



Original article

## Zingiber officinale supplementation suppresses eimeriosis and regulates goblet cell response



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### ABSTRACT

Coccidiosis affects both domestic and wild animals and negatively impacting industries worldwide. Medicinal plants are widely used against parasites. Using infected mice with *Eimeria papillata*, we assessed the anticoccidial impact of *Zingiber officinale* extract (ZE). The animals in the first group were just given distilled water, while the animals in the second group were given ZE. The parasite's oocysts were infected into the third and fourth groups. The fourth group was given ZE for five days. The oocysts in mice faeces were reduced after treatment with ZE. The total parasitic stages were reduced after treatments by about 50%. Also, gamonts, meronts and oocysts inside the jejunum were decreased after treatment with ZE.

The infection caused hypoplasia of goblet cells of jejunum. ZE was able to ameliorate the goblet cells decrease. Behavioral response of animals to infection and treatment was investigated. All of these improvements could be attributed to the existence of active chemical classes of substances identified using infrared spectroscopy. Additional experiments are required to identify the phytochemical compounds in ZE and to understand their fighting mechanism against the parasite.

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### 1. Introduction

Coccidiosis is an infectious disease caused by protozoan parasites belonging to the *Eimeria* genus. Animal morbidity and mortality are related with coccidiosis outbreaks, resulting in massive economic losses (Abudabos et al., 2017). Coccidiosis causes animals to become ill and lose weight as a result of symptoms including diarrhea and appetite loss. Coccidiosis can be treated with a range of medications, but usage of these medications contributed in multidrug resistance and an overabundance of parasite infections in tissues. Several medicinal plant extracts were proven for

their anticoccidial activity like pomegranate, garlic, mulberry, neem and others (Wunderlich et al., 2014; Thagfan et al., 2021).

The rhizome of *Zingiber officinale* (Ginger) is used as a delicacy or spice, and it is also widely used in medicine. It belongs to family Zingiberaceae (Li et al., 2021). One advantage of employing natural extracts like ginger is that it reduces the possibility of developing resistance; also, the residues of such natural items in meat are safe for human consumption and have no negative health consequences.

Numerous pharmacological properties of ginger have been identified, including analgesic, anti-inflammatory, gastrointestinal regulating agent, antibacterial, and antioxidant properties (Khan et al., 2012). Also, *Z. officinale* was effective against some parasites like *Giardia duodenalis* (de Almeida et al., 2022), leishmania (Mohammadi et al., 2021), *Schistosoma mansoni* (Abd El Wahab et al., 2021), and *Trypanosoma cruzi* (Sarto et al., 2021).

Moreover, *Z. officinale* was found to lower the oocyst number in faeces of chickens infected with *E. tenella* (Aljedaie and Al-Malki 2020). The anticoccidial efficacy of *Z. officinale* extract and its influence on goblet cells in infected mice jejunum were demonstrated

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in this study. Furthermore, behavioural responses during infection and after treatment were investigated.

## 2. Materials and methods

### 2.1. Methanolic extract preparation

*Zingiber officinale* rhizomes were obtained from the local market in Egypt. A taxonomist at Helwan University's Department of Botany validated the plant's botanical identity. Rhizomes were homogenized. The 70% *Z. officinale* methanolic extract (ZE) was prepared using the process cited in [Dkhil, \(2013\)](#).

### 2.2. Infrared spectroscopy

Fourier-transform Infrared Spectroscopy (FT-IR) to estimate the expected classes of compounds (see details in [Al-Quraishy et al. 2020](#)).

### 2.3. Infection and sampling

Male C57BL/6 mice, aged 10–12 weeks, were used. *E. papillata* oocysts were collected and processed from the faeces of infected mice (see [Dkhil et al., 2011](#)). Each mouse orally received  $10^3$  *E. papillata* sporulated oocysts. The number of oocysts/ g faeces was calculated. The animals were placed into four groups, each containing eight mice. The first group was given distilled water and acted as the infection-free control group. The second group received daily oral gavage inoculations of 500 mg/kg ZE ([Thomson et al., 2002](#)), the third and fourth groups, on the other hand, were

infected. with  $10^3$  oocyst of *E. papillata* inoculated by oral route ([Dkhil 2015](#)). The last group was treated with ZE for 5 days.

On day 5, oocyst shedding was quantified using a McMaster chamber and expressed as the number of oocysts per gramme of wet faeces. Following that, all mice were euthanized, and portions of the jejunum were preserved in formalin to count parasite stages and goblet cells.

### 2.4. Parasite number

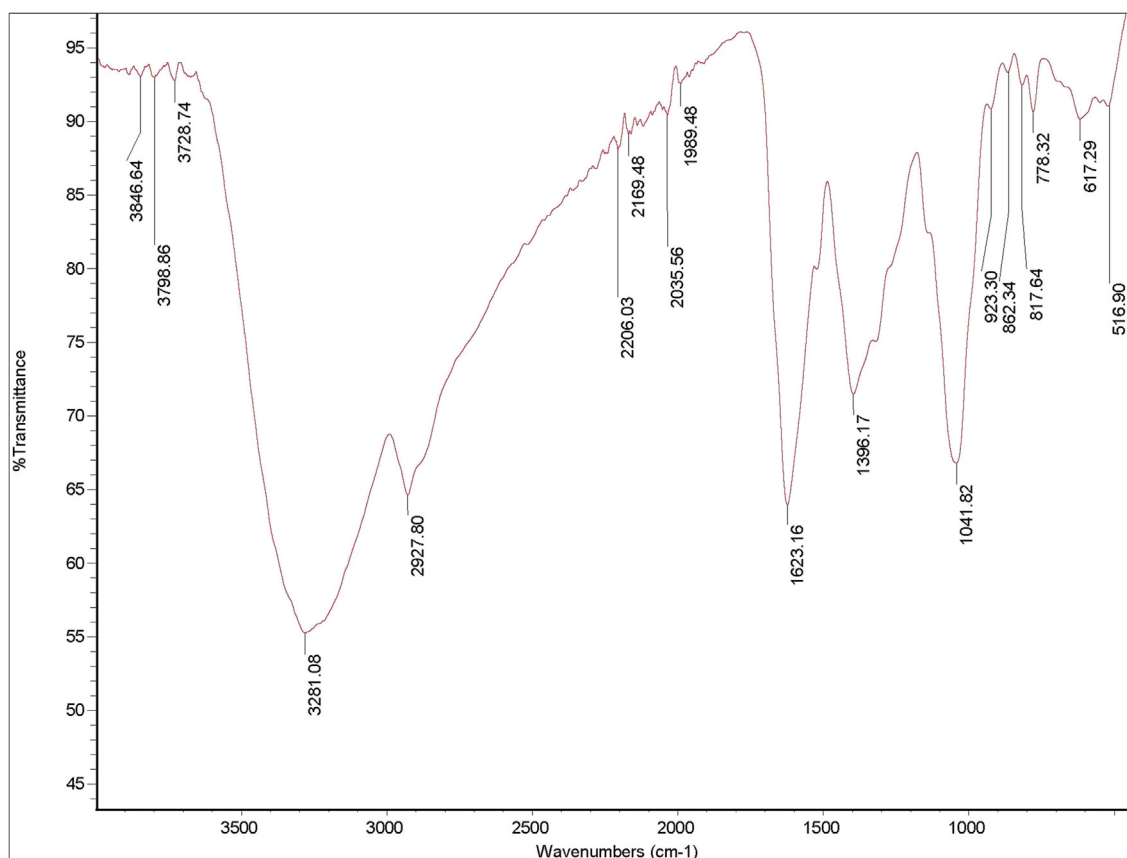
The Jejunum was embedded in paraffin wax and sectioned to  $4 \mu\text{m}$  thicknesses after being fixed in 10% buffered formalin. The hematoxylin and eosin was used to stain the sections. Each animal's oocysts were counted in ten villi using a light microscope.

### 2.5. Goblet cells

In Alcian blue stained sections, the number of goblet cells was counted and then the average number of goblet cells in each of the 20 villi was then calculated for each animal.

### 2.6. Animal behavior

Locomotor activity was determined as described by [Pontierii et al. \(2001\)](#) using the activity cage for mice. The measurement of mice grip strength was calculated using the Grip-Strength Meter as detailed in [Dkhil et al. \(2021\)](#). Rota-rod was also used to measure time to the rod, which indicated balance and activity (COMERIO).



**Fig. 1.** FT-IR spectroscopic analysis of *Z. officinale* extracts.

**Table 1**  
FT-IR spectrum of *Zingiber officinale* extract.

Absorption (cm <sup>-1</sup> )	Appearance	Transmittance (%)	Group	Compound Class
3846.64	medium, sharp	93	O–H stretching	alcohol
3798.86	medium, sharp	93	O–H stretching	alcohol
3728.74	medium, sharp	92.8	O–H stretching	alcohol
3281.08	medium	55	N–H stretching	aliphatic primary amine
2927.80	medium	64.5	C–H stretching	alkane
2206.03	weak	88.5	C≡C stretching	alkyne
2169.48	strong	89.5	S–C≡N stretching	thiocyanate
2035.56	strong	91	N=C=S stretching	isothiocyanate
1989.48	medium	93	C=C=C stretching	allene
1623.16	medium	64	C=C stretching	conjugated alkene
1396.17	medium	72	O–H bending	carboxylic acid
1041.82	strong	67	C–F stretching	fluoro compound
923.30	strong	91	C=C bending	alkene
862.34	strong	93.5	C–H bending	1,3-disubstituted
817.64	strong	93	C–Cl stretching	halo compound
778.32	medium	91	C=C bending	alkene
617.29	strong	90.5	C–Br stretching	halo compound
516.90	strong	91.2	C–I stretching	halo compound

**Table 2**  
*Zingiber officinale* effect on *E. papillata* developmental stages.

Group	Number of meronts	Number of gamonts (males and females)	Number of oocysts inside the epithelium
Infected	159.2 ± 40	25.5 ± 1	7.2 ± 3
Infected +ZE	80.2 ± 11*	16.2 ± 3.3*	1.6 ± 1*

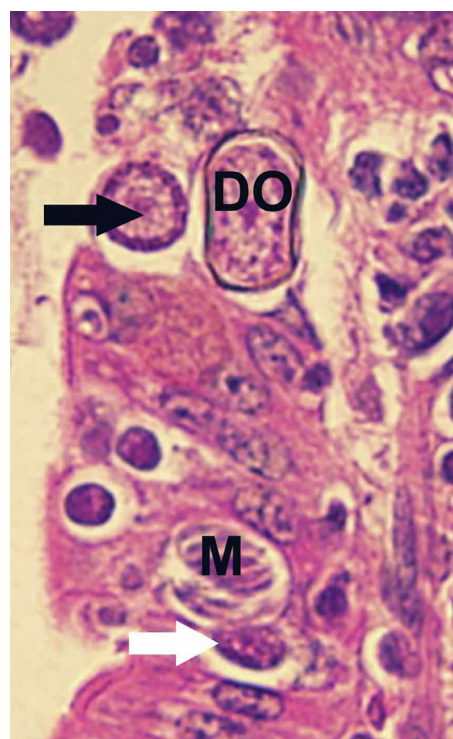
Data are mean ± SD in 10 villi. \*, Significance against infected group at p ≤ 0.05

2.7. Statistical analysis

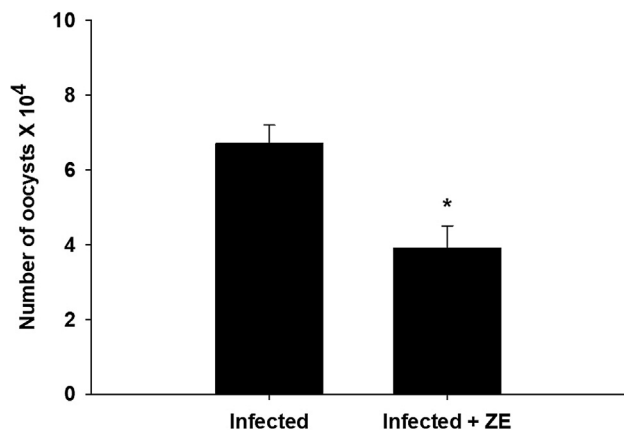
The Duncan method was used to make statistical comparisons between groups once the ANOVA analysis was completed in one way. At significance level of p ≤ 0.05.

3. Results

The FT-IR for ZE is shown in Fig. 1 and Table 1 was extracted from the IR Spectrum Table (sigmaaldrich.com). Different expected classes of compounds were present (alcohol, aliphatic primary amine, alkane, alkyne, thiocyanate, isothiocyanate, allene, conjugated alkene, carboxylic acid, fluoro compound, 1,3-disubstituted,



**Fig. 3.** Parasitic stages of *E. papillata* in jejunum. Male (white arrow) and female (black arrow) gamonts, meronts (M) and oocysts (DO).



**Fig. 2.** Oocysts count in faeces of infected and ZE treated mice. \*, The difference between the infected treated groups is significant at (p ≤ 0.05).

and halo compound) with absorbance from 516.90 to 3846.64 cm<sup>-1</sup>.

ZE decreased the number of oocysts released in faeces from 6.7 × 10<sup>4</sup> ± 0.5 to 3.9 × 10<sup>4</sup> ± 0.6 oocyst/g faeces (Fig. 2). Also, the number of gamonts, meronts and formed oocysts in the infected jejunum were reduced after treatment of mice by ZE to be 80 ± 11, 16.2 ± and 1.6 ± 1, respectively (Table 2, Fig. 3). The total number of parasite stages inside the infected villi was shown in Fig. 2 and Fig. 4. ZE was able to decrease this number to about 50% on day 5 post infection with oocysts of *E. papillata*.

In Alcian blue stained sections of the jejunum (Fig. 5), the number of goblet cells reduced after infection (4 ± 1 goblet cell/villus), but increased after treatment to 8 ± 1.3 goblet cell/villus (Fig. 6).



Fig. 4. Effect of ZE on total parasitic stages inside villi of *E. papillata* infected animals. \*, The significance vs infected group ( $p \leq 0.05$ ).

The behavioral activity of mice was assessed both during infection and after ZE treatment. We obtained no significant change on grip strength and time to the rod experiments. When compared to non-infected control animals, locomotor activity increased after infection and treatment with ZE (Table 3).

#### 4. Discussion

Coccidiosis is caused by *Eimeria* protozoan parasites and is one of the most dangerous illnesses endangering the commercial chicken industry. Anticoccidial agents from plant source are currently used as food additives for chicken and animals (Muthamilselvan et al., 2016). Here, *Z. officinale* extract was used for the treatment of the experimentally induced coccidiosis in mice. ZE possessed many active classes of compounds (Table 1).

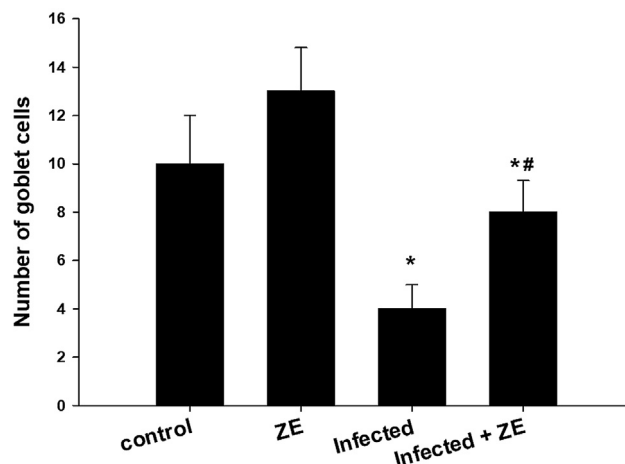


Fig. 6. The effect of ZE on the number of goblet cells in the jejunum of *E. papillata*-infected mice. Significance vs control (\*) and infected (#) at  $p \leq 0.01$ .

These compounds were able to reduce the number of expelled oocysts in the faeces as well as the parasitic stages in the intestine.

Ashraf et al. (2020) reported the anticoccidial efficacy of *Z. officinale* against *Eimeria* species of goats in central Kashmir. Also, Aljedaie and Al-Malki studied the efficacy of *Z. officinale* against *E. tenella* in chicken. According to Sharifi-Rad et al. (2017), ginger contains a variety of chemical components that have medicinal value.

One of the cellular immune responses in the intestine is the goblet cell response. It produces mucous. Mucus works as a first line of defense against infections, protecting the gut epithelial layer from pathogens (Uddin et al., 2021). Improvements in the induced hypoplasia of goblet cells due to infection with *E. papillata* have been investigated by the use of several medicinal plants like pomegranate, neem, garlic and grape (Wunderlich et al., 2014, Thagfan

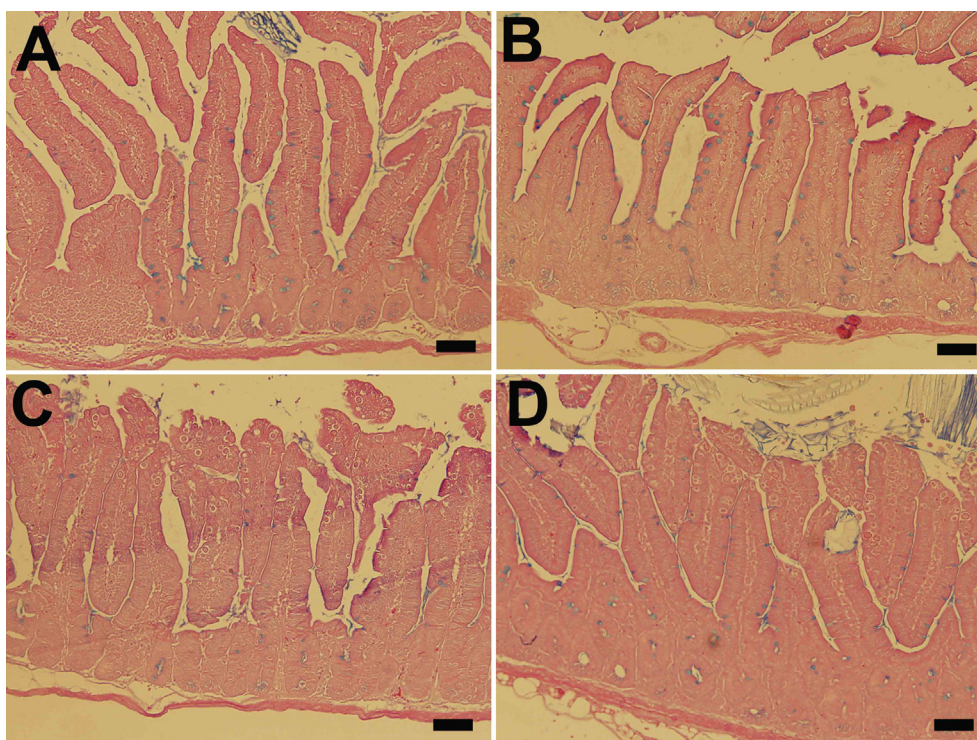


Fig. 5. Alcian blue stained sections showing goblet cells in control (A), ZE-treated group (B), infected (C) and infected-ZE treated (D) mice. Bar = 50  $\mu$ m.

**Table 3**  
Mice behavioral response during *E. papillata* infection and after treatment with *Zingiber officinale*.

Group	Activity		Grip strength records	Time on the rod
	Horizontal	Vertical		
Control	485 ± 49	27 ± 11	542 ± 112	57 ± 21
ZE	670 ± 95*	53 ± 9	530 ± 109	35 ± 11
Infected	701 ± 102*	45 ± 13	458 ± 93	26 ± 5
Infected + ZE	672 ± 114*	44 ± 10	462 ± 23	51 ± 25

\* , significance against control group.

et al., 2021). Active compounds in ZE may be the reason of the increased number after infection.

The behavior was studied because a signal could be sent to the neurological system to control behavior in some intestinal illnesses (Singh and Aballay 2019a, 2019b), and they discovered a neural route that triggers a behavioral host defenses against infection. Here, only the locomotor activity was changed after infection and treatment with ZE.

Because of its significant anticoccidial activities and capacity to modify the goblet cell response after infection, we concluded that ginger might be utilised as a food supplement in animals infected with *Eimeria*. Additional studies are required for the potential protective role in some organs as well as the biochemical and molecular mechanism of working where this study was focused on the parasitological effects.

#### Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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