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Original Article

Photoactivated Chlorophyllin and Acetylcholinesterase/ Cytochrome Oxidase Activity in *Fasciola gigantica* cercaria Larvae

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ABSTRACT

Objective: The effect of chlorophyllin (CHL), CHL + freeze dried cow urine (FCU) and FCU on acetylcholinesterase (AChE) and cytochrome oxidase activity in the nervous tissue of the host snail *Lymnaea acuminata*, *and* parasitic cercaria larvae of *Fasciola gigantica* were studied. **Methods:** Whole cercaria larvae and snail nervous tissue was homogenized and supernatant was used as an enzyme source. Enzyme activity was measured in 10-mm path length cuvette using incubation mixtures and change in optical density was recorded. **Results:** Sublethal 4 h LC_{50} 40% and 80% of these formulations in sunlight and laboratory conditions caused no significant change in AChE activity in the nervous tissue of host snail. It was noted that sublethal *in vivo* 8 h exposure to 40% and 80% of 4 h LC_{50} of single and binary combination of CHL+FCU, CHL and FCU significantly altered the AChE and cytochrome oxidase activity in the cercaria larvae. Binary combinations of CHL+FCU in sunlight were more effective in inhibiting both the enzymes. **Conclusion:** The present study reported that enzymes AChE and cytochrome oxidase were inhibited by chlorophyllin formulations and thus causing the larval death inside the snail body without affecting the snail itself which is an important bioindicator of aquatic ecosytem.

Key words: *Lymnaea acuminata*, *Fasciola gigantica*, Cercaria larva, Acetylcholinesterase, Cytochrome oxidase, Nervous tissue.

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INTRODUCTION

Fasciolosis is one of the most devastating food borne zoonosis caused by parasitic trematode *Fasciola*. *Fasciola* infection is prevalent across the globe, affecting humans as well as animals.[1-5] The parasite is transmitted by ingestion of metacercaria of *Fasciola* species found on the leaves of aquatic plants in contaminated fresh water. *Fasciola gigantica* caused endemic fasciolosis in cattle population of northern part of India.[6,7] and their intermediate hosts are *Lymnaea acuminata* and *Indoplanorbis exustus*. Scientists working on fasciolosis control programmed have advocated that control of the host snail population is one of the possible approaches to control fasciolosis.[8,9] Environmental changes on the planet earth have compelled us to use sustainable and non-hazardous methods of parasite control. In aquatic ecosystem of ponds and lakes host snails and *Fasciola* larvae encounter with each other. Snails are the important component of fresh water ecosystem. The larval stages rediae and cercariae are the division phases of *F. gigantica* inside the snail body.[10-12] If these larvae will be destroyed by plant larvicides without killing the infected snails, the rate of *Fasciola* infection can be reduced. In the last three decades, we are using plant derived larvicides against host snails for effective control of fasciolosis.[7,13,14] In this connection, we have recentlyobservedthatchlorophyllin(CHL)achlorophyll product possesses potential larvicidal activity against *F. gigantica* larvae in different wavelengths of visible light.[7,14] The mechanism of action of chlorophyllin in cercarial death is still unknown. In the last four decades, we have conclusively noticed that AChE in the host snails is one of the most sensitive indicator altered by synthetic as well as plant derived molluscicides.[15-17] In another study, we have also noted that freeze dried cow urine (FCU) formulations in sunlight is potent molluscicides.[18-21] The aim of the present study is to evaluate the effects of sublethal exposure of CHL and FCU formulations on acetylcholinesterase (AChE) and cytochrome oxidase activity in host snails as well as in cercaria larvae of *F. gigantica*.

MATERIALS AND METHODS

Test materials

Preparation of chlorophyllin

Chlorophyllin was prepared by the method.[22] Chlorophyll was isolated from spinach using 100% ethanol (for about 2 h at 55°C). To avoid transformation of chlorophyll into pheophytin by the acidic content of the cell vacuoles, 1 mg $CaCO_3$ /g plant material was added as a buffer. The extract was

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subsequently filtered, and petroleum benzene was added. After shaking the mixture, chlorophyll moved into lipophilic benzene phase. The two phases were separated using a separatory funnel and about 1 mL methanolic KOH was added to 50 mL of benzene phase. On agitation, the chlorophyll met the methanolic KOH and transformed to water soluble chlorophyllin (cleavage of the ester bond between the chlorophyllin and the phytol tail by saponification). The phytol tail is responsible for the lipophilic property of chlorophyll. Chlorophyll is found as chlorophyllin in the KOH phase.

Collection of cow urine

Geer breed cow urine was collected in sterilized bottles, from green grass grazing 3-5 years old healthy cows. Different formulations of cow urine were tested against the larvae of *F. gigantica.*

Preparation of freeze dried cow urine (FCU)

Geer cow urine was kept in sun light (8h/day)/ laboratory conditions for 15 days. After 15 days cow urine was freeze dried in lyophilizer. Binary combinations (1:1) of chlorophyllin with FCU kept for 15 days in sunlight (8h/day) and laboratory conditions were prepared at the time of treatment.

Animals

Adult *L. acuminata*, each measuring 2.6 ± 0.4 cm in length were collected locally from Ramgarh Lake and low lying submerged areas of Gorakhpur district of Uttar Pradesh, India. Cercariae shedding infected snails were separated from uninfected snails by the method of Largue *et al.*[23] and Sunita *et al.*[24]

In vivo treatment

The snails could acclimatize for 24 h in laboratory conditions and then treated with sublethal concentrations 40% and 80% 4 h LC_{50} of binary combinations (1:1) of chlorophyllin formulations (CHL, CHL+FCU and FCU) (Table 1). Six batches were set up for each concentration. Control group (for each batch) contained only dechlorinated tap water without any treatment. All the treatments were made in the dechlorinated water in laboratory conditions. Thereafter, enzymes AChE and cytochrome oxidase activity were determined. Effect of toxicant on host (snails *L. acuminata*) and parasite (*F. gigantica* larvae) were observed simultaneously. The treated animals were removed from the aquaria after 4 h and rinsed with water. After 4 h of treatment snails were dissected for procurement of cercariae and nervous tissues of snails. About 15 to 20 snails were dissected, which yielded about 70-100 mg nervous tissues. In each concentration 100 to 150 infected snails were used. Nearly 4000 to 6000 cercaria larvae were procured in dissection of a single infected snail. In control group, infected snails were not treated with cercaricides. Both cercariae as well as nervous tissues of infected snails (positive control) were used in the same manner as treated group for AChE and cytochrome oxidase activity measurement. Protein levels were estimated according to the method of Lowry *et al.*[25]

AChE activity

AChE activity was estimated in treated as well as control group of animals by the method of Ellman *et al.*[26] as modified by Singh *et al.*[27] Cercaria larvae as well as brains of the infected snails were taken for the measurement of acetylcholinesterase (AChE) and cytochrome oxidase activity. The brains of snails were removed separately and placed on ice cubes. Afterwards, they were placed on filter paper to remove the adherent water and not to be weighty. Snail nervous tissue (50 mg) was homogenized in 1.0 mL of 0.1M phosphate buffer, pH 8.0, for 5 min in ice bath, and then centrifuged at 1,000×g for 30 min at 4°C. The supernatant was used as an enzyme source. Enzyme activity was measured in a 10-mm path length cuvette using an incubation mixture consisting of 0.1 mL of enzyme source, 2.9 mL of 0.1M phosphate buffer (pH 8.0), 0.1 mL of chromogenic agent DTNB (5,5-dithio-bis-2- nitrobenzoic acid) and 0.02 mL of freshly prepared ATChI (acetylthiocholine iodide) solution in distilled water. The change in optical density at 412 nm was recorded for 3 min after every 3-min interval at 25°C. Enzyme activity has been expressed µ mole 'SH' hydrolysed/ min/ mg protein.

Homogenization of F. gigantica cercaria larvae

Fresh Cercaria (1×10^6) larvae were centrifuged at 4 °C for 2 min at 800×g. Then 1.0 mL 0.1M phosphate buffer pH 8.0 containing a cocktail of protease inhibitors (10 mM EGTA, 40 µg/mL leupeptin, 20 µg/mL pepstatin, 1.0 mg/mL bacitracin, 20 units/mL aprotinin and 1 mM benzamidine) was added (10 mL), followed by sonication at 50% duty cycle/output control 4 in a bath Ultrasonics sonicator, (Plainview, NY, U.S.A). Sonication was performed for 3×3 min at 4 °C. Each homogenate was used as enzyme source for AChE activity measurement. Protein levels were estimated according to the method of Lowry *et al.*[25]

Cytochrome oxidase

Activity of cytochrome oxidase was measured according to Cooperstein and Lazarow.[28] As modified by Singh and Agarwal.[29] Homogenate obtained from fraction of cercaria larvae (1×10^6) of *F. gigantica* in 1.0 mL of 1/30 mol/L phosphate buffer, pH-7.4 centrifuged at 10,000×g for 30 min at 4 °C. Supernatants were used an enzyme source. Enzyme activity at 25°C was measured in a 10-mm path length cuvette. Reduced cytochrome solution (3-mL) (1.7×10^{-5} mol/L) was taken into cuvette and 0.2 mL of cercaria larvae homogenate was added. The reactants were mixed by inverting the cuvette several times and absorption at 550 nm was monitored for 3 min. A few grains of potassium ferricyanide were added (to oxidize cytochrome c completely) and the extinction was redetermined. Enzyme activity has been expressed as the average enzyme activity/min/mg protein (arbitrary unit).

Acetylcholinesterase

Fractionated homogenate obtained from fractionation of cercaria larvae (1×10^6) of 0.1M phosphate buffer pH 8.0 centrifuged at 1000×g for 30 min at 4°C. The supernatant was used as an enzyme source 0.1 mL (5×10^{-4} M) of enzyme source, 2.9 mL of 0.1M phosphate buffer (pH 8.0 mL) 0.1 mL of chromogenic agent DTNB (5, 5-dithio-bis-2-nitrobenzoic acid) and 0.02 mL of freshly prepared ATChI (acetylthiocholine iodide) solution in distilled water was added in 10 mm path length cuvette. The change in optical density at 412 nm was monitored for 3 min interval at 25°C. Enzyme activity has been expressed µ mole 'SH' hydrolysed/ min/ mg protein.

Statistical analysis

Concentration mortality data for each group of cercaria larvae of *F. gigantica* were analyzed using the probit analysis program, POLO-PC (LeOra Software) Robertson *et al.*[30] Rank correlation coefficient was applied to observe significant variation in between treated and control group AChE and cytochrome oxidase activity.[31]

RESULTS

In control groups, AChE activity in the nervous tissues of *L. acuminata* in sunlight and in laboratory conditions was 0.91-0.92 µ mole 'SH' hydrolyzed/min/mg protein (Table 2). No significant change in AChE activity in sunlight and laboratory conditions was noted in control as well as treated (40 % and 80 % of 4 h LC_{50} of CHL, CHL+FCU and FCU) snails group (Table 2).

In untreated cercaria larva AChE activity in sunlight and laboratory conditions was 1.01-1.3µ mole `SH` hydrolyzed/min/mg protein (Table 3).

Table 1: Concentrations (mg/L) of different larvicides used in *in vivo* toxicity determination against *Fasciola* larva in sunlight and laboratory conditions.

Larvicides (mg/L)	CHL+FCU			CHL			FCU		
	LC_{50} of 4h	80% of $4hLC_{50}$	40% of $4hLC_{50}$	LC_{50} of 4h	80% of $4hLC_{50}$	40% of $4hLC_{50}$	LC_{50} of 4h	80% of $4hLC_{50}$	40% of $4hLC_{50}$
Sunlight	26.07	20.85	10.42	28.75	23.00	11.5	882.25	705.8	352.9
Laboratory	1094.82	875.85	437.92	360.34	288.27	144.13	1276.8	1021.4	510.4

Table 2: AChE activity (µ mole SH hydrolyzed/min/mg protein) in the nervous tissue of *L. acuminata* exposed to 40% and 80% of 4 h LC_{50} of active larvicides CHL+FCU, CHL and FCU in sunlight and laboratory condition.

Larvicides	4 h LC_{50}	Sunlight	Laboratory
Control		0.91±0.001 (100)	0.92±0.001 (100)
CHL+FCU	80%	0.89±0.001 (97.80)	.87±0.001 (94.56)
	40%	0.87±0.001 (95.60)	.85±0.001 (92.39)
CHL	80%	0.85±0.001 (93.40)	0.86±0.001 (93.47)
	40%	0.89±0.001 (97.80)	0.87±0.001 (94.56)
FCU	80%	0.89±0.001 (97.80)	0.87±0.001 (94.50)
	40%	0.86±0.001 (94.50)	0.86±0.002 (93.47)

Values are mean ± SE of six replicates. Values in paranthesis indicate % enzyme activity with untreated control taken as 100%.

Table 3: AChE activity (µ mole SH hydrolyzed/min/mg protein) in the cercaria larva of *F. gigantica* exposed to 40% and 80% of 4h LC_{50} of active larvicides chlorophyllin formulations CHL, CHL+FCU and FCU in sunlight and laboratory condition.

Larvicides	4 h LC_{50}	Sunlight	Laboratory
Control		1.01±0.004 (100)	1.3±0.00 (100)
CHL+FCU	80%	0.04±0.003* (3.9)	0.12±0.001* (9.23)
	40%	0.09±0.001* (8.91)	0.17±0.001* (13.07)
CHL	80%	0.12±0.001* (11.88)	0.22±0.002* (16.92)
	40%	0.14±0.001* (13.86)	0.26±0.001* (20)
FCU	80%	0.18±0.001* (17.82)	0.42±0.003* (32.30)
	40%	0.23±0.001* (22.77)	0.41±0.001* (39.23)

Values are mean ± SE of six replicates. Values in paranthesis indicate % enzyme activity with untreated control taken as 100%.

(*) significant (P<0.05), when rank correlation coefficient in between sublethal LC_{50} and corresponding AChE activity in the whole cercaria larvae of *F. gigantica* in treated group.

Table 4: Cytochrome oxidase (enzyme activity/min/mg protein) in the cercaria larvae of *F. gigantica* exposed to 60% of 4 h LC_{50} of active active larvicides chlorophyllin formulations CHL, CHL+FCU and FCU in sunlight and laboratory condition.

Larvicides	4 h LC_{50}	Sunlight	Laboratory
Control		13.12±0.01 (100)	12.41±0.001 (100)
CHL+FCU	80%	0.69±0.002* (5.25)	0.85±0.001* (6.84)
	40%	0.81±0.001* (6.17)	0.97±0.001* (7.81)
CHL	80%	0.92±0.001* (7.01)	1.61±0.002* (12.97)
	40%	1.64±0.001* (12.5)	1.85±0.001* (14.90)
FCU	80%	1.89±0.001* (14.48)	2.56±0.001* (20.62)
	40%	2.14±0.001* (16.31)	3.12±0.001* (25.14)

Values are mean ± SE of six replicates. Values in paranthesis indicate % enzyme activity with untreated control taken as 100%.

(*) significant (P<0.05), when rank correlation was applied in between sublethal LC_{50} and cytochrome oxidase activity in the whole cercaria larvae of *F. gigantica* in treated group.

Treatment with 80 % and 40 % of 4 h, LC_{50} of CHL+FCU, CHL and FCU for 4 h caused significant (P<0.05) inhibition in the AChE activity in the cercaria larvae (Table 3). Maximum inhibition upto 8.9 % and 3.9 % of control in AChE activity was observed in cercaria larvae found in the snails exposed to 40 % and 80 % of 4 h LC_{50} of CHL+FCU in sunlight (Table 3). Significant (P < 0.05) variation in AChE activity in cercaria larvae was noted in sunlight and laboratory conditions exposure of chlorophyllin. There was a significant (P < 0.05) rank correlation in between the treated concentration of larvicides and corresponding changes in the AChE activity in cercaria larvae (Table 3).

In untreated (control) cercariae, cytochrome oxidase activity was 13.12-12.41 / min / mg protein (Table 4). Significant (P < 0.05) decrease in cytochrome oxidase activity was noted in the whole cercaria larvae inside the host snails, exposed to 40 % and 80 % of 4 h LC_{50} of CHL+FCU, CHL and FCU. Maximum inhibition upto 6.17 % and 5.25 % of control in cytochrome oxidase activity was noted in the cercariae inside snails exposed to 40 % and 80 % of 4 h LC_{50} of CHL+FCU (Table 4). There was a significant rank correlation in between the cytochrome oxidase activity and corresponding concentrations of the CHL, CHL+FCU and FCU (Table - 4).

DISCUSSION

It is evident from the results section that in *in vivo* sub-lethal treatment of binary combinations of CHL+FCU, CHL and FCU caused no significant

change in AChE activity in the nervous tissue of snails *L. acuminata*. While significant inhibition of AChE activity was noticed in treated cercaria larvae in sunlight and laboratory conditions. It indicates that concentration toxic to cercaria larvae is safe against host snail *L. acuminata*. Presence of AChE is reported in several developmental stages of the parasite, i.e. in cercaria, schistosomula and adult worms.[32,33] Fried *et al.*[34] reported that AChE is an active enzyme in cercaria larvae of *Echinostoma revolutam* (Trematoda). AChE is widely used as a biomarker in various toxicological studies.[35,36] Seasonal variation in AChE activity in the nervous tissue of the clam *Tapes philippinarum*.[35] and snail *Cronia contracta* were noted in different Goa cost of India.[36] It is reported that acetylcholinesterase and cytochrome oxidases are very important enzymes in activation of cercaria.[37] The enzyme AChE plays an important role in animals exposed to various natural/unnatural stimulants and in nerve impulse transmission in both vertebrate and invertebrates.[38] Effect of sublethal concentrations of different larvicides formulations of CHL and FCU are potent inhibitors of acetylcholinesterase activity of cercaria larvae.

Cytochrome oxidase is an important enzyme of electron transport system, responsible for active movement of cercaria larvae.[37,39] A classical electron transport chain is reported in larval stages of trematode but is generally absent in adults.[39] Cercarian tails have more cytochrome oxidase activity to meet the energy demands for movement.[40] Earlier it has been reported that phyto cercaricides citral, ferulic acid, umbelliferone, azadirachtin and allicin caused significant inhibition of acetylcholinesterase (AChE) and cytochrome oxidase activity in the treated cercaria larvae of *F. gigantica*.[41] Kumar *et al.*[42] reported that chlorophyllin is a potent inhibitor of cytochrome P450 enzymes involved in bioactivation of several environmental carcinogens because of radical scavenging mechanism and suppress metabolic activation. There is a significant ($p<0.05$) decrease in the cytochrome oxidase activity in the treated cercaria. It indicates that inhibition of AChE and cytochrome oxidase may be the cause of cercarial death. Present study, clearly indicates that we can reduce the incidence of fasciolosis without killing the snails with different formulations of chlorophyllin in sunlight. Among all the formulations CHL+FCU in sunlight are more effective cercaricides as it caused highest inhibition of AChE and cytochrome oxidase in cercaria larvae.

CONCLUSION

It seems that inhibition of AChE and cytochrome oxidase by chlorophyllin formulations in cercaria larvae of *F. gigantica* inside the snail body is a major cause of larval death. Thus, the larvicidal activity of these natural compounds may be helpful in solving the problem of fasciolosis by killing the larvae inside the host. So, the present study is one of the promising methods to control fasciolosis without killing the snails.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest regarding publication of this manuscript.

ABBREVIATIONS

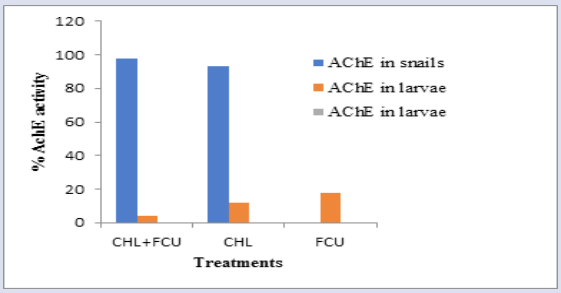
CHL: Chlorophyllin; **AChE:** Acetylcholinesterase; **FCU:** Freeze dried Cow Urine.

REFERENCES

1. Fox NJ, White PC, Mcclean CJ, Marion G, Evans A, Hutchings MR. Predicting impacts of climate change on *Fasciola hepatica* risk. PLoS One. 2011;6(1):e16126.
2. Charlier J, Hostens M, Jacobs J, Ranst BV, Duchateau L. Integrating fasciolosis control in the dry cow management: the effect of closantel treatment on milk production. PLoS One. 2012;7(8):e43216.
3. Ali NM. Development and evaluation of a dipstick assay in diagnosis of human fasciolosis. Parasitol Res. 2012;110(5):1649-54.
4. Usip LPE, Ibanga ES, Edoho HJ, Amadi EC, Utah E. Prevalence of Fascioliasis and the economic loss of condemned liver due to *Fasciola* infection in Cattle slaughtered at three abattoirs in Eket Urban, Akwa Ibom State of Nigeria. Glob Adv Res J Food Sci Technol. 2014;3(2):54-75.
5. Singh DJ, Singh VK, Singh DK. Photomediated Larvicidal Activity of Pheophorbide an against Cercaria Larvae of *Fasciola gigantica*. Scientifica. 2017;1-7.
6. Upadhyay A, Singh DK. Molluscicidal activity of *Sapindus mukorossi* and *Terminalia chebula* against the freshwater snail *Lymnaea acuminata*. Chemosphere. 2011; 83(4):468-74.
7. Singh DJ, Singh DK. Phytotherapy of chlorophyllin exposed *Lymnaea acuminata*: A new biotechnological tool for fasciolosis control. Parasite Epidemiol Control. 2016;1(2):20-5.
8. Shukla S, Singh VK, Singh DK. The effect of single, binary, tertiary combination of few plant derived molluscicides alone or in combination with synergist on different enzymes in the nervous tissues of the freshwater snail Lymnaea (Radix) acuminata (Lamark). Pesticide Biochem Physiol. 2006;85(3):167-73.
9. Kumar P, Singh VK, Singh DK. Bait formulations containing molluscicides and attractant amino acids against the harmful snail *Lymnaea acuminata*. Drug Invent Today. 2010;2(7):358-61.
10. Jaiswal P, Singh DK. Molluscicidal activity of Carica papaya and Areca catechu against the freshwater snail *Lymnaea acuminata*. Vet Parasitol. 2008;152(3):264-70.
11. Sunita K, Kumar P, Singh VK, Singh DK. *In vitro* phytotherapy of vector snails by binary combinations of larvicidal active components in effective control of fasciolosis. Rev Inst Med Trop Sao Paulo. 2013;55(5):303-8.
12. Singh DJ, Singh DK. Anthelmintic activity of chlorophyllin against different larval stages of *Fasciola gigantica*. Rev Inst Med Trop Sao Paulo. 2016;58:39.
13. Sunita K, Singh DK. Fasciolosis control: *in vivo* and *in vitro* phytotherapy of vector snail to kill *Fasciola* larva. J Parasitol Res. 2011;1-7.
14. Singh DJ, Singh DK. Toxicity of chlorophyllin in different wavelengths of visible light against *Fasciola gigantica* larvae. J Photochem Photobiol B: Biol. 2015; 144:57-60.
15. Rao IG, Singh A, Singh VK, Singh DK. Effect of single and binary combinations of plant-derived molluscicides on different enzymes activities in the nervous tissue of *Achatina fulica*. J Appl Toxicol. 2003;23(1):19-22.
16. Tripathi SM, Singh VK, Singh DK. Enzyme inhibition by the molluscicidal agent *Punica granatum* Linn. Bark and *Canna indica* Linn. Root. Phytother Res. 2004;18(7):501-6.
17. Kumar P, Singh VK, Singh DK. Kinetics of enzyme inhibition by active molluscicidal agents ferulic acid, umbelliferone, eugenol and limonene in the nervous tissue of snail *Lymnaea acuminata*. Phytother Res. 2009;23(2):172-7.
18. Tripathi R, Singh VK, Singh DK. Freeze-dried powder of cow urine reduces the viability of the snail *Lymnaea acuminata*. J Pest Sci. 2006;79(3):143-8.
19. Tripathi R, Kumar P, Singh VK, Singh DK. Cow urine and their formulations with plant molluscicides against reproduction of vector snail *Lymnaea acuminata*. Drug Invent Today. 2010;2(8):376-80.
20. Kumar S, Singh DK, Singh VK. Molluscicidal effect of freeze-dried cow urine of Indian breeds against *Lymnaea acuminata*. American-Euroasian J Toxicol Sci. 2011;3(2):70-4.
21. Kumar S, Singh DK, Singh VK. Binary combination of different Freeze-Dried Cow Urine (FCU) with some plant molluscicides against *Lymnaea acuminata*: Vector of Fasciolosis. Adv Life Sci. 2011;1(1):24-9.
22. Wohllebe S, Ulbrich C, Grimm D, Pietsch J, Erzinger G, Richter R, *et al.* Photodynamic treatment of *Chaoborus crystallinus* larvae with chlorophyllin induces necrosis and apoptosis. Photochem Photobiol. 2011;87(5):1113-22.
23. Largue C, Mcewan J, Poulin R, Keeney DB. Co-occurrences of parasite clones and altered host phenotype in a snail-trematode system. Int J Parasitol. 2007; 37(13):1459-67.
24. Sunita K, Kumar P, Singh VK, Singh DK. Larvicidal activity of Azadirachtin against *Fasciola* larvae. The Ecoscan. 2013;4:189-94.
25. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with folin phenol reagent. J Biol Chem. 1951;193(1):265-75.
26. Ellman GL, Courtney KD, Andres VT, Featherstone RM. A few and rapid colorimetric determination of acetylcholinesterase activity. Biochem Pharmacol. 1961;7(2):88-95.
27. Singh S, Srivastava MC, Tiwari JP. Anthelminthic activity of *Zanthoxylum alatum* (Tejbal). Indian Drugs. 1982;19:348-53.
28. Cooperstein SJ, Lazarow A. A microspectrophotometric method for the determination of cytochrome oxidase. J Biol Chem. 1951;189(2):665-70.
29. Singh DK, Agarwal RA. Action sites of cypermethrin, a synthetic pyrethroid in the snail *Lymnaea acuminata*. Acta Hydrochim Hydrobiol. 1991;19(4):425-30.
30. Robertson JL, Russell RM, Preisler HK, Savin NE. Bioassay with arthropods:

POLO computer programme for analysis of bioassay data. CRC press Taylor and Francis, Boca Raton, FL 2nd edition. 2007;1-224.
31. Sokal RR, Rohlf FJ. Introduction to biostatistics. *W.H. Freeman, San Francisco*, USA.
32. Bueding E. Acetylcholinesterase activity of *Schistosoma mansoni*. Br J Pharmacol Chemother. 1952;7(4):563-6.
33. Tarrab-Hazdai R, Levi-Schaffer F, Smolarsky M, Arnon R. Acetylcholinesterase of *Schistosoma mansoni*: antigenic cross-reactivity with *Electrophorus electricus* and its functional implications. European J Immunol. 1984;14(3):205-9.
34. Fried B, William BL, Harry SB. Histochemical Localization of Hydrolytic Enzymes in the Cercaria and Excysted Metacercaria of *Echinostoma revolutum* (Trematoda). Proceedings of the Helminthological Society of Washington. 1984;51(1):140-3.
35. Matozzo V, Tomei A, Marin MG. Acetylcholinesterase as a biomarker of exposure to neurotoxic compounds in the clam *Taps philippinarum* from the Lagoon of Venice. Marine Poll Bull. 2005;50(12):1686-93.
36. Gaitonde D, Sarkar A, Kaisary S, Desilua C, Dias CFM, Desousa SN, *et al.* Acetylcholinesterase activities in marine snail (*Cromia Contracte*) as a biomarker of neurotoxic contamination along the Goa coast, west coast of India. Ecotoxicol. 2006;15(4):353-8.
37. Schmidt GD, Roberts LS. Trematoda: From function, and classification of Digeneans foundation of Parasitology. MC Graw-Hill, New York, USA. 2000;6th edition.
38. Podolska M, Nadolna K. Acetylcholinesterase secreted by *Anisakis simplex* larvae (Nematoda: Anisakidae) parasitizing herring, *Clupea harengus*: an inverse relationship of enzyme activity in the host–parasite system. Parasitol Res. 2014;113(6):2231-8.
39. Tielens AGM, Fried B, Graczyk TK Eds. Biochemistry of trematodes in: Advances in trematode biology. CRC Press, Boca. Raton. 1997;309-43.
40. Skelly PJ, Stein LD, Shoemaker C. Expression of *Schitosoma mansoni* genes involved in anaerobic and oxidative glucose metabolism during the cercaria to adult transformation. Mol Biochem Parasitol. 1993;60(1):93-104.
41. Sunita K, Habib M, Kumar P, Singh VK, Husain SA, Singh DK. Inhibition of acetylcholinesterase and cytochrome oxidase activity in *Fasciola gigantica* cercaria by phytoconstituents. Acta Trop. 2016;154:19-24.
42. Kumar SS, Shankar B, Sainis KB. Effect of chlorophyllin against oxidative stress in splenic lymphocytes *in vitro* and *in vivo*. Biochim Biophys Acta. 2004;1672(2):100-11.

GRAPHICAL ABSTRACT



SUMMARY

- The present study demonstrates that photodynamic chlorophyllin alongwith FCU is a potent AChE and cytochrome oxidase enzyme inhibitor in cercaria larvae of *Fasciola gigantica*. It can be concluded that enzyme inhibitory effect of CHL and FCU is an effective biotechnological tool for fasciolosis control without killing the snail *Lymnaea acuminata.*

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