Original Article

A

Evaluation of Anti-Helmintic Activity of *Zingiber officinale* Roscoe Extract on *Fasciola hepatica* Miracidia *In vitro*

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Abstract

Aims: The control of fascioliasis has depended on the utilization of a predetermined number of anthelmintic drugs. Nonetheless, the resistance of antifasciolid is presently boundless, and there is a need for potential drug properties of medicinal plants as new medications against *Fasciola* spp. to interrupt the parasite transmission. **Materials and Methods:** This investigation is meant to assess the potential anti-fasciolicide impacts of *Zingiber officinale* roscoe hydroalcoholic extract against the *Fasciola* miracidia. The eggs of *Fasciola hepatica* were gathered from the livers contaminated goats and sheep and washed 20 times with chlorine-free water, and afterward incubated at different pH, temperature, and light density for embryonic development. Concentrated miracidia of *Fasciola* spp. were incubated at different concentrations (2, 5, and 10 µg/mL) of hydroalcoholic extract of *Z. officinale* at different time intervals. The data were analyzed using the ANOVA statistical test. **Results:** The extract at different concentrations showed antifasciolid effects in comparison to triclabendazole. This braking action was dose-proportional and further related firmly to the disclosure time ($P < 0.001$). In concentration of 10ug/ml extract killed the parasites at 105 ± 3 s ($P < 0.001$). **Conclusion:** *In vitro* antimiracidia activities of the hydro alcoholic extract of *Z. officinale* was satisfactory and potent fasciolicide effective, however, *in vivo* impact of this extract, remains for extra assessment. In this manner, these therapeutic plant extracts might be seen as confident origins of bioactive composites that could be matured against miracidia. This is the main work to evaluate the potential enemy of fasciolicide impact of certain plants against *Fasciola* miracidia.

Keywords: Anti-miracidia activity, *Fasciola hepatica*, hatch ability, *Zingiber officinale* roscoe

Introduction

The food-borne trematode infections represent a considerable gathering of neglected tropical diseases (NTDs) in over 10% of the world's population. Flukes of the biliary tract, as a more prevalent of food-borne trematodes, are pathogens are known well that occur in rural and urban tropical and subtropical areas have been a severe threat to human and animal health with major socioeconomic importance globally especially in developing countries.[1,2] Fascioliasis is a major zoonotic problem belonging to foodborne trematodes in ruminants as a result of two liver flukes named *Fasciola hepatica* and *F. gigentica* that affect the liver and other associated organs. Human fascioliasis has been happening at times from different geographical areas of the world.[3]

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Fascioliasis is an NTD that influences millions of human beings, even more than 70 countries. It is a harsh threat to the health of animals in many regions of the world, lead to major monetary damages to the livestock industry in many areas because of decreased production of over US$3 billion each year.[1]

Several factors such as resistance to anti-fascioliasis drugs, man-made environmental modifications, and climate alterations can influence the epidemiological pattern of the disease.[4] Control of fascioliasis is imperative for human health

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as well as an animal at the farm. At the movement, however, the effectiveness of disease control is breaking down in most infected areas. Alternative effective strategies for fasciola control are grazing management, biological control, and use of antifasciolid medications in the response of fascioliasis or its effects. Data recommends that chemotherapy procedures to control the infection are inefficient, also normally select for resistant pedigrees of the *Fasciola* spp. that globally recommended.[5] Because of high veterinary cost, limited available antiparasitic chemical compounds, drug resistance, and remain drug in milk and related toxicity, investigate on elective traditional properties herbs are justified.[2,6-8]

The medical treatment of fasciola infection is exceeding of manufacturing output of antiparasitic drugs, but occasionally with the use of medicinal plants.[9] At present, many disadvantages and limitations in using chemical drugs such as worldwide increasing resistance of fascioliasis to the drug of choice, that is triclabendazole (TCBZ) is very concerning because it threatened human health.[10,11]

Nowadays, a lot of researchers have made many efforts to find the solution for this problem and discover alternative anthelmintic derived from herbs, which has its benefit that is the stable characteristic of a natural active chemical compound the diversity which can preclude the anthelmintic resistance.[12] A review of the most effective medicinal plants for fascioliasis in traditional medicine has been considered in recent years. There are many herbs that have shown favorable anti-*Fasciola* spp. activities. The promising effects of *Lantana camara, Allium sativum, Bocconia frutescens, Lawsonia inermis, Cajanus cajan, Opuntia ficus, Artemisia mexicana*, and *Piper auritum,*[13,14] and mushroom such as *Ganoderma applanatum* and *Cantharellus cibarius* are recognized for their anti-fascioliasis effectiveness and inhibition miracicidal motility.[15] *Zingiber officinale Roscoe* is an important the tropical valued medicinal plant that is characterized by its adaptable medical activities like hepatoprotective activities, anti-inflammatory, and antibacterial.[16] Original research based on *in vivo* and *in vitro* assay revealed that *Z. officinale* and it,s competent to wield has an important nematocidal activity such as against, *Angiostrongylus cantonensis*, *Aniskis simplex*, and *Ascaridia galli*.[17,18] Similar works showed the Cestocidal activities of *Z. officinale* such as effects on *Hymenolepis* nana and *Echinococcus* protoscoleces either *in vivo* or *in vitro* assay.[19-21]

The anti-trematode activities of *Z. officinale* have been proven. It appeared that the *Z. officinale* advertised differently grade schistosomal effectiveness by decreasing eggs of *S. mansoni* burden and the size of hepatic granulomas in animals infected with this blood fluke.[22] In a similar study, *Z. officinale* has antischistosomal activities of the blood fluke *S. mansoni* harbored in mice and hand over a base for successive empirical evidence as well as clinical trials. The trematode concern and the egg frequency in the stool of animals treated with *Z. officinale* were significantly less than in control groups. Histopathological results demonstrated a decrease in granulomatous inflammation in number and diameter in the intestine as well as the liver of treated cases in comparison to control mice.[23] There is the theory that the mechanism of activity of *Z. officinale* could be peripheral and central, i.e., antihistaminic as well as an anticholinergic.[24] *Z. officinale* can therefore play an important role in preventing and treating various diseases, on the other hand, it can be used as an alternative to chemical drugs.

At present, there are no traditional medicinal plant extracts and natural products accessible for the treatment and control of fascioliasis. Accordingly, this research aimed to provide the fasciolicidal effects of the extract of *Zingiber officinale Roscoe* against *Fasciola* spp. miracidia *in vitro* in comparison to TCBZ.

Materials and Methods

F. hepatica miracidia development (embryogenesis in eggs).

Parasite collection

The adult fresh helminths were collected directly from the 70 infected gall bladders and livers of the sheep that were slaughtered in the abattoir of Arak City, Iran. The infected organs were transported to the research laboratory of parasitology for further assays. The removed worms were washed, with distilled water until they are separated from any attached particles for isolation and gathering the eggs.

Hatching of Fasciola eggs

For determining the effect of environmental parameters on the hatchability of *Fasciola* miracidia under laboratory conditions, the collected eggs were washed several times by PBS solution and centrifuged at 3000 rpm for 5 min. The supernatant was discarded and the precipitated eggs were washed three times. An amount of 100 µl of the residue containing approximately 1000 mature Fasciola eggs were considered for the next assay. The tubes containing *Fasciola* eggs were incubated in a dark cupboard in 10 mL of chlorine-free water at pH ranges (4–8), the temperature ranges (26°C–32°C), and intensity light ranges (840–1070) with three replicates for each examination.[15]

Ordinary pattern hatching of *Fasciola* miracidia observed with an inverted microscope (×40): There were blastomeres eggs, modulated eggs, embryo evolution, and finally developed miracidium. On the 1st day of the study, it was clear cellular growth. On the 12th day, the spots were amassed in a corner of the egg. The embryonic tissue differentiation was completed and the miracidia stage of *Fasciola* was observed inside the egg and on the 14th day, completely miracidium was formed.[25]

Preparation of plant extracts

The plant examined was selected based on using traditional herbal medicine.[26] The fresh *Z. officinale* plant was prepared and formal taxonomic identification of the plant material used in the current research was approved by the Research Center for Nutrition in Metabolic Diseases, Kashan University of Medical Sciences.

Assessment of activity extract

Antiparasitic effects of *Zingiber officinale* extract

The tests were performed with the various concentrations of *Z. officinale* extract after complete miracidial hatching. The eggs of *Fasciola* were incubated for 2 weeks in suitable conditions (pH, light, and temperature) to stimulate miracidial hatching.[27]

Then, 10 µl un-chlorinated water consist of 20 miracidia was dispensed in the wells and 50 µl of any extract at 2, 5, and 10 µg/ml concentrations were added to the wells. We used un-chlorinated water and TCBZ as negative and positive control groups. Each treatment was done in a triplicate assay for each positive and negative experimental group. The assessment of each extract against *F. hepatica* miracidia was analyzed under the light microscope at ×40 coupled to a digital camera in 5 min intervals (1, 5, 15, 20, and 25 min). At each evaluation time, the activity of antimiracidia of the plant extracts was measured by the comparison dead/immobile and alive miracidia with the subsequent formula.[25]

$$\text{Effectiveness rate} = \frac{\begin{array}{c}\text{No. of Miracidia in}\\ \text{the control group -}\\ \text{No. of Miracidia in}\\ \text{the tested group}\end{array}}{\begin{array}{c}\text{No. of Miracidia in}\\ \text{the control group}\end{array}} \times 100$$

In the present study, the time of survival (s) and speed of miracidium (mm/s) followed by hatching before and after treatment was calculated and considered as the efficiency of investigating extracts.

Calculating the speed of miracidium by specifically designed software

So far, the velocity of the *Fasciola hepatica* miracidium has not been precisely determined, for the first time, we designed and used specific software to evaluate the effectiveness of the medication. The software can track parasites and calculate speed, displacement, and distance.

Analysis of data

The obtained data of *Fasciola* miracidia were analyzed by one-way ANOVA and t-tests, followed by the Tukey posttests to determine the differences among the groups. In analyzing the data, SPSS statistical program (version 18, SPSS, IBM, Armonk, NY, USA).

Results

The anti-fasciolicide activity of *Z. officinale* hydroalcoholic extract was examined on time of survival and speed of *Fasciola* miracidia at different concentrations (2, 5, and 10 µg/ml) in comparison with positive (triclabendazole) and negative (chlorine-free water in combination with medication) control groups 1–10 min after treatment. The results showed the extract stopped the survival time as well as the speed of miracidia *Fasciola.* This braking action of the hydroethanolic extract was dose-proportional and further related firmly to the disclosure time ($P < 0.001$). No killed parasite was shown in the negative control groups. An important positive relation was detected between the concentration of extract and fatality, time of treatment, and fatality of the miracidia ($P < 0.001$). The inhibition portrait realized everywhere the trial for extract was related to that achieved with total the TCBZ in 2 ug/ml concentrations ($P < 0.001$). As the concentrations of the extract increased, the survival time and the speed of miracidia decreased. A concentration of 10ug/ml *Z. officinale* hydroalcoholic extract killed the miracidia at 105 ± 3 s time. However, in 2ug/ml concentration this time was 520 ± 4 s [Table 1]. By increasing the concentration of the extract, the speed of the parasite was significantly reduced. Thus, at a concentration of 10ug/ml the speed of miracidia 0.08 ± 0.01 (mm/s) was determined [Table 2].

Discussion

The utilization of medicinal plants is getting well known because of the toxicity and side effects of modern medicines. Medicinal herbs assume a significant act in the expansion of capability therapeutic substances.[28] *Z. officinale* extract has been proved to have phytochemical composing

Table 1: Comparison hydroalcoholic extract of *Zingiber officinale* to triclabendazole against the survival of *Fasciola hepatica* miracidia

Concentration ug/ml	Extract	Time of survival (s)		
		Maximum	Minimum	Mean±SD
2	*Z. officinale*	524	516	520±4
	Triclabendazole (positive control)	62	59	60.1±7.51
5	*Z. officinale*	252	250	275±6.11
	Triclabendazole (positive control)	0	0	0
10	*Z. officinale*	108	102	105±3
	Triclabendazole (positive control)	0	0	0
Negative control	597			
Comparison groups (*P*)	<0.001			

SD: Standard deviation, *Z. officinale: Zingiber officinale*

Table 2: Comparison hydroalcoholic extract of *Zingiber officinale* to triclabendazole against the speed of *Fasciola hepatica* miracidia

Concentration ug/ml	Extract	Speed of miracidia (mm/s)		
		Maximum	Minimum	Mean±SD
2	*Z. officinale*	0.84	0.08	0.82±0.02
	Triclabendazole (positive control)	0.36	0.32	0.34±0.02
5	*Z. officinale*	0.79	0.76	0.77±0.02
	Triclabendazole (positive control)	0.07	0.05	0.06±0.01
10	*Z. officinale*	0.09	0.07	0.08±0.01
	Triclabendazole (positive control)	0.03	0.01	0.02±0.01
Negative control			1.01	
Comparison groups (*P*)			<0.001	

SD: Standard deviation, Z. officinale: Zingiber officinale

that advantageous to be anthelmintic. Phytochemical constituents that act as an anthelmintic in *Z. officinale* are flavonoids, saponins, tannins, terpenoids, and phenol.[29] The present results go beyond previous reports, showing the anthelmintic activity of *Z. officinale*. A similar conclusion was reached by Moazeni and Khademolhoseini (2016) that demonstrated *Z. officinale* extract decreased the miracidia creation of *F. hepatica* eggs. This study showed that *F. hepatica* eggs are sensitive to the alcoholic extract of *Z. officinale* at different concentrations.[30] The present work is the first study that investigates the impressive miracicidal activities of *Z. officinale* extracts, although the latter is more promising by monitoring biological parameters. The present work shows that the extract examined has a good effect against *Fasciola* embryonic development. This result highlights that little is known about the impact of anthelmintic treatment intervention using herbal medicine. The mechanism of this inhibitory activity is not well known and maybe the outcome of embryonic lyses utilizing the extracts' capability to penetrate the egg of the Fasciola shells.[31-33] The entering extracts may prevent the explanation of proteins filaments and microtubules in the cytoplasm as well as cell growth during embryogenesis.[34] However, more studies necessary to be done in explaining the mechanism function of these extracts. The obtained results can be known that *Z. officinale* extract has anthelmintic activity-dependent concentration and exposure time. The findings are directly in line with previous findings. Pereira *et al*. evaluated the possible anti-fasciolicide activity of *Momordica charantia* extract on the eggs of *F. hepatica* liver flukes in different concentrations and times.[25] The miracicidal activities extracts of *Cantharellus cibarius* and *Ganoderma applanatum* have been reported. When in comparison to the potency of other natural origins, these extracts can be treated to have good miracicidal capabilities similar to that of albendazole, which is used antifasciolid. Consequently, these herbs can be respected as encouraging origins composites that could be evolved into a miracicide accordingly gift efficient, low cost, toxic-free, and ecologically sociable recourses to fabricate drugs for the management of fascioliasis in indigenous districts.[15]

Recently, some investigations developed a screening assay to evaluate the impacts of herbal extracts on the development and motility of *Haemonchus contortus* larval stages, a very common parasite and one of the most gastrointestinal pathogenic roundworms of small ruminant livestock. It is responsible for edema, anemia, and the death of infected goats and sheep. It is shown that the extracts originated from Picria fel-terrae Lour at concentrations of 3–5 mg/ml have an extensive inhibitory effect on the development and motility of *H. contortus* larvae, which could be associate with the attendance of dynamic elements in these extracts.[35] *In vitro* ovicidal effects of the methanolic extract of *Z. officinale* at different concentrations and various disclosure times on *F. hepatica* eggs were conducted satisfactorily.[30]

There is little evidence on the miracidia outcome of herbal plants opposite *F. hepatica* is accessible. In this study, the potency of the alcoholic extract on miracidia of *F. hepatica* was considered. The present study showed, the *Z. officinaleon* extract at a concentration of 10ug/mL, after 10 min, reduced the speed and survival time miracidia of *F. hepatica*. Our results revealed that liver fluke *F. hepatica* miracidia are impressionable to the hydroalcoholic extracts of *Z. officinaleon*. Furthermore, chemical researches are required to separate the biological compounds accountable for miracidia activities presented in the *Z. officinale*. Notwithstanding, *in vivo* miracidia potency of these plant extracts desires additionally review moreover. According to the current study, the study extracts offer a chance for novel agents. The promising tramatodicidal effect of the extract of *Z. officinale* has not nevertheless been explored, and the present element most likely has some pharmacological effect contra adult *F. hepatica*. More investigations are going to target other phases of the life cycle of this liver fluke trematode. However, *in vivo* anthelmintic performance of this extract needs more studies as well. According to the results of the present work, *Z. officinale* offers freedom for uncommon combinations. The possible trematodicidal effectiveness of the extract of *Z. officinale* has not yet been surveyed, and this composite most likely has a few pharmacological activities against other stages of *Fasciola spp*. Our results demonstrated that *Z. officinale* offers an opportunity for strange compounds

and casts a new light on the control of fascioliasis in endemic areas.

Conclusion

Traditional plant-based are encouraging therapeutic tools for the management of fasciola infection in humans and animals. The present work showed the promising anti-miracicidal effects of the extract of *Zingiber officinale Roscoe* on the embryonic development of *F. hepatica* eggs. The *Z. officinale* has a good miracicidal capability like TCBZ, which is presently used as a choice drug for the treatment of fasciolosis. Extensive knowledge on *Z. officinale* can be justified by new pharmacological studies highlighting the chemical nature of this plant, its effects on various parameters, and the mechanisms of the detected biological activities.

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Conflicts of interest

There are no conflicts of interest.

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