*.

MATERIALS AND METHODS

Collection of Adult Snail

Adult snail *L. acuminata* (2.26±0.25 cm in length) were collected locally and used as experimental animals from the lakes and low-lying submerged fields in Muhammadabad Gohna, Mau. The snails were acclimatized in the laboratory for 72h in dechlorinated tap water at 26±1 °C. Batches of 50 snails were kept in glass aquaria containing 6L dechlorinated tap water. Snails were treated according to the method of Singh and Agarwal (1986; 1989). Food was not provided to treated snail during the experiment. The same method had been used in a large number of studies (Singh and Agarwal 1986; 1989) and there was no mortality in the controls group. The oxygen concentration was normal in control animals as there was no sign of stress oxygen deficiency. The test method was standardized in the laboratory, in which 50 snails/6L water was used, which causes no oxygen stress in test snails (Singh and Agarwal 1986). The pH of the water was 7.2-7.4 and dissolved oxygen, free carbon dioxide, and bicarbonate alkalinity were 6.4-7.2, mg/l, 5.1-6.4mg/l, and 104.0-106.0mg/l, respectively. Dead snails were removed immediately from aquaria to avoid any contamination.

Preparation of Root Powder and Column Purifications

The freshly dried root of *Potentilla fulgens* was procured from the local market in Muhammadabad Gohna, Mau, (UP) India. The dried root of *P. fulgens* was

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pulverized separately in the electric grinder and the crude powders thus obtained, were then sieved with the help of a fine mesh cloth. These fine powder gradually mixed well in one thousand milliliters of ethanol organic solvent were subjected to silica gel (60-120 mesh, Qualigens Glass) chromatography through a 5 × 45 cm glass column. Five-milliliter column fractions eluted with ethanol were collected. Ethanol was evaporated under a vacuum machine and the remaining solids column extract obtained was used sub-lethal (20 and 60% of 96h LC₅₀, Kumar *et al.*, 2018a) concentration for the endogenous level of free amino acids, protein, and nucleic acids in the nervous tissue of *L. acuminata*.

Exposure of Snail with Sub-Lethal Concentration

Adult snail *L. acuminata* were kept and exposed in glass aquaria containing 6L dechlorinated tap water. Each aquarium contains 50 snails. The snails were treated with sub-lethal (20 and 60% of 96h LC₅₀) concentrations as reported by Kumar *et al.*, (2018a). The control groups of snails were kept in similar laboratory conditions without treatment. Each experiment was replicated at least 6 times for statistical analysis.

Withdrawal of Treated Snail

To study the effect of withdrawal from treatment, the snails were first exposed to the above doses for 96h following which these were transferred to freshwater. This water was changed every 24h for the next seven days, after which biochemical parameters were estimated in the nervous tissue of the snail.

Biochemical Estimations

The biochemical estimation was made in the nervous tissues of the experimental snails. Treated/control snails were removed from the aquaria, nervous tissues were dissected out, the adherent tissue was removed and the organs were put on a filter paper for absorption of water. 20-25 snails had to be dissected to obtain 100 mg of nervous tissue.

Estimation of Amino Acid

The estimation of total free amino acids was done by the method of Spies (1957). The nervous tissues were homogenized in 96% ethanol (10:1 w/v) in an electrical homogenizer for 5 minutes and centrifuged at 8000 g for 20 minutes. In 0.1ml of supernatant, 0.1ml of distilled water, and 2.0 ml of ninhydrin reagent were added and mixed thoroughly. The reaction mixture was kept in a boiling water bath for exactly 12-15 minutes. Two milliliters of 50% ethanol were added to the above solution after cooling. A violet colour developed which was measured at 575nm. Glycine was used to determine

the standard curves. Free amino acids have been expressed as µg/mg tissues.

Estimation of Protein

Quantitative estimation of protein was made according to the method of Lowry *et al.*, (1951). The nervous tissues were homogenized (1mg/ml, w/v) in 10% TCA using an electrical homogenizer for 5 minutes. Standard curves were prepared with different concentrations of bovine serum albumin. Results have been expressed as µg/mg tissues.

Estimation of Nucleic Acids (DNA, RNA)

Estimation of nucleic acids (DNA and RNA) was performed by the method of Schneider (1957). Homogenate (1.0 mg/ml, w/v) of nervous tissues was prepared in 5% TCA at 90°C. The result suspension centrifuged (5000g x 20 minutes) and a clear supernatant solution was taken for the nucleic acids estimation. Standard curves were drawn at different concentrations of calf thymus DNA to determine the standard curve. Yeast RNA was used to the determine standard. Results have been expressed as µg/mg tissue.

Statistical Analysis

The data have been expressed as mean ± SE of at least six replicates. The student's "t" test was applied between controls and treated groups to analyzed significant changes (p<0.05). The two-way analysis of variance was applied to detect the effect of dose and time-dependent effect on biochemical parameters (Sokal and Rohlf, 1973).

RESULTS

Exposure for 24h and 96h to 20% and 60% of 96h LC₅₀ of column purified fraction of root powder of *Potentilla fulgens* caused significant changes in the endogenous levels of total free amino acids, protein, and nucleic acid in the nervous tissue of snail *L. acuminata*. The levels of total free amino acids on sub-lethal 20% and 60% at 96h exposure in nervous tissue were 48.28 µg/mg and 53.26 µg/mg, respectively (Table-1). The exposure of *L. acuminata* for 96h at sub-lethal (20% and 60% of 96h LC₅₀) the protein levels in nervous tissue was 49.85 µg/mg and 50.23 µg/mg, respectively. Likewise, the total DNA levels in the nervous tissue after 96h exposure at sub-lethal 20% and 60% exposure were 48.13 µg/mg and 52.21 µg/mg, respectively. The exposure of 20 and 60% of 96h LC₅₀ of column purified fractions caused a further decline in the levels of RNA were 46.13 µg/mg and 55.26 µg/mg, respectively. Seven days after the snails have withdrawn from the 96h (20% and 60%) treatment it was found that there was a significant

recovery in the levels of total free amino acids, protein, and nucleic acid (DNA, RNA) in the nervous tissue of snail *L. acuminata* (Table-1).

Table 1: Biochemical changes in snail *L. acuminata* in the nervous tissue after exposure for 24h, 48h, 72h, and 96h for the level of total protein, amino acids, DNA, and RNA in sub-lethal (20 and 60%) exposure of 96h LC₅₀ of column purified fraction of *Potentilla fulgens*. In snail the effect of withdrawal for 7 days exposure for 96h

Biochemical estimations	Amino acids, Proteins, DNA and RNA level (µg/mg tissue #)						
	Control	24h**		96h**		Effect of withdrawal after 7 days	
		20%	60%	20%	60%	20%	60%
Amino acids	42.31±1.51 (100)	43.04±0.46* (101.7)	46.54±0.51* (108.8)	48.28±1.11* (114.11)	53.26±0.83* (125.8)	41.90±1.22+ (99.03)	48.96±1.18+ (115.71)
Proteins	38.92±1.70 (100)	40.51±1.19* (104.08)	43.26±1.61* (111.15)	49.85±1.20* (128.08)	50.23±1.25* (129.05)	44.12±1.73+ (113.36)	48.21±1.29+ (123.86)
DNA	39.32±0.18 (100)	42.60±0.85* (108.34)	44.91±0.35* (114.21)	48.13±1.38* (122.40)	52.21±0.91* (132.78)	46.71±1.26+ (118.79)	50.67±0.28+ (128.86)
RNA	32.64±0.62 (100)	39.00±0.41* (119.48)	41.13±0.58* (126.01)	46.13±0.82* (141.32)	55.26±0.73* (169.30)	41.93±0.29+ (128.46)	51.01±0.23+ (156.28)

** Kumar *et al.*, (2018a), Values are mean ±SE of six replicates. Values in parentheses indicate percent change with controls taken as 100%. *- Significantly (P<0.05) different from controls then student's t-test was applied.

+ - Significantly (P<0.05) different from 20% and 60% of the 24h and 96h treated groups when student's t- test was applied. Two-way analysis of variance demonstrated that, in snails, changes were dose and time-dependent.

#100 mg of nervous tissues were used for each biochemical estimation in snails *L. acuminata*

DISCUSSION

Earlier it was reported that the dried root powder, organic extract (ethanol, acetone, methanol, chloroform, and ether), and ethanolic column purified of *Potentilla fulgens* are a potent source of the molluscicides/anti-reproductive against freshwater snail *Lymnaea acuminata* and *Indoplanorbis exustus* (Kumar *et al.*, 2018a; Kumar *et al.*, 2018b; Kumar, 2021b). The present study is the extension of the same work, in which the effects of sub-lethal dose of column purified fraction of root powder of *P. fulgens* are determine on different biochemical parameters have been investigated. Thus levels of total free amino acids, protein, nucleic acids (DNA, RNA) in the nervous tissue have been studied in treated and untreated (control) snail *L. acuminata*. It is clear from the result section that exposure of *L. acuminata* to sub-lethal doses of column purified fraction of root powder of *P. fulgens* decreases the rate of the total free amino acids, protein, and amino acid (DNA, RNA) levels in the nervous tissue of *L. acuminata*. Hemalatha *et al.*, (2013) have been reported the ethanolic root extract of *P. fulgens* preventing gastric ulcers in rats due to antihistaminic and H⁺ K⁺ -ATPase inhibitory activities. It may be possible that the different active components of *P. fulgens* in the snail body could change the different biochemical parameters. Kumar, (2020) has been reported that the root extract of *P. fulgens* have anthelmintic activity against the sporocyst, redia and cercaria larvae of *Fasciola gigantica*. Ray *et al.*, (2010) have 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, a lysosomal enzyme, Aruna *et al.*, (1979) plays an important role in catabolism pathological necrosis autolysis. *P. fulgens* root extract is rich in polyphenolic components, with the maximum quantity of phenolic tannins (Hemalatha *et al.*, 2013). Jaitak *et al.*, (2010) have been reported the root extract of *P. fulgens* contain a high amount of tannin and flavonoid. The synthesis of protein in any of the tissue can be affected in two ways by a chemical; (I) it either affects the RNA synthesis at the transcription stage or (II) it somehow affects the uptake of amino acids in the polypeptide chain. Both these possibilities may account for the lower protein content in the affected tissue. In the first case, the RNA synthesis would be inhibited resulting in reduced RNA as well protein content. In the second case, only the protein content would be affected (Tariq *et al.*, 1977).

Data emerging from the result section demonstrated that treatment with sublethal dose of column purified fraction of *P. fulgens*, there was a sharp decline in the levels of total free amino acid, protein, DNA, and RNA. The changes, however, in the case of all the treatments were time and dose-dependent. With reduced RNA levels there is bound to be a fall in protein

levels because of a reduction in the synthesis of new protein. The result section demonstrated that seven days after the snails were withdrawn from 96h column purified fraction of *P. fulgens* sub-lethal treatment was found that there was near-complete recovery in the levels of total free amino acids, protein, DNA, and RNA in the nervous tissue of *L. acuminata*. This finding demonstrates that the effects of the phytochemicals are reversible.

CONCLUSION

In conclusion, it can be stated that the plant-derived phytochemicals cause an effect on the nervous tissue of the snail where it is a decrease in the levels of free amino acids, proteins, DNA, and RNA in the nervous tissue of the host snail *L. acuminata*.

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