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 zoonotic disease. The definite host of liver fluke is cattle, is mainly related to plant-borne trematode digenetic 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, antiluercogenic, 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*.

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

Organic solvent extracts

Five gram dried powder of Potentilla 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 Potentilla 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 used for the fractions of dried root powder of Potentilla 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, MgSO₄-0.29 mM, KCl-4.01 mM, CaCl₂-0.40 mM, NaNHCO₃-17.8 mM, Glucose-22.3 mM, Streptomycin sulphate-6900 unit 10 mg/l and Benzyl penicillin-9900 units/l was prepared by the method of Hajare, et al. [18]. 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, MgSO₄-0.29 mM, KCl-4.01 mM, CaCl₂-0.40 mM, NaNHCO₃-17.8 mM, Glucose-22.3 mM, Streptomycin sulphate-6900 unit 10 mg/l and Benzyl penicillin-9900 units/l was prepared by the method of Hajare, et al. [18]. H-F solution contain NaCl-119.82 mM, MgSO₄-0.29 mM, KCl-4.01 mM, CaCl₂-0.40 mM, NaNHCO₃-17.8 mM, Glucose-22.3 mM, Streptomycin sulphate-6900 unit 10 mg/l and Benzyl penicillin-9900 units/l was prepared by the method of Hajare, et al. [18].

In vitro toxicity determination

In vitro toxicity of dried root powder, organic extract (acetone, ether, chloroform, methanol, and ethanol), and column purified-fraction of Potentilla 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 Potentilla fulgens against Fasciola gigantica. Ten Fasciola 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 Fasciola 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 (LC₅₀) 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 Potentilla fulgens and their different preparations against Fasciola gigantica was concentration and time-dependent. The lethal concentration (LC₅₀) 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 LC₅₀ of ethanol extract of dried root powder of Potentilla fulgens against Fasciola gigantica were 5.22, 5.02, 4.88, and 3.43 mg/ml, respectively. The column purified fractions of dried root powder of Potentilla fulgens were more toxic. The 2h, 4h, 6h, and 8h exposure the LC₅₀ 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 LC₅₀ 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 Potentilla fulgens are potent sources of anthelmintic components against Fasciola gigantica. It may be possible that active phytochemicals of the medicinal plant, Potentilla fulgens are entering the body of fluke Fasciola gigantica and cause mortality. In the treated group, all the preparations of Potentilla fulgens are cause significant toxicity in vitro against Fasciola gigantica (Table 1). But no mortality was observed in the control group. It indicates that the active phytochemicals of Potentilla fulgens are entering through the tegument layer which caused paralysis and mortality of the fluke. The toxicity of different preparations of Potentilla fulgens is time as well as concentration dependant as evident from the LC₅₀ values and exposure period (Table 1). Higher toxicity of ethanol extract was observed among all organic extracts which indicate that the anthelmintic components Potentilla fulgens are more soluble in ethanol organic solvent.

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

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     | Potentilla fulgens dried root powder | Acetone extract | Ether extract | Chloroform extract | Methanol extract | Ethanol extract | Column purified |
|------------------|------------|--------------------------------------|-----------------|--------------|--------------------|-----------------|----------------|----------------|
|                  | LC50       | 2h                                   | 4h              | 6h           | 8h                 | 2h              | 4h            | 6h            | 8h          |
|                  | 8.35       | 7.76                                 | 7.21            | 6.45         | 6.95               | 5.22            | 3.25          |               |
|                  | LCL        | 6.42                                 | 6.12            | 5.58         | 5.72               | 4.25            | 2.12          |               |
|                  | UCL        | 9.24                                 | 8.45            | 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          |               |
|                  | LC50       | 7.12                                 | 6.45            | 5.37         | 6.12               | 5.58            | 6.01          | 5.02          | 2.65        |
|                  | LCL        | 6.89                                 | 5.35            | 4.61         | 5.37               | 4.65            | 4.12          | 1.75          |               |
|                  | UCL        | 8.23                                 | 7.91            | 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          |               |
|                  | LC50       | 6.45                                 | 5.54            | 5.13         | 4.55               | 4.71            | 3.24          | 0.66          |               |
|                  | LCL        | 5.96                                 | 4.55            | 4.07         | 4.55               | 4.57            | 5.88          | 7.32          | 2.13        |
|                  | UCL        | 7.12                                 | 6.35            | 5.37         | 4.95               | 4.57            | 4.12          | 2.46          | 0.56        |
| 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          |               |
|                  | LC50       | 5.78                                 | 4.87            | 5.66         | 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.

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 anthelmintic 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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