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Research Article

Medicinal plant *Potentilla fulgens* and its effect *in vitro* against *Fasciola gigantica*

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Keywords: *Potentilla fulgens*; *Fasciola gigantica*; Fascioliasis; Anthelmintic activity

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Abstract

Fascioliasis is a one of the most important serious parasitic zoonotic disease which caused by trematode giant liver fluke *Fasciola hepatica* and *F. gigantica* among cattle's and humans. The infection of *Fasciola* can be control by the use of phytochemicals as anthelmintic components. The anthelmintic activities of dried root powder of medicinal plant *Potentilla fulgens* and their different preparations (organic extracts and column purified fraction) are uses *in vitro* against liver fluke *F. gigantica*. The dried root powder, different organic extract, and column fractions were time and concentration-dependent. Among all the organic extracts, ethanol extract was high toxic than other organic extracts. The toxic effect of ethanolic extract of *P. fulgens* after 2h exposure the LC_{50} value is 5.22 mg/ml against *F. gigantica.* The column purified fraction of dried root powder of *P. fulgens* shows more toxicity. The 2h LC_{50} of column purified fraction was 3.25 mg/ml whereas in 8h exposure the LC_{50} is 1.24 mg/ml. The phytochemicals of the *P. fulgens* may be used as anthelmintic components against liver fluke *F. gigantica*.

Introduction

The liver fluke *Fasciola hepatica* and *F. gigantica* is the causative agent of fasciolosis in cattle and human populations [1]. These diseases are parasitic in livestock with over 700 million production animals at risk of infection [2]. Fascioliasis is mainly related to plant-borne trematode digenetic zoonotic disease. The definite host of liver fluke is cattle, sheep, buffaloes, and goats which have an impact on the development, growth rate, the productivity of the ruminants and it's considered economically significant [3,4]. Fasciolosis is very common in the cattle population of the eastern part of Utter Pradesh, India [5-10]. In humans, the adult fluke of *Fasciola* causes a variety of symptoms such as long-standing fever, malaise, weight loss, eosinophilia, pain under the right costal margin, and anemia due to feeding on the blood [11].

Anthelmintic synthetic drugs can be used for the control of liver fluke infection, but it causes adverse effect on the host as well as develops resistant/residual effect in the fluke. Synthetic drugs are not easily available in some of the remote rural areas of developing countries [12]. The plant products or active phytochemicals are may be an effective anthelmintic component. Therefore, the use of the common medicinal plant as anthelmintic offers an alternative source that can solve these problems and it may be more acceptable due to eco-friendly and easily available for the users [13]. The medicinal plant *Potentilla fulgens* are commonly used as antihyperglycemic, antioxidant, antitumor, anti-hyperlipidemic, antiulcerogenic, anti-inflammatory, anthelmintic larvicidal, and molluscicidal [14-17]. The present study aims to evaluate the different preparations of medicinal plant *P. fulgens in vitro* treatments and their anthelmintic efficacy against the giant liver fluke *F. gigantica.*

Material and methods

Collection of liver fluke (*F. gigantica*)

The adult fluke *F. gigantica* (3.2 ± 0.18 cm in length) were collected from the infected bile ducts of the freshly slaughtered buffaloes from slaughterhouse district Gorakhpur (UP), India. The live fluke was kept in freshly prepared Hedon-Fleig (H-F) solution at 37 ± 2 ^{0}C in a BOD incubator until use.

Plants

The dried root of the *Potentilla fulgens* was procured from the local market in Muhammadabad Gohna, district Mau (UP), India, and authenticity by Dr. A. K. Singh, Department of Botany, S.G.N. Government P.G. College, Muhammadabad Gohna, Mau, Uttar Pradesh.

Preparation of crude plant products

The freshly dried root of *P. fulgens* washes through fresh-eater and dry under sunlight till to dry and cut into small pieces. The dried root pieces of *P. fulgens* were pulverized in the electric grinder machine and the crude powders thus obtained were used for the *in vitro* anthelmintic activity against *F. gigantica*.

Organic solvent extracts

Five gram dried powder of *P. fulgens* were extracted with 500 ml each of 98.1% acetone, 97.8% ether, 98.6% chloroform, and 94.7% ethanol at room temperature for 48h. The solvents were removed under a vacuum machine and the following remaining dried parts were used for the determination of *in vitro* anthelmintic activity of *P. fulgens* and extract obtained 350 mg-ethanol, 360 mg-chloroform, 385 mg-ether, and 395 mg-acetone.

Column purified-fractions

One thousand milliliters of 94.7% ethanol were uses for the fractions of dried root powder of *P. fulgens* were subjected to silica gel through 5 × 45 cm column chromatography (60-120 mesh, Qualigens Glass, Purchases from Precious Electrochemidus Private Limited, Bombay, India) for different fractions. One hundred milliliter fractions eluted with ethanol were collected. Ethanol from the column purified fractions was evaporated under a vacuum machine and the remaining solids column extracts were used for the determination of *in vitro* anthelmintic activity against *Fasciola*.

Hedon-Fleig (H-F) solution

The Hedon-Fleig (H-F) solution is used in this study which was prepared by the method of Hajare, et al. [18]. H-F solution contain NaCl-119.82 mM, $MgSO_4$-0.29 mM, KCl-4.01 mM, $CaCl_2$-0.40 mM, $NaHCO_3$-17.8 mM, Glucose-22.3 mM, Streptomycin sulphate-6900 unit 10 mg/l and Benzyl pencillin-9900 units/l at 38 ± 2 ^{0}C in BOD incubator.

In vitro toxicity determination

In vitro toxicity of dried root powder, organic extract (acetone, ether, chloroform, methanol, and ethanol), and column purified-fraction of *P. fulgens* were performed in the petri dish by the method of Kumar and Singh, [19]. Six Petri dishes were set for each concentration of different preparations of the *P. fulgens* against *F. gigantica*. Ten *F. gigantica* were kept in each Petri dish (10 cm × 1.5 cm) containing 50 ml H-F solution. Flukes were exposed to different concentrations of the different preparations (Table 1). The mortality of adult *Fasciola* was observed after 2h, 4h, 6h, and 8h exposure. The control group of the experiment was kept in an equal volume of H-F solution in a Petri dish under similar laboratory conditions but without treatment. The mortality of *Fasciola* was established by the opening of the sucker and contraction of the body. Usually in H-F solution in *in vitro F. gigantica* can survive up to 48h. Mortality of fluke was observed after 2h up to 8h were counted in treated and control groups.

In vitro, anthelmintic toxicity data were observed every 2h up to 8h. The Lethal Concentration (LC_{50}) values, lower and upper confidence limits (LCL and UCL), slope values, and t-ratio value were calculated by the POLO computer program [20].

Results

In vitro anthelmintic activity of dried root powder of *P. fulgens* and their different preparations against *F. gigantica* was concentration and time-dependent. The lethal concentration (LC_{50}) values of dried root powder at 2h, 4h, 6h, and 8h were 8.35, 7.12, 6.45, and 5.78 mg/ml, respectively (Table 1). Whereas, among all the organic extract (acetone, ether, chloroform, methanol, and ethanol) the ethanol extract were more toxic. The 2h, 4h, 6h, and 8h LC_{50} of ethanol extract of dried root powder of *P. fulgens* against *F. gigantica* were 5.22, 5.02, 4.88, and 3.43 mg/ml, respectively. The column purified fractions of dried root powder of *P. fulgens* were highly toxic. The 2h, 4h, 6h, and 8h exposure the LC_{50} value of the column purified-fractions were observed 3.25, 2.65, 1.94, and 1.24 mg/ml, respectively (Table 1). The slope values given in table 1 were steep and the separate estimates of LC_{50} values based on each of the six replicates of the experiments were found to be within the 95% confidence limits of lethal concentrations. The t-ratio value is greater than 1.96 that indicates the significant anthelmintic efficacy of the various treatments (Table 1).

Discussion

The present study is demonstrated that the dried root powder, organic extracts, and column purified fractions of *P. fulgens* are potent sources of anthelminthic components against *F. gigantica*. It may be possible that active phytochemicals of the medicinal plant, *P. fulgens* are entering the body of fluke *F. gigantica* and cause mortality. In the treated group, all the preparations of the *P. fulgens* are cause significant toxicity *in vitro* against the *F. gigantica* (Table 1). But no mortality was observed in the control group. It indicates that the active phytochemicals of *P. fulgens* are entering through the tegument layer which caused paralysis and mortality of the fluke. The toxicity of different preparations of *P. fulgens* is time as well as concentration dependant as evident from the LC_{50} values and exposure period (Table 1). Higher toxicity of ethanol extract was observed among all organic extracts which indicate that the anthelmintic components *P. fulgens* are more soluble in ethanol organic solvent.

In vitro toxicity of the root powder of the *P. fulgens* and their different extract preparations are significant and cause antilarvicidal activities against sporocyst, redia, and cercaria larva of the *F. gigantica* [21]. In *in vitro* treatment at 2h exposure, the highest toxicity was noted against sporocyst,

Table 1: *In vitro* anthelmintic activity of dried root powder of *P. fulgens* and their different organic extract, column purified against *F. gigantica* at different exposure periods.

Exposure periods	Values	*In vitro* exposure of Helminthicides (mg/ml)						
		Potentilla fulgens dried root powder	Acetone extract	Ether extract	Chloroform extract	Methanol extract	Ethanol extract	Column purified
2h	LC_{50}	8.35	7.76	7.21	6.45	6.95	5.22	3.25
	LCL	6.42	6.12	6.58	5.45	5.72	4.25	2.12
	UCL	9.24	8.45	8.93	7.82	8.64	7.93	4.65
	Slope-value	1.30 ± 0.21	1.12 ± 0.20	1.27 ± 0.11	1.18 ± 0.19	1.23 ± 0.27	1.19 ± 0.17	1.25 ± 0.16
	t-ratio	3.45	4.32	3.55	4.11	3.23	3.96	4.12
4h	LC_{50}	7.12	6.45	6.37	5.86	6.01	5.02	2.65
	LCL	6.89	5.35	5.37	4.61	4.65	4.12	1.75
	UCL	8.23	7.91	8.32	6.94	7.74	7.53	3.24
	Slope-value	1.14 ± 0.18	1.15 ± 0.21	1.26 ± 0.29	1.27 ± 0.11	1.19 ± 0.24	1.11 ± 0.23	1.31 ± 0.25
	t-ratio	3.11	4.43	3.76	4.26	3.76	3.23	4.23
6h	LC_{50}	6.45	5.54	6.12	5.12	5.76	4.88	1.94
	LCL	5.96	4.55	5.13	4.55	4.71	3.24	0.66
	UCL	7.12	6.35	8.93	7.32	6.77	5.35	2.13
	Slope-value	1.35 ± 0.20	1.33 ± 0.19	1.27 ± 0.22	1.18 ± 0.35	1.26 ± 0.16	1.39 ± 0.26	1.35 ± 0.21
	t-ratio	4.54	3.14	4.54	3.23	3.65	4.87	4.53
8h	LC_{50}	5.78	4.87	5.86	4.95	4.64	3.43	1.24
	LCL	4.86	3.24	4.32	3.91	3.74	2.46	0.56
	UCL	6.31	6.51	7.43	5.88	6.27	4.99	2.02
	Slope-value	1.26 ± 0.19	1.22 ± 0.24	1.26 ± 0.17	1.34 ± 0.29	1.17 ± 0.24	1.34 ± 0.26	1.32 ± 0.26
	t-ratio	3.57	4.56	3.11	4.12	4.23	3.54	4.13

Ten *Fasciola gigantica* in six batches were exposed *in vitro* (H-F solution) on different concentrations of the above anthelmintic preparations. Mortality of *Fasciola* was determined after every 2 h exposure period. LCL: Lower Confidence Limits; UCL: Upper Confidence Limits

redia, and cercaria the LC_{50} value of column extract were 62.42, 59.25, and 45.11 mg/ml, respectively. It may be due to the uptake of the active moiety which progressively increases in the fluke body with an increase in the exposure period. It may be possible that the phytochemicals of the *P. fulgens* in the *F. gigantica* could change the different enzyme activity and cause effects. Many numbers of medicinal plants are have been used for the treatment of parasitic infection in animals and humans [22]. Kumar and Singh, [19] have been reported that in *in vivo* the common species *Allium sativum*, *Ferula asafoetida*, *Syzygium aromaticum* and their active components like allicin, ferulic acid, umbelliferone, and eugenol have anthelmintic activities against *F. gigantica*. The binary combinations (1:1 ratio) of the active components the allicin, ferulic acid, umbelliferone, and eugenol are more effective *in vitro* against the liver fluke *F. gigantica* [23]. The ethanolic root extract of *P. fulgens* is preventing gastric ulcers in rats due to H^+ K^+-ATPase inhibitory and antihihistaminic activities [24]. The dried root powder of *P. fulgens* and their different organic extracts, column purified fractions are a potent source of the molluscicides which cause significant mortality against liver fluke vector snails *Lymnaea acuminata* and *Indoplanorbis exustus* [15-17]. Roy, et al. [25] have been reported that the alcoholic extract of dried root powder of *P. fulgens* has significantly reduced the vital tegumental enzymatic activity of the alkaline phosphatase, acid phosphatase, and adenosine triphosphatase (ATPase) in cestodes parasite *Raillietina echinobothrida* and trematodes *Gastrothylax crumenifer*. The root extract of *P. fulgens* has flavonoids and tannin in high amounts [26]. *In vitro* and *in vivo* studies have been evidence to support the anthelmintic effect which feeds the tannins and other polyphenols against abdominal and intestinal parasitic nematodes [27,28]. Athanasiadou, et al. [29] have been evaluated that tannin shows anthelmintic activities against infected sheep with *Trichostrongylus colubriformis* and causing larval mortalities. The compound of tannin in *in vitro* inactivates the enzyme activities in *Trichostrongylus colubriformis* larvae which are responsible for hatching and development [30]. Previously in different studies have been evaluated that the root extract of *P. fulgens* possesses antitumor [31], antioxidant [26], anthelmintic [25], and gastroprotective [24]. The root extract of *P. fulgens* in *in vitro* inhibit enzyme activities of amylase, α-glucosidase, β-glucosidase, and lipase in the liver, kidney, and eye lens of the diabetic mice [32].

Conclusion

It can be concluded from the present study that the dried root powder of *P. fulgens* and their different organic extract and column purified fractions may be used as potent anthelminthic components for the control of liver fluke *F. gigantica*. This study also revealed a further study that the phytochemicals of the *P. fulgens* at the molecular level elucidate in the parasitic life of the liver fluke *F. gigantica*.

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