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MEDICINAL PLANT: *GLYCYRRHIZA GLABRA* AND ITS EFFICACY IN *IN VITRO* AGAINST *FASCIOLA GIGANTICA* LARVA

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ABSTRACT
Fascioliasis is an economically important disease, which caused by Fasciola (liver fluke) among cattle and human. Class trematoda has Fasciola hepatica and F. gigantica which are worldwide. The intermediate host of Fasciola is Lymnaeidae snail. Fasciola is a hermaphrodite and their larval stages (sporocyst, redia and cercaria) are completed within the host snail. Infection of this disease can be control by destroy the larval stages. The aim of this study is to use of different preparation of Glycyrrhiza glabra plant in in vitro against sporocyst, redia and cercaria larva of F. gigantica. Each experimental setup of Petri dish contains 10 larvae. The dried root powder, organic extracts and column purified fractions were exposed in 10 ml dechlorinated water against sporocyst, redia and cercaria within 2h, 4h, 6h and 8h separately. The column purified fraction were shows after 2h LC_{50} 51.15, 48.42 and 55.69 mg/ml and 8h LC_{50} 45.95, 40.15, and 45.62 mg/ml efficacy against sporocyst, redia and cercaria, respectively.

Keywords: *Glycyrrhiza glabra, Fascioliasis, Sporocyst, Redia, Cercaria.*

INTRODUCTION

Fasciola hepatica and *F. gigantica* is a causative agent of Fascioliasis. The species of *F. gigantica* is one of the major trematode in tropical and subtropical region of the world (Nyindo and Lukambagire, 2015), which caused zoonosis. In eastern part of the Uttar Pradesh, India *F. gigantica* is a causative agent of fascioliasis (Kumar and Singh, 2006, Kumari *et al*., 2012, Kumar *et al*., 2018). In the ruminants it is generally live in gall bladder and biliary duct of definite host (primary host), but some time it also observed in ectopic place like eye, skin, spleen (Rokni *et al*., 2014). It causes economic loss due to wasting infected organs, decreasing milk, wool and meat production. In world population around 17 million people are infected with fascioliasis (Mas-Coma et al. 2009a) and high pathogenicity situations as ophthalmological and neurological affections giving rise to permanent sequelae and even fatal cases (Mas-Coma et al., 2014b) speak about the public health importance of this disease. Human fascioliasis caused by eating metacercaria encysted leaves that are eaten as contaminated food, vegetables and water (Mas-Coma et al., 2014a; Bahram et al., 2020). Effect of fascioliasis in endemic area is comparatively higher in children and women, even at early age (Mas-Coma et al., 2018).

Snail and Slugs are causes agricultural loss (Kumar, 2020) and snails *Lymnaea acuminata* is an intermediate host of *Fasciola* which transmitted zoonotic disease (Kumar et al., 2018; Vishwakarma and Kumar, 2021a) in livestock keeper and human. *F. gigantica* complete their life cycle between snail and sheep which are very complex and have five larval stages such as miracidium, sporocyst, redia, cercaria and metacercaria. The sporocyst, redia and cercaria larva are found inside the body of host snail *L. acuminata*. Fascioliasis can be control by strategical approaches to break their life cycle by killing the sporocyst, redia and cercaria larva inside the host snail. Before the treatment of different larval stages of *Fasciola* inside the host snail an approaches should be established in *in vitro* treatment of the larva. In *in vitro* treatment of larvae by plant products may be new approaches for killing the *Fasciola* larva, which are ecologically safer and biodegradable. Phytochemicals have sufficient larvicidal properties in *in vitro* (Sunita and Singh, 2011; Vishwakarma and Kumar, 2021b) against *Fasciola* larva. *Glycyrrhiza glabra* are commonly known as Licorice or Mulethi, which are worldwide medicinal plant (Titei et al., 2017). It is a native herbs to the Southwest and Central regions of Asia and the Mediterranean basin (Bell et al., 2011; Karkanis et al., 2018) and it found at the high or low altitudes, up to 1200 meter above sea level (Durak, 2014). Traditionally *G. glabra* are uses as anti-inflammatory, anti-ulcer, laxative, antibacterial, anti-protozoan, anti-tumor, antimicrobial, anti-malarial and Hepato-protective (Sangam, et al., 2017; Zang,2020). The aim of the present study is to evaluate the efficacy of dried root powder, organic extracts and column purified fraction in *in vitro* against *Fasciola* larva.

MATERIAL AND METHODS

Collection of host snails:

Snails *Lymnaea acuminata* (2.6±0.20 cm in length) were collected locally from pond and low lying areas of Muhammadabad Gohana, Mau, UP, (India). The snails were allowed to acclimatize for 24 hours in laboratory condition (pH 7.3-7.5) before dissection and collection of different *Fasciola* larva.

Identification of infected snails and collection of larva:-

Infected snails *L. acuminata* is often shows more resources to growth than uninfected snails, which can grow in large size than uninfected (Mouritsen and Jensen, 1994; Gorbushin, 1997; Probst and Kube, 1999), locomotion is slow than uninfected ones, appeared yellowish in color, foots are more swollen and shedding cercaria were appeared at the mouth of snails and shell morphology is changed in infected snails (Hay, *et al.,* 2005; Lagrue, *et al.,* 2007; Kumari Sunita et al., 2013). Each infected snail was dissected in a glass Petri dish containing 10 ml of dechlorinated water at 23°C-25°C under dissecting microscope. Sporocyst, redia and cercaria larva were separated in different Petri dish which containing 10 ml of dechlorinated water. These larvae were kept in dechlorinated tap water where they survive up to 48h in laboratory condition.

Plant products:

The dried root of *G. glabra* were purchased from local market Muhammadabad Gohana Mau, U.P. (India) and were authenticated by Dr. A. K. Singh, Department of Botany, S.G.N. Govt. P.G. College Muhammadabad Gohana Mau, U.P. (India). The roots washed with water, dried under shade after complete drying, the root materials were cut into small pieces and then grind in an electric grinder machine and the crude powder thus obtained, were then sieved with the help of fine mesh cloth. Finally, the powder was stored in a suitable container in laboratory condition at 25^0C-27^0C in incubator.

Extraction of plant products:

Ten gram dried root powder *G. glabra* were extracted with 200 ml of 98% ether, 99.7% chloroform, 98% methanol, 98% acetone and 95% ethanol at room temperature for 24 hours. Each preparation was filtered separately through sterilized Whatman No. 1 filter paper and the filtered extracts where subsequently evaporated under vacuum. The residues, thus obtained, were used for the determination of larvicidal activity. The root powder of *G. glabra* yielded 330 mg ether, , 225 mg chloroform, 375 mg methanol, 370 mg acetone, and 105 mg ethanol extracts.

Column extraction:

One liter of ethanol extract fraction of dried root powder of *G. glabra* was 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 pump and the remaining solids obtained were used for the determination of*in vitro* larvicidal activity of each fraction.

***In vitro* treatment of plant products:-**

In *in vitro* larvicidal activity of organic extract (ether, chloroform, methanol, acetone and ethanol) and column was performed in Petri dish by the method of Sunita and Singh (2011). Ten sporocyst, redia and cercaria larva were separated in different Petri dish containing 10 ml dechlorinated tap water. Exposure of dried root powder, different organic extracts and column purified were made directly in Petri dish under laboratory condition which containing 10 sporocyst/redia/cercaria. Mortality of sporocyst, redia and cercaria were observed after 2h, 4h, 6h and 8h of treatments. Dead larvae stop physical activity and it remove from Petri dish in each intervals. In control group, no treatments were given in Petri dish. Usually in *in vitro* conditions (control group) survival of 48h in dechlorinated water. Counting of larvae in control and treated groups was performed with the help of a light microscope. Each experiment was repeated six times for statistical calculation.

Lethal value (LC_{50}), lower and upper confidence limit (LCL and UCL), Slop-values, t-ratio, g value and heterogeneity factor were calculated by the help of POLO computer programmed of Robertson *et al.,*2007.

RESULTS

The exposure of dried root powder, different organic extract ether, chloroform, methanol, acetone and ethanol and column purified of *G. glabra* in *in vitro* against sporocyst, redia and cercaria larva of *F. gigantica* was time and concentration dependent (Table 1-3). *In vitro* exposure of dried root powder of *G. glabra* was more effective against cercaria (2h LC_{50} 70.25 mg/ml and 8h LC_{50} 60.32 mg/ml) (Table-3). The exposure of ethanol extract against sporocyst, redia and cercaria after 2h $LC_{50}$was 53.49, 51.50 and 58.00 mg/ml, respectively (Table 1-3). Whereas, 8h LC_{50} of ethanol extract against sporocyst, redia and cercaria was 47.15, 42.90 and 48.78 mg/ml, respectively. The column purified fractions of dried root powder of *G. glabra* against sporocyst, redia and cercaria in 2h exposure LC_{50} was 51.15, 48.42 and 55.69 mg/ml and 8h exposure LC_{50} was 45.92, 40.15 and 45.62 mg/ml, respectively (Table 1-3). The column purified was more effective against sporocyst, redia and cercaria. Maximum effects of column purified of *G. glabra* were observed against redia larva (8h LC_{50} 40.15 mg/ml) (Table-2).

DISCUSSION

The larvicidal activity of dried root powder, organic extracts and column purified fractions of *Glycyrrhiza glabra* in *in vitro* against sporocyst, redia and cercaria was more effective. The primary phytochemical constituents of *G. glabra* are glycyrrhizin, liquiritin, glabridin, triterpene, isoliquiritin, glycyrrhetic acid, saponins, flavonoids (Sangam and Sheela, 2017; Karkanis et al. 2018; Batiha et al. 2020; Murck, 2020). The ethanolic extract of root powder of *G. glabra* have antimicrobial activity due to presence of sponins, alkaloid, flavonoids, tannin, glycosides and phenolic compounds (Karami et al., 2013) against *Staphylococcus aurens*, *Candida albicans*, *Bacillus subtilis* (Demizu et al., 1988; Mitscher et al., 1980), while methanolic extract shows antibacterial property against *Agrobacterium tumefaciens*, *Bacillus cereus*, *Bacillus subtilus* and *Pseudomonas syringae* (Ercisli, et al., 2008). The phytochemicals of *G. glabra*are uses as anti-inflammatory, anti-cariogenic, antiulcer, antibacterial, antifungal, anti-viral, anti- allergic, antioxidant, immune stimulatory, anti-protozoan, anti-tumor (Martins, et al., 2015, Karkanis, et al., 2018), hepatoprotective, immunomodulatory, neuro-protective, memory enhancement, anti-diabetic, anti-asthmatic, haematinic, cerebro-protective, anti-tussive, hair growth promoting (Anagha, et al., 2013; Sangam, et al., 2017; Zang,2020).

In *in vitro* larvicidal study of different preparation of *G. glabra* are effective against sporocyst, redia and cercaria larva of *F. gigantica*. Anti-larvicidal activity of *G. glabra* may be due to the presence of different phytochemicals in the root products which are easily diffuses in larvae and progressively increase along with exposure period and cause mortality by the various enzyme action. The highest mortality of larvae was observed in ethanolic extract, which indicate that phytochemicals of this plant easily dissolved in the ethanol.

The steep slope values of toxic study indicate that a small increase concentration of various treatments caused mortality in the sporocyst, redia and cercaria (Table 1-3). Value 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 adequately. A t-ratio value greater than 1.96 clearly indicated that the regression is significant. 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 92) since it is less than 0.5.

CONCLUSION

The study of *Glycyrrhiza glabra* in *in vitro* larvicidal activity of dried root powder, different organic extract and column fraction against sporocyst, redia and cercaria was more effective. The exposure of all the treatment were analyzed that its time and concentration dependents against sporocyst, redia and cercaria. Whereas, ethanol extract shows higher larvicidal activity among all extract which may be due to presence of one or more phytochemicals are present in the ethanol extract which might be responsible for larvicidal activity. *In vitro* studies of *G. glabra* were revealed that the phytochemical of this plant, easily diffuses in the larval body and causes mortality. Therefore, it propagates for the further study that how the phytochemical of *G. glabra* act at the molecular level in the *Fasciola* larva. The phytochemicals are may be useful in the control of fascioliasis.

Conflict of Interest:
The authors have no conflict of interest.

Table 1. *In vitro* larvicidal activity of dried root powder, different organic extract and column purified fractions of *Glycyrrhiza glabra* against the sporocyst larva of *Fasciola gigantica.*

Larvicidal (mg/ml)	Exposure	LC_{50}	LCL	UCL	Slope - value	t-ratio	g-value	Heterogeneity
Glycyrrhiza glabra dried root powder	2h	74.83	70.69	94.16	0.18±0.39	2.47	0.43	0.11
Ether extract		67.91	52.42	89.34	0.21±0.63	2.83	0.37	0.17
Chloroform extract		66.37	53.72	93.22	0.56±0.84	2.33	0.21	0.10
Methanol extract		71.42	67.42	91.88	0.26±0.68	2.39	0.26	0.13
Acetone extract		63.70	50.93	74.53	0.16±0.73	2.21	0.34	0.23
Ethanol extract		53.49	45.88	69.12	0.23±0.68	2.55	0.46	0.14
Column purified		51.15	41.62	64.30	0.21±0.74	2.61	0.48	0.10
Glycyrrhiza glabra dried root powder	4h	72.56	65.76	90.84	0.29±0.11	2.69	0.21	0.19
Ether extract		65.82	51.22	88.25	0.56±0.33	2.87	0.37	0.12
Chloroform extract		64.53	50.78	91.15	0.78±0.29	2.54	0.49	0.21
Methanol extract		69.10	61.32	89.56	0.60±0.18	2.27	0.22	0.30
Acetone extract		61.62	48.99	73.44	0.93±0.54	3.05	0.28	0.19
Ethanol extract		51.53	40.68	67.35	0.53±0.33	2.37	0.31	0.16
Column purified		49.20	36.22	62.92	0.67±0.29	2.93	0.49	0.14
Glycyrrhiza glabra dried root powder	6h	69.91	61.99	87.69	0.74±0.36	2.67	0.44	0.21
Ether extract		63.01	48.33	85.72	0.35±0.67	2.51	0.39	0.17
Chloroform extract		62.90	49.18	88.98	0.54±0.99	3.14	0.33	0.15
Methanol extract		67.58	59.28	86.42	0.97±0.17	2.76	0.31	0.36
Acetone extract		58.92	45.42	70.85	0.64±0.10	2.13	0.29	0.14
Ethanol extract		48.95	38.74	65.49	0.84±0.23	2.78	0.37	0.19
Column purified		47.32	34.79	60.50	0.89±0.37	2.17	0.24	0.18
Glycyrrhiza glabra dried root powder	8h	67.38	53.25	85.43	0.65±0.12	2.83	0.32	0.15
Ether extract		60.97	46.15	81.69	0.77±0.48	2.57	0.39	0.20
Chloroform extract		61.10	45.99	84.50	0.91±0.63	2.34	0.43	0.17
Methanol extract		65.20	57.82	85.22	0.89±0.24	2.91	0.32	0.13
Acetone extract		56.45	43.52	77.92	0.80±0.56	3.19	0.47	0.22
Ethanol extract		47.15	36.84	60.83	0.57±0.30	2.99	0.44	0.13
Column purified		45.92	32.18	56.15	0.48±0.19	2.33	0.40	0.19

LCL- lower confidence limits, UCL-upper confidence limits.
Six batches of 10 sporocyst larva were exposed different concentration of the above larvicide treatments. Mortality of larva was recorded every 2h .Concentration given are the final concentration (W/V) in the Petri-disc water.

Table 2. *In vitro* larvicidal activity of dried root powder, different organic extract and column purified fractions of *Glycyrrhiza glabra* against the redia larva of *Fasciola gigantica.*

Larvicidal (mg/ml)	Exposure	LC_{50}	LCL	UCL	Slope - value	t-ratio	g-value	Heterogeneity
Glycyrrhiza glabra dried root powder	2h	71.25	66.50	83.05	0.77±0.31	2.31	0.25	0.19
Ether extract		65.42	57.22	71.48	0.84±0.51	2.74	0.31	0.83
Chloroform extract		62.95	53.67	72.39	0.34±0.16	2.48	0.29	0.27
Methanol extract		67.36	61.48	71.37	0.29±0.07	3.14	0.70	0.43
Acetone extract		60.22	48.15	69.97	0.77±0.53	3.11	0.66	0.37
Ethanol extract		51.50	41.57	62.34	0.91±0.67	2.39	0.61	0.28
Column purified		48.42	37.91	59.47	0.34±0.57	2.55	0.79	0.55
Glycyrrhiza glabra dried root powder	4h	67.92	60.32	74.25	0.36±0.44	2.40	0.27	0.30
Ether extract		62.74	54.90	68.81	0.55±0.21	2.79	0.81	0.46
Chloroform extract		60.18	51.38	68.22	0.67±0.33	2.20	0.21	0.29
Methanol extract		64.22	58.23	69.50	0.81±0.45	3.01	0.29	0.68

Acetone extract		58.62	46.73	67.46	0.19±0.24	3.42	0.37	0.24
Ethanol extract		47.82	38.72	60.16	0.97±0.34	2.69	0.22	0.56
Column purified		46.25	35.22	56.15	0.68±0.71	2.93	0.40	0.37
Glycyrrhiza glabra dried root powder	6h	64.69	57.72	69.16	0.59±0.41	2.04	0.55	0.18
Ether extract		60.18	52.34	67.10	0.24±0.31	2.75	0.33	0.47
Chloroform extract		58.32	50.56	66.38	0.87±0.63	2.44	0.21	0.59
Methanol extract		61.98	55.62	67.54	0.94±0.29	2.87	0.74	0.27
Acetone extract		56.48	44.97	64.82	0.74±0.54	2.99	0.29	0.39
Ethanol extract		45.20	36.41	55.39	0.34±0.61	2.16	0.33	0.20
Column purified		43.92	33.69	53.17	0.27±0.07	3.03	0.50	0.43
Glycyrrhiza glabra dried root powder	8h	62.15	51.26	67.42	0.55±0.69	3.19	0.37	0.76
Ether extract		57.95	48.62	64.59	0.91±0.71	3.08	0.20	0.35
Chloroform extract		56.50	48.16	65.20	0.90±0.55	2.81	0.49	0.22
Methanol extract		59.42	51.76	64.27	0.37±0.18	2.59	0.67	0.48
Acetone extract		51.35	41.35	61.72	0.53±0.79	2.43	0.40	0.50
Ethanol extract		42.90	32.27	51.68	0.60±0.37	2.96	0.29	0.22
Column purified		40.15	30.18	51.73	0.29±0.55	2.41	0.38	0.39

LCL- lower confidence limits, UCL-upper confidence limits.

Six batches of 10 sporocyst larva were exposed different concentration of the above larvicide treatments. Mortality of larva was recorded every 2h .Concentration given are the final concentration (W/V) in the Petri-disc water.

Table 3. *In vitro* larvicidal activity of dried root powder, different organic extract and column purified fractions of *Glycyrrhiza glabra* against the cercaria larva of *Fasciola gigantica.*

Larvicidal (mg/ml)	Exposure	LC_{50}	LCL	UCL	Slope - value	t-ratio	g-value	Heterogeneity
Glycyrrhiza glabra dried root powder	2h	70.25	57.29	81.92	0.54±0.37	3.47	0.60	0.22
Ether extract		68.34	59.16	83.20	0.69±0.29	2.33	0.34	0.29
Chloroform extract		66.74	56.93	77.41	0.93±0.44	2.72	0.74	0.43
Methanol extract		69.53	58.40	79.10	0.87±0.26	3.64	0.25	0.40
Acetone extract		66.15	53.79	77.22	0.67±0.52	3.18	0.37	0.36
Ethanol extract		58.00	43.92	69.14	0.73±0.31	2.56	0.40	0.49
Column purified		55.69	40.78	66.39	0.59±0.20	2.89	0.29	0.21
Glycyrrhiza glabra dried root powder	4h	65.92	54.76	79.52	0.94±0.39	2.18	0.53	0.28
Ether extract		64.26	56.64	80.29	0.55±0.68	2.67	0.50	0.35
Chloroform extract		62.84	51.39	75.61	0.74±0.32	3.14	0.38	0.22
Methanol extract		67.42	54.82	77.34	0.44±0.81	3.39	0.21	0.49
Acetone extract		61.92	50.32	74.71	0.37±0.19	2.91	0.56	0.63
Ethanol extract		54.73	41.27	65.67	0.55±0.63	2.46	0.63	0.50
Column purified		51.33	36.39	63.91	0.79±0.33	2.84	0.47	0.44
Glycyrrhiza glabra dried root powder	6h	62.17	51.39	77.41	0.86±0.57	2.36	0.40	0.29
Ether extract		61.82	52.88	78.20	0.72±0.21	2.48	0.21	0.46
Chloroform extract		60.95	49.35	74.22	0.38±0.19	3.19	0.30	0.33
Methanol extract		62.68	52.10	71.93	0.44±0.25	3.33	0.43	0.75
Acetone extract		58.47	48.79	70.17	0.58±0.63	3.67	0.36	0.39
Ethanol extract		51.38	39.22	63.46	0.82±0.34	3.02	0.22	0.28
Column purified		47.92	33.42	61.77	0.52±0.30	2.49	0.41	0.41
Glycyrrhiza glabra dried root powder	8h	60.32	47.95	72.22	0.60±0.27	2.93	0.30	0.57
Ether extract		59.62	50.31	71.96	0.77±0.62	2.87	0.37	0.22
Chloroform extract		57.48	47.27	72.41	0.99±0.35	3.08	0.64	0.39
Methanol extract		60.15	50.27	69.76	0.83±0.47	2.37	0.52	0.25
Acetone extract		57.24	46.82	68.49	0.34±0.58	2.20	0.44	0.42
Ethanol extract		48.78	36.74	60.15	0.29±0.48	3.19	0.29	0.20
Column purified		45.62	30.87	56.22	0.87±0.18	2.74	0.36	0.37

LCL- lower confidence limits, UCL-upper confidence limits.

Six batches of 10 sporocyst larva were exposed different concentration of the above larvicide treatments. Mortality of larva was recorded every 2h .Concentration given are the final concentration (W/V) in the Petri-disc water.

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