

New approach to control of fasciolosis: Neutralization of *Fasciola hepatica* miracidia with methanol extracts of three *Artemisia* species at preserved vitality of *Galba truncatula* snails

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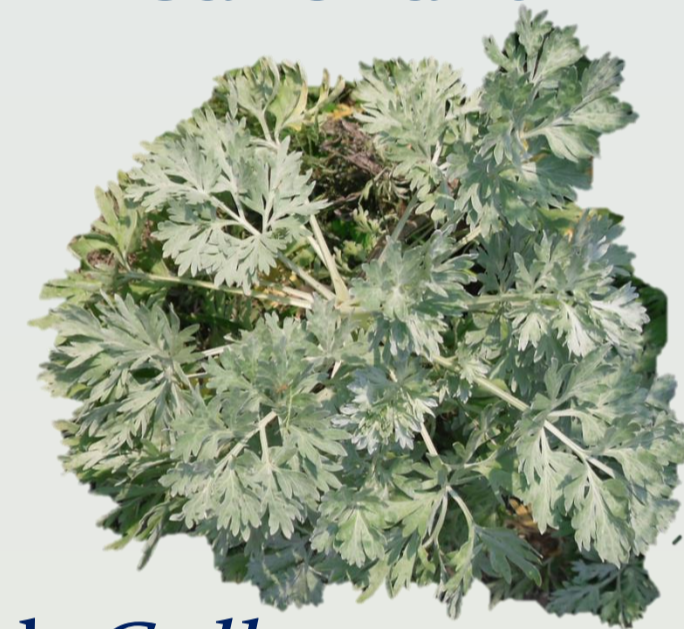
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AIM OF STUDY

- Assessment of the potential of medicinal plants to prevent *Fasciola hepatica* infection in *Galba truncatula* snails in the aquatic environment

Fasciola hepatica is a causative agent of fasciolosis, a widespread parasitosis with a significant negative effect on the livestock industry. Freshwater snails serve as an intermediate host in the complex life cycle of the parasite. Molluscs become infected after penetration of the ciliated swimming larva, the miracidium, through the soft body. In this study we tested the targeted miracidial treatment as an opportunity for the control of fasciolosis. The effectiveness of the methanolic extracts of *Artemisia absinthium* L., *Artemisia lerchiana* Stehm and *Artemisia santonicum* L. (Asteraceae) were examined for anti-miracidial and molluscicidal action.



MATERIAL AND METHODS

- Parasites and snails:** *Fasciola hepatica* miracidia and *Galba truncatula* snails were obtained from the laboratory maintained life cycles.
- Plant material and preparation of extract:** Plant material was collected from the natural localities of the studied species. Air-dried, ground plant parts was extracted with 80% methanol by classical maceration for 24 h two times. The extract was analyzed for chemical composition by GC/MS. The chemical composition of the studied extracts and fractions were analyzed by gas chromatography-mass spectrometry (GC-MS) and high-performance thin-layer chromatography (HPTLC). Phenolic acids (chlorogenic, 4(p)-hydroxybenzoic, vanillic and quinic), flavonoid aglycones (artemetin - quercetagenin 3,6,7,3',4'-pentamethyl ether), fatty acids (linoleic acid, C18:2 as dominant) were identified in the extract of *A. absinthium*. *A. lerchiana* and *A. santonicum* belong to *Artemisia* maritima group and have similar chemical profiles. However, a few differences were found in the extracts. Multi-component flavonoid profiles of both species were detected. Highly methylated flavonoid aglycones derivatives of quercetagenin, apigenin, and luteolin were identified. The high content of unsaturated fatty acids (C18:3, C18:1, C18:2) was found, too. Also chlorogenic, quinic, azelaic, protocatechuic, and caffeic acids as well as polyols, triterpenes were established in the extracts.
- Design of experiments:** Three groups of 100 miracidia and 10 snails each were constituted per treatment. Each snail was individually exposed to 10 freshly hatched miracidia in 3 ml water in the absence (control) or presence of *Artemisia* extracts at final concentrations of 50, 100 to 200, and 400 µg/ml, for 2 h (exposure period), at room temperature.

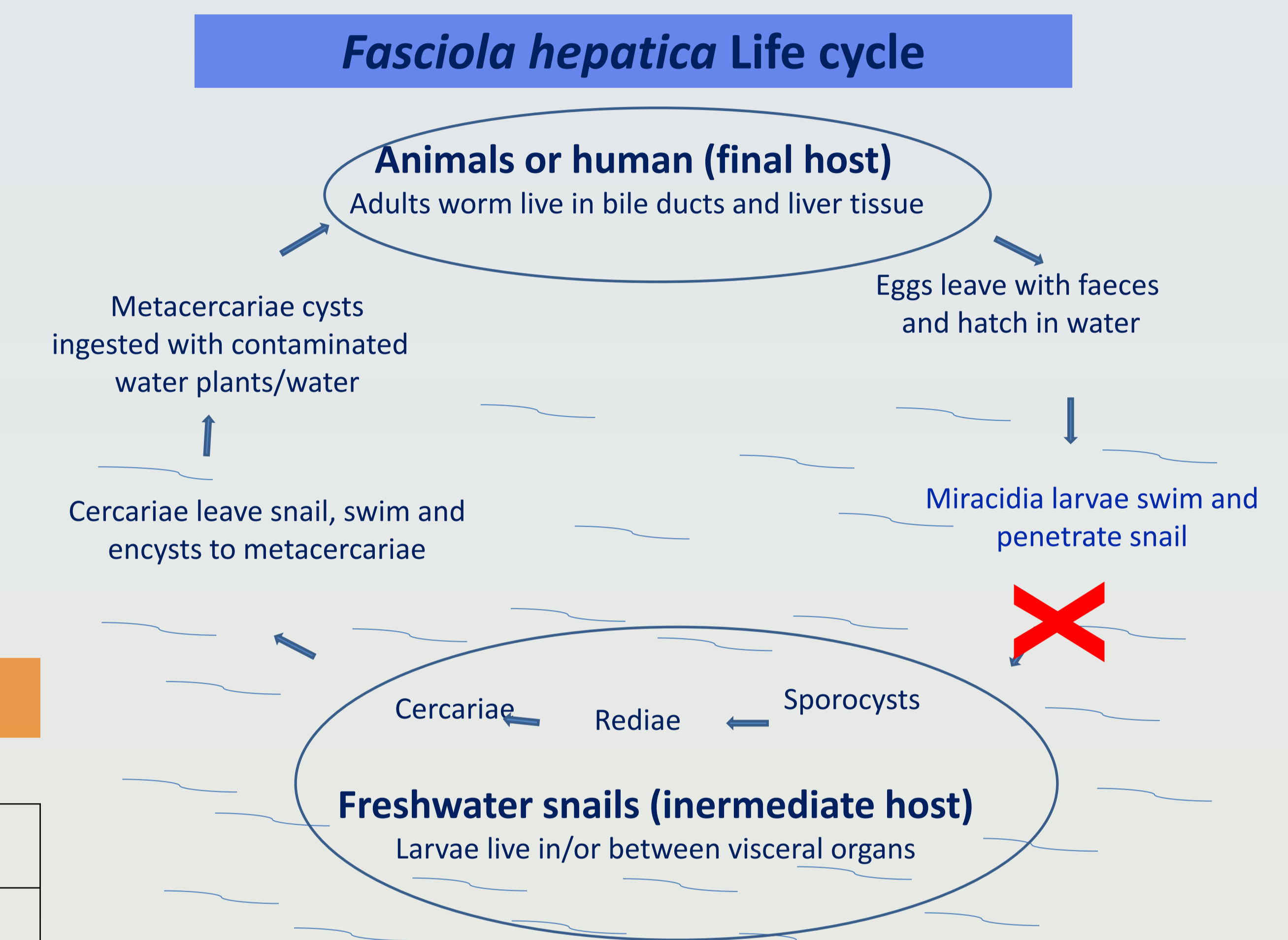
RESULTS

Concentration of <i>Artemisia absinthium</i> extract (µg/ml)	No of examined miracidia/snails	No of live miracidia/snails after treatment			
		15 min	30 min	60 min	120 min
0 (Control)	300/30	46/30	41/30	41/30	37/30
50.0	300/30	295*/30	293*/30	12*/30	0/30
100.0	300/30	297*/30	277*/30	0/30	0/30
200.0	300/30	201*/30	106*/30	0/30	0/30
400.0	200/20	0/20	0/20	0/20	0/20

* The larvae with reduced motility.

Concentration of <i>Artemisia lerchiana</i> extract (µg/ml)	No of examined miracidia/snails	No of live miracidia/snails after treatment			
		15 min	30 min	60 min	120 min
0 (Control)	300/30	46/30	41/30	41/30	37/30
50.0	300/30	19*/30	0/30	0/30	0/30
100.0	300/30	0/30	0/30	0/30	0/30

Concentration of <i>Artemisia santonicum</i> extract (µg/ml)	No of examined miracidia/snails	No of live miracidia/snails after treatment			
		15 min	30 min	60 min	120 min
0 (Control)	300/30	46/30	41/30	41/30	37/30
50.0	300/30	45*/30	0/30	0/30	0/30
100.0	300/30	0/30	0/30	0/30	0/30



CONCLUSION

- The obtained results clearly demonstrated the effectiveness of used concentrations of *Artemisia* extracts on the viability of invasive larvae of the parasite and its intermediate snail host.
- Preliminary data presented for the neutralization of *F. hepatica* miracidia with natural plant products at preserved vitality of *G. truncatula* snails have a potential value for future application and outlined a new approach to control of fasciolosis.

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