Research Article

Evaluation the Ovicidal Effect of *Zingiber officinale* Methanol Extract Against Eggs of *Fasciola* spp. *In Vitro*

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Abstract | The purpose of this study was to compare the effects of *Zingiber officinale* methanolic extracts on eggs of *Fasciola spp.* to both positive (Albendazole) and negative (chlorine-free water) control groups *in vitro*. Microscopic examination of the treated *Fasciola spp.* eggs with ginger methanolic extract revealed that the various concentrations of *Z. officinale* methanolic extract used in our study were highly sensitive to it and that their activities differed noticeably from those of the positive (albendazole) and negative (chlorine-free water) controls. The ovacidal influence of ginger extract at a dosage of 5mg/ml with 24, 48, and 72 hr. treatment times was 90.1, 95.8, and 99.2% respectively, compared to the positive and negative control groups, which had ovacidal effects of 83.1 and 16.88%, respectively. But, the ovicidal efficacy of ginger methanolic extract at a dosage of 10 mg/ml is 97.4, 100, and 100% respectively, with treatment times of 24, 48, and 72 hr. While, using ginger extract at doses of 20, 25, and 50 mg/mL with treatment times of 24, 48, and 72 hr. respectively, resulted in 100% ovicidal effectiveness.

Keywords | Ovicidal, *Zingiber officinale* extract, *Fasciola* spp. Eggs

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INTRODUCTION

One of the most significant parasitic zoonotic diseases in the world is fascioliosis, which is caused by the *F. hepatica* and *F. gigantica* obligatory endoparasitic trematodes of the Phylum Platyhelminthes-Class Trematoda. These two species are localized in the liver, bile ducts, and gall bladder of a variety of mammals, including cows, buffaloes, sheep, goats, and occasionally equine (Al-Sultan et al., 1999; Shrimali et al., 2016). Also, blood-feeding *Fasciola* spp. have a two-host life cycle (Fadl et al., 2011). *Lymnaea* spp., a species of freshwater snail, comprise the majority of the intermediate hosts where its asexual stage develops. Ruminants, who are the specific hosts who contracted the infection after consuming metacercaria, go through the sexual stage (Oleiwi et al., 2017; Mikaeel, 2020). Besides, Fasciolosis has a wide geographic spread as food-borne zoonotic disease. *F. gigantica* is responsible for the large liver fluke in tropical areas, which is notably prevalent throughout Asia and Africa, because *Lymnaea natalensis*, its intermediate host, is widely distributed. Whereas, *Lymnaea truncatula*, the intermediate host of *F. hepatica,* is only found in a few temperate locations, including Australia, the Americas and Europe (Kanyari et al., 2010; Hassone and Salah, 2019). In addition, Fasciolosis have posing a serious health threat and causing enormous economic losses in livestock industries through death of infected animals, decrease in production, growth retardation, condemnation of affected livers, costs of treatment, and expense for control measures in ruminants (Al-Kubaisee et al., 1999; Abdulwahed and Al-Amery, 2019).

On the other hand, due of growing contraindications in the use of synthetic medications, researchers have recently

focused a lot of emphasis on exploring for biologically active plants that can be employed as novel anti-parasitic medicines. Due to their anthelmintic effectiveness and lack of side effects, the use of unprocessed medicinal plants ensures positive health consequences for both humans and animals. The identification of novel compounds with a distinctive structure, high activity, and selectivity can also result from natural product screening; these molecules can subsequently be further improved utilizing synthetic techniques (Al-Bayaty et al., 2006). Moreover, these extracts frequently disrupt the major objectives of parasites, including membrane integrity, microtubules, DNA (intercalation, alkylation) and brain signal transmission (El-Sayed et al., 2012). When examined for anti-parasitic action, a variety of medicinal plant extracts were discovered to be superior to the medicines already in use (Muhammed, 2015). Holistic remedies made from plant extracts are probably going to replace conventional methods of parasite control in veterinary care (Bauri et al., 2015). There are a variety of plants that can be used as antiparasitic, antibacterial and antifungal agents, such as *Z. officinale* (El-Sayed and El-Saka, 2015).

MATERIALS AND METHODS

Preparation of *Fasciola Spp.* eggs

The abattoirs of Samawah in Al-Muthanna province, Iraq, were the source of buffalo gallbladders that were naturally infected with *Fasciola* spp. The collection of samples was done between March 2022 and December 2022. The abattoirs were visited three times per week, and between 2 and 5 buffaloes were slaughtered daily. The team tested the gallbladders to see how plant extract affected *Fasciola* mircidium's capacity to hatch in a controlled environment. After being aseptically placed into glass cylinders, the bile liquid was allowed to set for 30 minutes. The eggs fell to the cylinders' bottoms. The eggs were collected, numerous times rinsed in PBS solution, and then centrifuged for five minutes at 3000 rpm. The supernatant was discarded, and PBS was used to wash the precipitated eggs multiple times. The eggs were collected, mixed with normal saline in a dark glass container, and stored at 4°C until they were required once again (Moazeni and Khademolhoseini, 2016).

Preparation of ginger (*Zingiber officinale*) extract

The local herbal market in Muthanna province, south of Iraq, was where we bought the fresh ginger rhizomes. The rhizomes were cut into slices, then dried for a week in the shade, after that mechanically ground into a powder using an industrial electric blender 400 ml of pure methanol and 100 g of dry ginger powder were combined gradually for an hour with a magnetic stirrer to create the methanolic extract. For 24 h the resulting solution was kept at room temperature. The mixture was mixed once more, filtered using Whatman cellulose filter paper, and the solvent was finally evaporated in a rotary evaporator. The leftover semisolid substance was disposed of and stored at 4°C in a clean glass container for future use (Moazeni and Nazer, 2010).

McMaster's egg counting technique

Using a modified McMaster Counting Chamber, the amount of eggs found in the bile fluid collected from the Fasciolosis-infected buffaloes was determined (Urquhart et al., 1996; Taylor et al., 2016).

Experimental effecting of *Z. officinale* (Ginger) extract on *Fasciola* spp. eggs *in vitro*

Fasciola eggs were incubated with extracts of ginger at various times at doses of 5, 10, 20, 25, and 50 mg/ml. In each experiment, a test tube containing 450 ml of ginger extract with different concentrations was introduced to 50 µl of egg-rich sediment containing roughly 500 unembryonated eggs. At 37°C, the tubes were incubated for 24, 48, and 72 hr. After that, a pipette was used to extract the ginger solution's supernatant while avoiding the eggs that had hardened up. Each tube's eggs were placed into specialized little plastic containers containing 3 mL dechlorinated tap water after being thoroughly cleaned. The containers were incubated at 28 °C in the dark for 14 days before being exposed to light for two hours to encourage miracidium hatching. In a control group, 500 eggs from one container were incubated at 28 °C without being exposed to ginger extract. Moreover, albendazole (ABZ; 5 mg/ml) was created by dissolving 25 mg of albendazole in 5 ml of distilled water with 5% DMSO. This was utilized as a positive group. Due of its better effectiveness against *Fasciola* spp. eggs compared to other anti-*Fasciola* medications, albendazole was employed as the positive control (Alvarez et al., 2009). The eggs were split into three groups: Those with live miracidia, those with growing cells, and the dead eggs. This allowed researchers to assess the ovicidal action of the ginger extract on each category. Using the proportion of eggs that did not successfully grow and hatch, ovicidal activity was calculated (Vargas-Magaa et al., 2014). It is expressed in the following way:

$$Effectiveness\ rate\ (\%) = \frac{Number\ of\ eggs\ not\ hatches}{Total\ number\ of\ eggs} \times 100$$

RESULTS AND DISCUSSION

The anti-fasciolicide activity of *Z. officinal*e methanolic extract was seen *in vitro* at various concentrations (5, 10, 20, 25 and 50 mg/mL) for varied times in comparison to positive (Albendazole) and negative (chlorine-free water) control groups (24, 48 and 72 h). The eggs were incubated

at 28 °C for 14 days to investigate the effect of the ginger extracts on the miracidial development. Tables 1, 2, and 3 shows the findings of microscopic examinations of the eggs exposed to various doses and durations of ginger extract, respectively. The results revealed that *Fasciola* spp. eggs are susceptible to *Z. officinale* methanolic extract at the various concentrations used in our experiment, and that their activities were dramatically different from the negative group (chlorine-free water) and positive (albendazole) controls. According to the Table 1, the ovicidal influence of ginger extract at a dosage of 5 mg/ml with treatments lasting 24, 48, and 72 hr is 90.1, 95.8, and 99.2%, respectively, in comparison to the positive and negative control groups, which are 83.1 and 16.88%, respectively. But, the ovicidal efficacy of ginger extract at concentrations of 10 mg/ml with treatments times 24, 48, and 72 hr is 97.4, 100, and 100%, respectively, as seen in the Table 2. In addition, ginger extract was used at doses of 20, 25, and 50 mg/mL with treatments lasting 24, 48, and 72 hours, respectively, to achieve 100% ovicidal efficacy as displayed in the Table 3. On the other hand, in our experimental study, the eggs were split into three groups: those containing live miracidia, containing growing cells, and dead eggs, as shown in Figure 1. This allowed us to assess the ovicidal activity of the ginger extract.

Table 1: Results the effects of methanolic extracts of *Z. officinale* against eggs of *Fasciola* spp. after 24 h *in vitro*.

Concentration of drug and ginger extract (mg/ml)	No. of examine eggs	After 14 days					
		Eggs containing live miracidia	%	Developing eggs	%	Dead eggs	%
5	418±11.52	26±1.00 a	6.23	15±1.00 a	3.59	377±2.88 a	90.1
10	426±1.00	11±1.00 b	2.58	0	0	415±2.00 a	97.4
20	438±1.00	0	0	0	0	438±1.00	100
25	411±1.00	0	0	0	0	411±1.00	100
50	426±1.00	0	0	0	0	426±1.00	100
5 Albendazole positive control	431±1.00	0	0	70.33±2.51c	16.8	360±2.08a	83.1
Distal water negative control	453±3.60	328±1.00 c	72.1	51±1.00 b	10.7	75±2.00c	16.8

Table 2: Results the effects of methanolic extracts of *Z. officinale* against eggs of *Fasciola* spp. after 48 hr *in vitro*.

Concentration of drug and ginger extract (mg/ml)	No. of examine eggs	After 14 days					
		Eggs containing live miracidia	%	Developing eggs	%	Dead eggs	%
5	412±1.00	11±1.00 a	2.9	5±1.00 a	1.21	396±1.00 a	95.8
10	422±1.00	0	0	0	0	422±1.00	100
20	441±1.00	0	0	0	0	431±8.386	100
25	426±1.00	0	0	0	0	426±1.00	100
50	414±1.00	0	0	0	0	414±1.00	100
5 Albendazole positive control	431±1.00	0	0	70.33±2.51c	16.8	360.33±2.06b	83.1
Distal water negative control	453±3.60	328 b	71.9	50±1.00 b	10.7	75±1.00c	16.8

Table 3: Results the effects of methanolic extracts of *Z. officinale* against eggs of *Fasciola* spp. after 72 hr *in vitro*.

Concentration of drug and ginger extract (mg/ml)	No. of examine eggs	After 14 day					
		Eggs containing live miracidia	%	Developing eggs	%	Dead eggs	%
5	421±1.00	3±1.00 a	0.71	0	0	418±0.00 a	99.2
10	435±1.00	0	0	0	0	435±1.00	100
20	416±1.00	0	0	0	0	416±1.0	100
25	423±1.0	0	0	0	0	419±7.234	100
50	446±1.00	0	0	0	0	446±1.00	100
5 Albendazole positive control	431±1.00	0	0	73±1.00 a	16.8	360.33±2.08b	83.1
Distal water negative control	453±3.60	328 b	71.9	51±1.00 b	10.7	75±2.00c	16.8

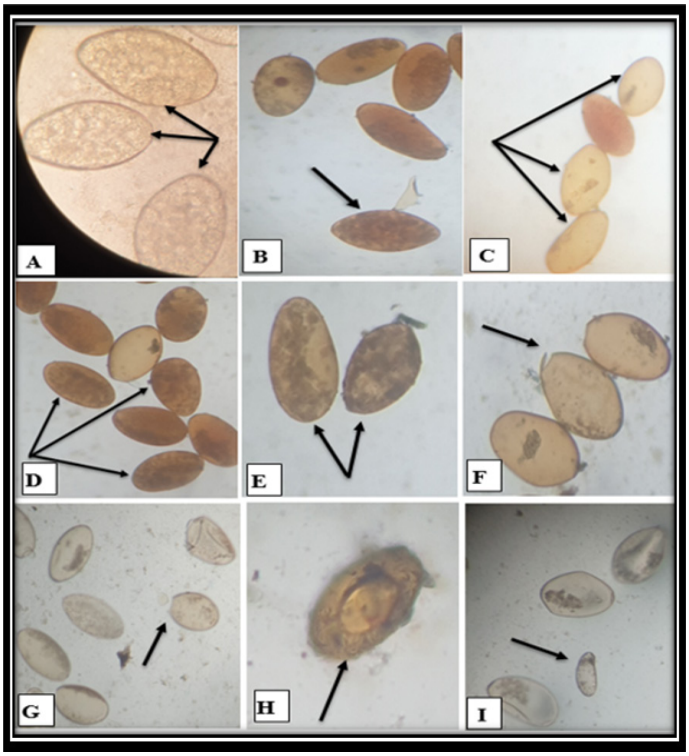

Figure 1: Different developmental stages of *Fasciola* spp. eggs at control and treated groups. (A, B) Developing eggs (Morula stage or cell division). (C) Empty egg. (D, E) Eggs with lysed embryo (dead eggs). (F, G) Hatched eggs. (H) Eggs containing live Miracidia. (I) empty egg beside its hatched mircidium (X10).

The use of therapeutic herbs is becoming increasingly common due to the toxicity and side effects of current pharmaceuticals. The discovery of powerful pharmaceutical chemicals is greatly aided by herbal remedies (Ashraf et al., 2020). *In vitro*, the results of analyze the ovicidal activity of *Z. officinale* methanolic extracts against eggs of *Fasciola* spp. at various concentrations and times were agreement with those study by Moazeni and Khademolhoseini (2016), Ghafar et al. (2021), who reached for a similar conclusion that *F. hepatica* eggs are susceptible to various concentrations of *Z. officinale* extracts, and that *Z. officinale* also reduced the production of miracidia in *F. hepatica* eggs. These investigations also displayed that the anthelmintic activity of *Z. officinale* extract depends on exposure time and concentration. These findings are explained by the chemical components of *Z. officinale* extract which act as anthelmintic are tannins, flavonoids, saponins, terpenoids and phenol (Abdullahi et al., 2017; Ghafar et al., 2021). Additionally, the precise mechanism underlying this activity is unknown, but it might be caused by embryonic lysis that takes advantage of the extracts ability to penetrate the *Fasciola* egg shells. Subsequently, the extracts that have entered the egg may have prevented the development of cells during embryogenesis and the expression of proteins filaments and microtubules in the cytoplasm (Arafa et al., 2015; Hegazi et al., 2018). Moreover, mention Gomes et al. (2016) Saponin, one of the phytochemical components of *Z. officinale* extract, acts on cell membrane instability and increases permeability, which results in embryonic lysis. These explanations could be supported by results other study by Moxon et al. (2010), those who performed proteome study on *F. hepatica* eggs and found that from the onset of embryogenesis through the development of the miracidium, a complicated protein expression profile is present. They discovered that a large number of proteins expressed early in embryogenesis play a direct role in cytoskeleton structure and cellular proliferation. Therefore, it is conceivable that phytochemical components in the *Z. officinale* impede the early phases of embryogenesis and thereby interfere with the protein expression profile. On the other hand, the results of the efficiency of Albendazole against *Fasciola* spp. eggs *in vitro* in the current study were consistent with those study by Arafa et al. (2015); Pereira et al. (2016), who showed that *Fasciola* spp. eggs are sensitive for Albendazole *in vitro* and the effectiveness of Albendazole against *Fasciola* spp. eggs depends on the drug concentration, McKellar and Scott (1990), who demonstrated that albendazole activity is restricted to flukes older than 12 weeks, disagree with these findings. The metabolic situation of treated animals, where pathological liver alterations can impair the bioavailability of anthelmintics, poor application, erroneous dose owing to inaccurate weighing, and other factors can all affect an anthelmintic's effectiveness (Babják et al., 2021). In addition, due to the eggs sensitivity to Albendazole, the drug concentration has an impact on the percentage of eggs that hatch. Albendazole have ability the inhibitory effect to prevent the growth and development of *Fasciola spp.* eggs suggests that it can penetrate through the egg shell (Arafa et al., 2015). There are two mechanisms that benzimidazoles (BZs) work against nematode eggs, and these include stopping embryonation and preventing hatching (Weston et al., 1984). The ovicidal action of ABZ is similar to that of BZs on nematodes is also correlated. In addition to having an affinity for L-tubulin, ABZ can also pierce the egg shell and accumulate inside. High solubility in lipids is related to the highest action, and this allows ABZ to easily penetrate the eggs' shell and hinder the growth and development of the embryo. It has been hypothesized that the hydrophobic nature of the drug determines how well it inhibits egg hatching (Robles-Pérez et al., 2014).

CONCLUSIONS AND RECOMMENDATIONS

Based on the data, results of the current study concluded that *Fasciola* spp. eggs are highly sensitive to *Z. officinale* methanolic extract at the different concentrations and times *in vitro* and that their activities were significantly

different from those of the positive (albendazole) and negative (chlorine-free water) controls.

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NOVELTY STATEMENT

The novelty of the study is focus on physiologically active for *Zingiber officinale* extract that can be employed as novel anti-parasitic pharmaceuticals due to the lack of an approved vaccination for any parasitic disease and the lack of readily accessible, secure, and efficient medicines for some diseases or parasites that are resistant to synthetic treatments, it is imperative to seek into alternate sources of anti-parasitic medications.

AUTHORS CONTRIBUTION

These authors each contributed equally.

Conflict of interest

The authors have declared no conflict of interest.

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