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STUDY THE ETHANOLIC EXTRACT EFFECT OF *ZINGIBER OFFICINALE* ON *CYSTICERCUS TENUICOLLIS* SCOLICES WITH EXPERIMENTAL INFECTION OF DOGS

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ABSTRACT
The aim of this experiment was the estimation of the lethal activity of Ginger extract on scolices of *Cysticercus tenuicollis*. A different concentration of ginger extract have been used in vitro, which were (62.5, 250 and 500) mg/ml. After that, the treated scolices, with ginger extract, given to the puppies and observe scolices' growth to adult worms. The scolices collected from sheep omentum infected with *Cysticercus tenuicollis* cysts, and then treated with the different concentration of ginger extract for the period of one to 24 hours. Four groups of puppies used in this study, the first, second and third group infected with scolices treated with ginger extract (62.5mg/ml, 250mg/ml and 500mg/ml) respectively, while the last group kept as a control and infected with fresh untreated scolices. In spite of we used different concentrations to treat the scolices in vitro, we did not note any scolicidal effect of ginger extract on *C. tenuicollis* scolices. Nevertheless, the necropsied puppies showed that the scolices treated with 500mg/ml and 250mg/ml did not developed to adult worm, which means there is an effect of ginger extract on the growth and development of scolices to adult worm.

KEYWORDS: *Zingiber officinale*, ginger, *Taenia hydatigena*, *Cysticecus tenuicollis*, sheep .

INTRODUCTION

Cestodes of the Taeniidae family that infect the dogs as a definitive host are transmitted to a wide range of intermediate host species when they cause cysticercosis, echinococcosis or coenurosis[1]. *Taenia hydatigena* is a globally distributed parasite, which infects canids including dogs and wild carnivores as definitive host[2]. While the intermediate host of the mature metacestode is sheep, goats, cattle, dromedaries, antelope rarely pigs[3] and monkey[4]. Those infect with the larval stage of tapeworm (*Cysticercus tenuicollis*)[5]. The mature cysticerci frequently found attached to the omentum, mesentery, peritoneum and less commonly on the pericardium and pleura [6]. Commonly, *Taenia hydatigena* infection is not so pathogenic in dogs [3]. The metacestode infection due to *C. tenuicollis* is significant because it causes large economic losses due to the condemnation of infected meat or offal [7] .

Recently, plants and microorganisms used as a source of anti-solicidal compound[8]. *Zingiber officinale* (ginger) is a perennial herb, it is belong to the Zingiberaceaes' family. It adds flavor to the food[9]. Ginger have a high historical importance due to its health benefits. Many antioxidant constituents involved in ginger, *i.e.* phenolic derivatives (zingerone), volatile oil and oleoresin (gingerols and shogaols) [10]. The ethanolic extract of ginger was used as scolicidal agent for *Echinococcus* protoscoleces[11]. There is unavailable study reported for the effect of ginger on *C. tenuicollis*. Therefore the aim of the present study is to estimate the effect of different concentration of ethanolic extracts of ginger (*Zingiber officinale*) and test it as scolicidal agent for *Cysticercus tenuicollis* scolices.

MATERIALS & METHODS

Viability test

In the present study, a sufficient number of *C. tenuicollis* cysts collected. The collection of the cysts was randomly during inspection of the sheep carcasses in the abattoir, washed with normal saline, and transferred into the laboratory for examining them then checking their viability. Parasites classify as viable if a defined cystic structure with liquid content was still present, and as a degenerated if the cyst replaced by semi-solid content or an inflammatory scar like calcified nodule[12]. *Cysticercus tenuicollis* cysts initially identified according to their feature such as a long-necked single scolex, virtually translucent cyst fluid and rostellar hook morphology[13]. To test the viability of the scolices microscopically, we used 0.1% aqueous solution of eosin stain (1g of eosin powder in 1000 ml distilled water), the view under microscope was after five minutes [14] and after 15 minutes [15], and dead scolices appeared stained, while the viable ones remained colorless.

Preparation of alcohol ginger extract

Ginger powder (*Zingiber officinale*) collected from a local market in Baghdad city, Iraq, then extracted with 70% ethanol. A100g based on dry weight powdered of rhizomes (*Z . officinale*) added to adequate amount ethanol 70% (500ml). By using a magnetic stirrer, we shake the mixture for two hours [11]. The products squeezed through gauze to remove any particles remained. The suspension filtered through filter paper, and then the crude ethanol extracts were drying at 37 ^{0}C by incubator. The alcohol free residue of each extracts weight to give 5g of ginger. The semisolid extract stored at 4^{0}C until use [11].

Determination of ginger effect on scolices in vitro

The scolicidal tests carried out based on [15]. In the present study, we tested three concentrations (62.5, 250 and 500 mg/ml) of extracts for 1, 12 and 24h on scolices. Different weights took to prepare the required concentrations, therefore 0.625g, 2.5g and 5g dissolved in 10ml distilled water to get (62.5mg/ml, 250mg/ml and 500mg/ml) respectively. To follow regular procedure, the three prepared concentrations took in six test tubes (two test tube for each concentration), in each test tube two scolices placed, incubated all of them in 37 ^{0}C for one hour after we had shaken gently. The extract discarded and replaced with 1ml of eosin stain (0.1%) with gently shaking. Here we had two test tube of each concentration containing two scolices immersed in 1 ml of eosin stain, the eosin stain discarded after 5 min from 1 test tube and after 15 min from other test tube. In addition, we repeated the above procedure for different incubation period (12 hours and 24 hours). The scolices that used as a control kept in a normal saline only. To improve the experiment part of this study, the procedure repeated three times.

Collection and treating of *Cysticercus tenuicollis* for infection

The cysticerci of *C. tenuicollis* collected from the carcasses of sheep, they found attached to the visceral organ, separated and placed in a clean nylon bag and transferred to the laboratory for treatment with the ginger extracted. On the same day, some steps done, firstly the outer layer of tissues which surrounding the *C. tenuicollis* removed. Secondly, the scolex and part of the neck separated from the cystericus body, finally the scolices treated with the extract by placed them in the prepared extract as mentioned in 2.2 with rate of two scolices per tube of 2.5 ml containing extract and placed in the incubator for 24 hours. On the next day, each puppy received eight scolices placing in a piece of breed with cooked meat soup.

Experimental animals

The typical definitive hosts used as experimental animals were dogs, ten animals at the age of two weeks, local breed, kept in the experimental unit of the faculty of veterinary medicine at Baghdad University from January to April 2017. The hosts grown in conditions that excluded spontaneous infection and complied with the requirements of human treatment of experimental animals. After two weeks of shelter, all puppies examined for intestinal parasite by routine fecal examination to confirm they were non-infected[17]. They fed on milk and bread only to insure they did not get any infection with other worms for sex weeks before experimental infection[18]. The cysticerci (infective *Cysticercus tenuicollis*) retrieved in a hygienic assessment during a regular sheep slaughtering in a slaughterhouse. The experiment designed to divide the puppies and numbered them into four groups, each group consist of two dogs, three groups of them infected with treated *C. tenuicollis* scolices with ginger extract, and the last group infected with untreated scolices which considered as control group. The first, second and third group infected with metacestodes which treated with different concentration of ginger extract (62.5, 250 and 500 mg/ml) respectively. The last group of puppies infected with non-treated metacestodes, which were freshly isolated from slaughtered sheep and kept as controls. Each dog received eight metacestodes (table 2). The prepatent period determined by fecal daily examination for proglottids and egg excretion. Blood samples collected twice during the study for blood parameter .

Dogs necropsy and worms collecting

After observing the gravid proglottids in the feces, which was after forty days of infection. The first group have been killed was group no. 1 which infected with metacestode that treated with 62.5mg/ml of ginger extract. After one week, the second group was killed which infected with metacestode treated with 250mg/ml of ginger extract. On the next week, the third group of dogs killed, which administered scolices, treated with 500mg/ml of ginger extract and then the control group killed. After each killing step, the intestines opened and worms collected from them if present. The collected worms saved in 70% ethanol, measure their lengths, stain them and study the morphological features .

Statistical Analysis

The data succumbed to the Statistical Analysis System (SAS). The program was used to effect of difference factors in study parameters. Chi-Square: $\chi 2$ test was used to significant compare between percentages in this study[19].

RESULTS

Viability test

A total of 115 cysts of *C. tenuicollis* collected and examined for the viability of their scolices inside the cyst by relying on that mentioned in table (1).

TABLE 1: Distribution of sample according to Cyst viability test

Cyst viability	Number of examined cyst	Percentage (%)
Live	109	94.78
Dead	6	5.22
Total	115	100%
Chi-square	---	14.319 **

** (P<0.01).

The number of live scolices were (109 ≈ 94.78%), while the number of died scolices were (6 ≈ 5.22%). There is a significant differences between them (P<0.01). The live *Cysticercus tenuicollis* appeared to be surrounded by a semi-transparent, white yellowish membrane, the second layer was transparent, thin and contained clear watery fluid, and the evaginated scolex of *Cysticercus tenuicollis* swim in the fluid and these scolex did not stain with eosin stain (Fig. 1, 3). On the other hand, the dead cysticerci appeared calcified, contained few amount of fluid and their scolices stained with eosin stain (Fig. 2, 4).

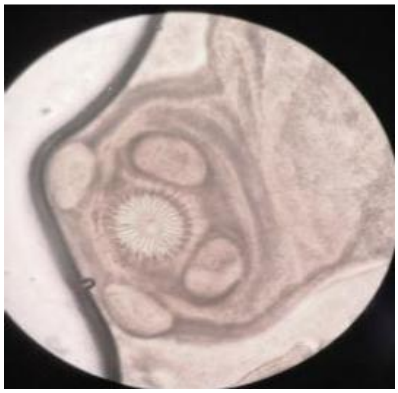

FIGURE 1: live unstained scolex after staining with 0.1% eosin (x10)

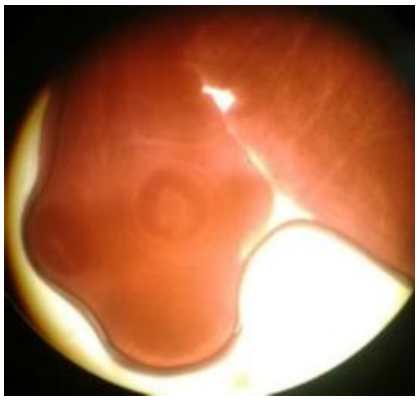

FIGURE 2: Dead stained scolex of Cysticercus tenuicollis after staining with 0.1% eosin (x10)

FIGURE 3: life cyst of Cysticercus tenuicollis semi-transparent thin wall with adequate of fluid with live swimming scolex

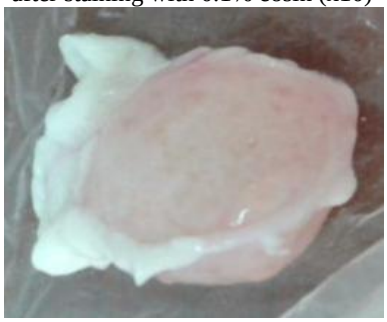

FIGURE 4: calcified cyst of *Cysticercus tenuicollis* has opaque, thick wall, few amount of fluid with dead scolex

Determination of ginger effect in vitro

There is no scolicidal effect of different concentration of Zingiber officinale, which use to inactivate the scolices in vitro. The scolices did not show any signs suggesting that they killed, including they did not stain with eosin stain. The mortality rates of scolices following exposure to ginger extract at different concentration (62.5, 250 and 500) mg/ml were zero after a period of (1 , 12 and 24) hours of applications, the treated scolices appeared live unstained with eosin stain under light microscope (fig 5,6,7).

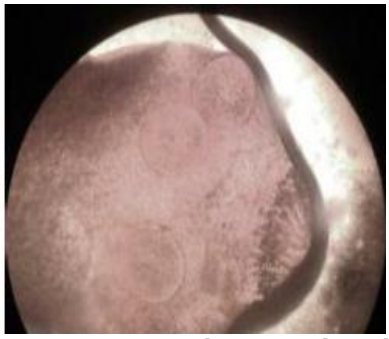

FIGURE 5: Scolex treated with 62.5mg/ml ginger extract unstained with eosin stain (x10)

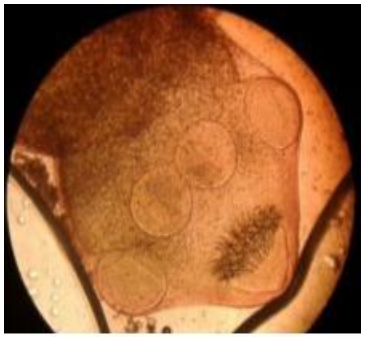

FIGURE 6: Scolex treated with 250mg/ml ginger extract unstained with eosin stain (x10)

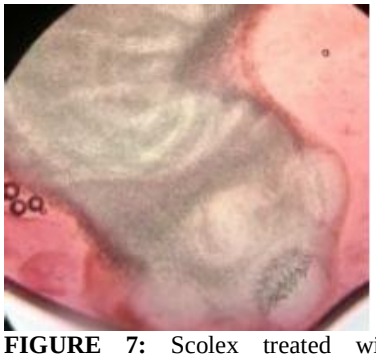

FIGURE 7: Scolex treated with 500mg/ml ginger extract unstained with eosin stain (x10)

Isolation of worms

The parasite recovered at necropsy from the dogs and the infection was 100% in the first group and control group. Eight worms obtained from each group (fig8, 9). While, there is no worms obtained from others infected groups, that means, there is an effect of ginger extract on growth and development of scolices to adult worms. The length obtained from worms of group no.4 range between 7 – 34 cm while the length of worms collected from control group ranged between 9-86 cm, table(2). The collected stained worms appear in fig. (10, 11, 12, and 13).

TABLE 2: recovered worms from necropsied dogs

Group no.	No. of given *Cysticercus tenuicollis*	no. of obtained *Taenia hydatigena*	Average of *Taenia hydatigena* (cm)
1	8	8	24
2	8	Zero	-
3	8	Zero	-
4	8	8	52

DISCUSSION

The present study revealed that the most of examined cysts were viable (98.78%) while the dead degenerated and calcified cysts were (5.22%), this agrees with previous studies [13, 20]. The mortality rates of scolices exposure to different concentration of ginger extract were zero. This result in contrast with those used the ginger extract to inactivation and killing the protoscolices of hydatid cysts in vitro, these results may be due to the nature of *Cysticercus tenuicollis* membrane and its evaginated Scolex, which have resistance capacity to extract of ginger compared with protoscolies of hydatid cysts. Al-Bayati confirmed for the first time that parasite had various specific cell which may related to the nature of this parasite and at last the total nature of parasitic bladders have a wide range of similarities with known connective tissue and its constituents of various cells, fibrous and other matrix components[21]. On the other hand, the recovered worms from experimental infected dogs isolated from the group, which infected with treated metacestodes by 62.5mg/ml of extract, and also from control group have been isolated and there was no worms developed from treated metacestodes at 250, 500mg/ml of ginger extract. This result revealed the inactivation effect of ginger extract to scolices of *C. tenuicollis*, which prevent them to develop and grow, so the scolices digested in gastrointestinal tract of dogs and exerted. *Zingeber officinale* contains about 1-2% of volatile oil and 5.8% of resinous matter, starch and mucilage. The volatile oil contains monoterpenes, sesquiterpenes and sesquiterpene alcohol zingiberol, gingerol and shagoals. Most of the pharmacologicaly active constituents reside in the volatile oils [22].This present result came in harmony with previous reports were using medicinal plants to kill protoscolices of *Echinococcus granulosus*[23,24,25,26].

Accoding to the results of our study, Z. *officinale* showed lower scolicidal power in comparison with the methanolic extract of *Z. officinale* (at 100 mg\ml) on protoscolices activity of hydatid cyst [8]. In addition, *Z. officinale* showed lower scolicidal power than the study reported by Baqer *et al*, that revealed the viability rate of hydatid cyst was zero percent at concentration of 150 mg\ml after 60 min of exposure to ethanolic extract of ginger [16]. Also our results were less than the results of the study that investigated the protoscolicidal agent of ethanolic extract against *Echinococcus* protoscolices at 50mg\ml concentration after 10min of exposure [11]. This may be due to the above explained in detail the nature of *C. tenuicollis* cyst and its scolices . The duration of the prepatent period evaluated at 40 days after scolices administration for our experimental infection, which is agree with the data of Gemmel study that showed the prepatent period for *T. hydatigena* was 42 to 56 days [27], and disagree with various prepatent periods given by different authors. The prepatent period for this parasite reported as 51 days [2] or 56 days [28,29]. NevaiLemarie and Guralp reported this period as 8-12 and 10-12 weeks respectively [30,31]. In our study, the prepatent period seen to be shorter when it compared to other studies.

CONCLUSION

In conclusion, there is an effect of ethanolic extract of ginger on growth and development of scolices of Cysticercus tenuicollis. The optimal concentration of the extract having high effect is 250mg/ml.

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