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Research Paper

In vivo Anthelmintic Activity of Medicinal Plant *Asparagus racemosus* Against Larva of *Fasciola gigantica*

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A B S T R A C T

Trematode infections are causes zoonotic disease by *Fasciola hepatica* and *F. gigantica* among cattle and human. The control of fascioliasis by treatment of sporocyst, redia and cercaria larva in *in vivo* which can break the life cycle and check the zoonotic disease. The present study was designed for treatment of *F. gigantica* larva in host snail *Lymnaea acuminata* in *in vivo* at different exposures. The dried root powder of *Asparagus racemosus* and their different organic extract (ether, chloroform, methanol, acetone and ethanol) and column purified fractions was observed up to 8h at different concentration against *Fasciola* larva. *In vivo* mortality of larva was observed at 2h, 4h, 6h and 8h of treatment of infected snails at different concentration. The dried root powder of *A. racemosus* were more effective (2h LC_{50} 71.39 mg/l and 8h LC_{50} 63.22 mg/l) against cercaria larvae. The 8h LC_{50} of ethanol extract against sporocyst, redia and cercaria was observed 51.90, 48.05 and 49.76 mg/l, respectively. Among all organic extracts, ethanolic extract was more effective than other extract. The LC_{50} of 8h exposure of column purified fraction of *A. racemosus* was 50.37, 45.92 and 46.30 mg/l against sporocyst, redia and cercaria, respectively.

***Key words*:** Fascioliasis, *Fasciola gigantica, Asparagus racemosus,* Sporocyst, Redia, Cercaria, Anthelmintic activity

Fascioliasis is a zoonotic disease of domestic ruminants, which caused by *Fasciola hepatica* and *Fasciola gigantica* among cattle and humans which surpasses all the zoonotic infection worldwide [1-2]. The impact of fascioliasis is very high due to its pathogenicity [3]. In past three decades human fascioliasis has gained notice as a disease of primary importance. Human affection by these trematodes with progressive description of many human endemic areas and an increase of human infection reports [4]. Human fascioliasis classified as a food-borne trematode infection, which commonly acquired by eating metacercaria encysted on leaves that are eaten as vegetables [4]. In India, infection of fascioliasis in human was observed in state of Assam, Bihar, Maharashtra, Uttar Pradesh, Arunanchal Pradesh and West Bengal [5-9]. In eastern part of Uttar Pradesh (India), infection of fascioliasis is very common in cattle population [10-14]. Some species of snails are also causing greater economic loss in agricultural sectors [15]. *Lymnaea acuminata* is a Fresh water host snail which is a vector of *Fasciola* species [16-17]. The development of digenetic trematode *F. gigantica* is a very complex, because it undergoes in five larval stages where initial infection of the host snail occurs by the free-swimming miracidium larva, its transform in a parasite primarily sporocyst stage, followed by asexual reproduction and produce sporocyst, redia and finally the eventual formation and release of cercaria larva which is next free-swimming stage in their life cycle. Therefore, one of the strategically approaches to control fascioliasis is to break their life cycle at larval stages of *Fasciola* in *in-vivo* treatment of host snail by the use of phytochemicals. The synthetic anthelmintic larvicides are causes adverse impact on non-target organism and environment.

The phytochemicals of plant products have sufficient anthelmintic larvicidal activity in *in vivo* [18] against trematodes. *Asparagus racemosus* is a one of the medicinal plants which is commonly distributed in tropical and subtropical part of India. It has effective phytochemicals which can used in treatment of various diseases [19]. The phytochemicals of *A. racemosus* shows hypertensive response, antioxytoxic, anticancer, anti-abortifacient, antidysentric, antifungal, antibacterial, antioxidant, antiulcer, spasmodic, hypoglycemic and anticoagulant activities [20]. The aim of the present study is to evaluate in *in vivo* phytotherapy of infected host snails through *F. gigantica* by the use of dried root powder of *A. racemosus* and their different organic extracts, column purified fractions against sporocyst, redia and cercaria larva.

MATERIALS AND METHODS

Collection of vector snails

Adult snail *L. acuminata* (2.6±0.21 cm in length) were collected locally from low lying areas and pond of Muhammadabad Gohna, Mau, (U.P.) India. The snails were acclimatize for 24 hours in laboratory condition. The pH of

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the aquarium water was 7.1-7.3 and dissolved oxygen, free carbon dioxide and bicarbonate alkalinity were 6.3-72 mg/l, 5.4- 6.3 mg/l and 104.0-103.0 mg/l, respectively.

Plant material

The roots of *Asparagus racemosus* were purchased from local market of Muhammadabad Gohna 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. The roots washed with fresh water, dried under shade after complete drying, the plant materials were cut into small pieces and then grind in an electric grinder machine and the crude powder thus obtained, were then sieved with help of fine mesh cloth. Finally, the root powder was stored in a sterilized container in laboratory condition.

Preparation of plant extracts

The dried root powder of *A. racemosus* were extracted with 10 gram in 200 ml of 98% ether, 99.7% chloroform, 98% methanol, 98% acetone and 95% ethanol, separately 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 root powder of *A. racemosus* yielded 350 mg ether, 250 mg chloroform, 325 mg methanol, 405 mg acetone and 200 mg ethanol extracts. The residues, thus obtained, were used for the determination of *in vivo* larvicidal activity.

Column extraction

One liter of ethanol extract fraction of dried root powder of *A. racemosus* 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 and remaining solids obtained and each fraction were used for determination of larvicidal activity.

Selection of infected snails

Infected snails *L. acuminata* is often followed by the allocation of more resources to growth with the result the infected snails [21-23] which can grow in large size than uninfected, 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 [24-26].

In vivo toxicity essay

In *in vivo* toxicity of dried root powder of *A. racemosus* and their organic extract (ether, chloroform, methanol, acetone, ethanol) and column purified fraction of ethanol at different concentration where determine toxic effects against larva of *F. gigantica* in infected snail *L. acuminata*. After 2h, 4h, 6h and 8h treatment of infected snails where dissected, then live and dead sporocyst, redia and cercaria larva were counted under binocular microscope. Death of larvae was stabilized by immediate arrest of locomotion and movement. It was continuously monitor up to 48h in all treatments to ensure death. Present mortality of larvae at each concentration for 2h, 4h, 6h and 8h was used for the determination of LC_{50} values.

The values of 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 [27].

RESULTS AND DISCUSSION

The larvicidal properties of dried root powder of *Asparagus racemosus* and their different organic extract (ether, chloroform, methanol, acetone and ethanol) and column purified *in vivo* larvicidal properties against larva of *F. gigantica* (sporocyst, redia and cercaria) was time and concentration dependent (Table 1-3). In *in vivo* exposure after 2h and 8h of dried root powder of *A. racemosus* were more effective against cercaria larva of *F. gigantica* and LC_{50} was 71.39 and 63.22 mg/l, respectively (Table-3). The 8h LC_{50} of ethanol extract against sporocyst, redia and cercaria was 51.90, 48.05 and 49.76 mg/l, respectively (Table 1-3). Maximum effect of ethanolic extract was observed against redia. The column purified fractions of dried root powder of *A. racemosus* against sporocyst, redia and cercaria in 2h LC_{50} was 57.44, 53.76 and 56.00 mg/l and 8h LC_{50} was 50.37, 45.92 and 46.30 mg/l, respectively (Table 1-3). Maximum effects of column purified of *A. racemosus* were observed against redia larva (8h LC_{50} 45.92 mg/l) of *F. gigantica*. Significant ($p<0.05$) negative regression was observed in between exposure period and LC_{50} of dried root powder, different organic extracts and column purified of the *A. racemosus* against *F. gigantica* larva.

Table 1 *In vivo* larvicidal activity of dried root powder of *A. racemosus* and their different organic extract, column purified fractions against sporocyst larva of *Fasciola gigantica*

Exposure periods	Values	Larvicidal (mg/l)						
		Asparagus racemosus dried root powder	Ether extract	Chloroform extract	Methanol extract	Acetone extract	Ethanol extract	Column purified
2h	LC_{50}	79.93	76.84	75.04	77.34	71.62	60.34	57.44
	LCL	66.02	62.22	63.81	66.43	60.39	59.49	46.81
	UCL	93.74	89.57	85.23	86.99	81.22	72.47	69.50
	Slop-value	0.22±0.37	0.56±0.19	0.42±0.21	0.50±0.61	0.18±0.32	0.37±0.43	0.93±0.67
	t-ratio	3.50	2.47	2.22	2.91	2.56	3.05	2.29
	g-value	0.38	0.26	0.44	0.37	0.51	0.30	0.45
	Heterogeneity	0.17	0.11	0.21	0.10	0.27	0.18	0.14
4h	LC_{50}	77.31	73.42	72.99	75.01	68.93	57.50	56.12
	LCL	63.87	60.94	61.79	63.21	58.11	50.67	43.44
	UCL	91.04	86.29	82.26	84.05	78.47	65.73	66.71
	Slope-value	0.74±0.08	0.60±0.39	0.38±0.41	0.56±0.29	0.48±0.72	0.29±0.67	0.34±0.11
	t-ratio	2.87	2.56	2.94	3.57	3.18	2.89	2.17
	g-value	0.29	0.58	0.44	0.39	0.48	0.27	0.35

	Heterogeneity	0.19	0.23	0.47	0.13	0.28	0.18	0.31
6h	LC_{50}	75.24	69.50	68.42	71.91	65.47	54.18	53.84
	LCL	61.22	58.47	59.01	60.77	55.97	46.88	41.03
	UCL	88.91	82.02	81.39	80.76	76.44	63.99	62.81
	Slope-value	0.28±0.53	0.44±0.63	0.91±0.31	0.60±0.27	0.49±0.79	0.54±0.32	0.78±0.18
	t-ratio	3.48	3.09	3.26	3.42	2.68	2.40	3.10
	g-value	0.40	0.61	0.23	0.35	0.49	0.53	0.34
	Heterogeneity	0.22	0.39	0.18	0.14	0.29	0.16	0.23
8h	LC_{50}	69.05	67.83	66.22	68.79	63.02	51.90	50.37
	LCL	59.47	54.18	53.71	57.42	52.37	40.07	37.69
	UCL	85.07	80.05	80.92	79.18	74.83	60.32	59.91
	Slope-value	0.55±0.07	0.46±0.24	0.30±0.81	0.66±0.47	0.18±0.31	0.92±0.84	0.26±0.49
	t-ratio	2.76	2.99	2.61	2.18	2.49	3.17	2.85
	g-value	0.48	0.29	0.40	0.33	0.38	0.52	0.47
	Heterogeneity	0.18	0.12	0.27	0.21	0.19	0.23	0.14

Six batches of 10 infected snails were kept in different concentration of the above preparations. In *in vivo* mortality of sporocyst was recorded every 2h up to 8h. Concentration given are the final concentration (W/V) in the aquarium water.
LCL- lower confidence limits, UCL-upper confidence limits

Table 2 *In vivo* larvicidal activity of dried root powder of *A. racemosus* and their different organic extract, column purified fractions against redia larva of *Fasciola gigantica*

Exposure periods	Values	Larvicidal (mg/l)						
		Asparagus racemosus dried root powder	Ether extract	Chloroform extract	Methanol extract	Acetone extract	Ethanol extract	Column purified
2h	LC_{50}	75.84	74.18	71.69	74.37	69.42	56.22	53.76
	LCL	61.39	69.28	60.20	63.50	57.49	48.47	41.39
	UCL	89.47	81.72	83.43	85.74	80.22	69.00	66.83
	Slop-value	0.43±0.31	0.58±0.23	0.24±0.38	0.78±0.29	0.94±0.45	0.89±0.51	0.76±0.32
	t-ratio	2.29	3.14	2.91	2.74	2.34	2.56	2.76
	g-value	0.25	0.40	0.42	0.33	0.50	0.74	0.40
	Heterogeneity	0.48	0.40	0.19	0.22	0.34	0.45	0.29
4h	LC_{50}	73.05	71.92	68.72	72.04	66.27	53.38	51.04
	LCL	62.20	59.84	57.66	60.57	56.40	44.00	40.93
	UCL	86.71	82.22	80.04	82.91	75.74	62.87	66.90
	Slope-value	0.38±0.71	0.66±0.34	0.41±0.13	0.86±0.79	0.45±0.72	0.33±0.18	0.45±0.32
	t-ratio	2.44	2.20	3.05	2.78	3.14	2.44	2.92
	g-value	0.35	0.53	0.74	0.70	0.64	0.37	0.95
	Heterogeneity	0.40	0.28	0.34	0.56	0.24	0.47	0.30
6h	LC_{50}	70.91	68.37	67.01	69.12	63.92	50.25	47.18
	LCL	59.72	57.34	58.42	57.36	52.04	41.77	36.98
	UCL	81.14	77.05	75.22	80.59	74.20	59.48	57.66
	Slope-value	0.76±0.21	0.84±0.53	0.18±0.29	0.42±0.07	0.67±0.34	0.52±0.44	0.32±0.57
	t-ratio	2.67	2.89	2.56	2.34	2.47	2.91	3.24
	g-value	0.58	0.44	0.68	0.38	0.77	0.60	0.51
	Heterogeneity	0.51	0.27	0.38	0.46	0.54	0.44	0.30
8h	LC_{50}	67.34	66.52	64.89	65.80	61.00	48.05	45.92
	LCL	56.02	54.37	59.83	53.67	50.01	40.59	38.02
	UCL	78.94	72.40	75.07	77.91	73.48	56.22	54.70
	Slope-value	0.99±0.71	0.44±0.62	0.63±0.48	0.59±0.21	0.16±0.37	0.22±0.40	0.37±0.07
	t-ratio	3.19	2.27	2.90	3.36	3.07	2.93	2.55
	g-value	0.49	0.27	0.22	0.48	0.78	0.33	0.40
	Heterogeneity	0.21	0.35	0.31	0.44	0.29	0.51	0.38

Six batches of 10 infected snails were kept in different concentration of the above preparations. In *in vivo* mortality of sporocyst was recorded every 2h up to 8h. Concentration given are the final concentration (W/V) in the aquarium water.
LCL- lower confidence limits, UCL-upper confidence limits

In *in vivo* larvicidal study of *Asparagus racemosus* clearly indicate that their dried root powder, different organic extract and column purified fractions have toxic efficacy against sporocyst, redia and cercaria larva of *F. gigantica*. The primary chemical constituents of *A. racemosus* are [28] essential oils, arginine, asparagines, tyrosine, flavonoids, resin and tannin. The phytochemicals investigation of root powder of *A. racemosus* shows the presence of tannins, saponins, sterols, flavonoids and alkaloids [29]. The root extracts of *A. racemosus* have saponins which possess abundant biological properties which include anti-oxidant, anti-diabetic, anti-ulcerogenic, anti-amnesic, anti-tussive and hepatoprotective

effects [30]. [31] has been reported that the primary chemical constituents of *A. racemosus* are essential oils, asparagines, tyrosine, arginine, flavonoids (quercetin, kaempferol and rutin), tannin and resin. In *in vivo* methanolic extract of root powder of *A. racemosus* shows antibacterial efficiency against *Shigella dysenteriae*, *Vibrio cholerae*, *Psedomonas putida*, *Staphylococcus aurens*, *Escherichia coli*, *Shigella flexneri*, *Salmonella typhi*, *Salmonella typhimurium*, *Shigella sonnei* and *Bacillus subtilis* [32-33]. Extract of the *A. racemosus* have been investigated for different properties like anti-oxidant, antidiarrhaeal, anti-oxytoxic, anti-tumor, immune-modulator, anti-inflammatory, anti-tussive and galactogouge [34-36]. *A. racemosus* are uses in the treatment of rheumatism, diarrhea, diabetes, jaundice, brain complaints, urinary disorders, blood diseases, bronchitis and cough [37-38]. It also uses the treatment of the depression, inflammation, ulcer, cancer, hepato-toxicity, lithiasis, and diabetes [39]. The ethyl acetate extract of the roots of *A. racemosus* has anti-plasmodial activity against *Plasmodium falciparum* with an IC_{50} value of 29µg/ml [40].

Table 3 Table 3 *In vivo* larvicidal activity of dried root powder of *A. racemosus* and their different organic extract, column purified fractions against cercaria larva of *Fasciola gigantica*

Exposure periods	Values	Larvicidal (mg/l)						
		Asparagus racemosus dried root powder	Ether extract	Chloroform extract	Methanol extract	Acetone extract	Ethanol extract	Column purified
2h	LC_{50}	71.39	73.52	70.27	72.49	68.22	58.67	56.00
	LCL	60.53	62.10	59.91	61.87	56.42	47.22	45.55
	UCL	82.72	81.57	78.42	81.39	77.00	67.14	64.79
	Slop-value	0.74±0.29	0.28±0.37	0.30±0.42	0.77±0.56	0.64±0.37	0.22±0.56	0.34±0.18
	t-ratio	3.94	3.62	3.04	2.91	2.18	2.59	3.10
	g-value	0.27	0.91	0.68	0.22	0.45	0.50	0.25
	Heterogeneity	0.48	0.29	0.34	0.42	0.22	0.18	0.27
4h	LC_{50}	68.78	70.09	68.62	70.78	64.57	55.05	54.82
	LCL	59.14	62.04	58.22	59.10	53.39	44.90	42.34
	UCL	77.92	79.86	77.07	80.49	73.97	64.08	63.72
	Slope-value	0.50±0.39	0.25±0.63	0.48±0.19	0.35±0.27	0.59±0.71	0.48±0.54	0.83±0.61
	t-ratio	2.95	2.77	3.16	3.29	3.78	3.32	3.45
	g-value	0.76	0.44	0.39	0.64	0.59	0.34	0.68
	Heterogeneity	0.14	0.33	0.40	0.25	0.51	0.44	0.30
6h	LC_{50}	66.91	67.83	65.70	66.04	62.99	52.57	50.47
	LCL	58.22	59.77	54.03	56.38	51.91	41.18	44.80
	UCL	79.31	75.39	76.47	73.55	70.83	60.47	59.68
	Slope-value	0.72±0.21	0.18±0.40	0.99±0.73	0.55±0.61	0.49±0.37	0.24±0.30	0.44±0.19
	t-ratio	2.68	2.94	2.25	2.57	2.18	3.09	3.17
	g-value	0.35	0.16	0.79	0.18	0.22	0.40	0.36
	Heterogeneity	0.23	0.45	0.40	0.18	0.12	0.37	0.29
8h	LC_{50}	63.22	65.14	62.93	63.15	60.35	49.76	46.30
	LCL	55.39	54.92	51.17	51.09	49.95	38.82	36.22
	UCL	74.08	74.38	73.26	72.58	69.52	58.15	55.98
	Slope-value	0.38±0.43	0.72±0.51	0.94±0.79	0.18±0.49	0.38±0.20	0.50±0.29	0.84±0.63
	t-ratio	2.76	2.87	2.99	2.43	3.18	3.39	2.19
	g-value	0.55	0.46	0.32	0.18	0.81	0.59	0.24
	Heterogeneity	0.41	0.28	0.14	0.76	0.57	0.19	0.33

Six batches of 10 infected snails were kept in different concentration of the above preparations. In *in vivo* mortality of sporocyst was recorded every 2h up to 8h. Concentration given are the final concentration (W/V) in the aquarium water.
LCL- lower confidence limits, UCL-upper confidence limits

Anthelmintic *in vivo* larvicidal study of root powder of *Asparagus racemosus* and their different organic extracts, column purified revealed that active toxic phytochemical components which may be easily diffused in the body of *F. gigantica* larva (sporocyst, redia and cercaria) through body fluid of host snail and caused mortality. In *in vivo* toxicity of *Asparagus racemosus* phytochemicals may be either due to the uptake of the active moiety which progressively increase the amount of phytochemicals in the larval body with increase in exposure period or it might be possible that the phytochemicals of *Asparagus racemosus* could change into more toxic form in larval body due to the various enzymatic actions. The ethanolic extract of dried root powder of *Asparagus racemosus* have higher toxicity among all the organic extracts which indicates that the larvicidal phytochemicals are maximum soluble in the ethanol. Maximum numbers of active constituent of *Asparagus racemosus* were present in ethanolic extract [41].

It is evident from result section, the steep slope values indicate that a small increase in the concentration of various treatments, which caused mortality in the sporocyst, redia and cercaria (Table 1-3). A t-ratio value is greater than 1.96 which 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. The 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.

CONCLUSION

In *in vivo* larvicidal activity of dried root powder of *A. racemosus* and their organic extract, column purified fraction was studied against sporocyst, redia and cercaria larva of *F. gigantica* for the control of fascioliasis. All the treatment were analyzed against sporocyst, redia and cercaria larva of *F. gigantica* which was time and concentration dependent, whereas ethanolic extract of dried root powder of *A. racemosus* showed significant larvicidal properties which may be due to the presence of one or more active phytochemicals in this plant which might be responsible for larvicidal activity. *In vivo* study of *A. racemosus* against *F. gigantica* larva (sporocyst, redia and cercaria) concluded that the ethanolic extract showed significant anthelmintic larvicidal activity which can be used for the phytotherapy of infected snail which will be helpful for breaking the life cycle of *F. gigantica*. It also revealed towards the further more study, that how their phytochemicals act at molecular level in *Fasciola* larva.

Conflict of interest

The authors have no conflict of interest.

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