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# *In vitro* Evaluation of *Allium cepa* Ethanol Extract in Inhibiting the Growth of *Toxocara canis* Eggs

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## ABSTRACT

**Background:** Toxocariasis is one of the most significant infectious disease that is caused by *T.canis* which primarily infect dogs. Dogs become infected by consuming contaminated food that contains either eggs or larvae (L2) encysted in the paratenic hosts. These eggs can resist harsh conditions.

**Methods:** Investigating the effects of onions (*Allium cepa*) ethanol extracts at varying concentrations (5, 10, 20, 30 and 50 mg/ml) for periods of time (24 and 48 hours) against the unembryonated *T. canis* eggs growth *in vitro* was aimed of this study. The positive control group was Drontal® Plus, while the negative control was water.

**Result:** Upon microscopic, the results indicated that eggs of *T. canis* treated with *A. cepa* extract *in vitro* are sensitive to the extract at different doses and that their activities are time- and concentration-dependent. The highest was observed at dosages of 30 and 50 mg/ml for 24 and 48 hrs. respectively, where the efficacy was 100%. At doses of 20 mg/ml, the extract shows 98.8 and 100% ovicidal efficiency for 24 and 48 hrs. respectively. When compared to the Drontal® Plus group and water group, which were 100 and 11.8%, respectively, the treated *T. canis* eggs with doses of 5 and 10 mg/ml of *A. cepa* extract demonstrated ovicidal activity at 24 and 48 hrs., by 88.4, 93.9, 94.3 and 98.6%, respectively.

**Key words:** *A. cepa*, Dog, Drontal® Plus, *In vitro*, Ovicidal, *T. canis*.

## INTRODUCTION

Dogs play a vital role in people life, their versatility makes it capable of performing a wide range of security scenarios such as detection dogs specialize in sniffing drugs, explosives and as guards for house and even personal protection. Besides, looking after dogs can contribute to children's development, making them more confident and energetic, while also providing senior citizens with meaningful companionship (Waggoner *et al.,* 1997; Jezierski *et al.,* 2013). Dogs are susceptible to a number of zoonotic diseases, the two most common parasite illnesses being toxocariasis and echinococcosis. In various parts of the world, these disease are pose serious threats to public health and economic problems (El Maghrbi *et al.,* 2019; Al Malki, 2021).

Toxocariasis is one of the most significant infectious disease, that is caused by *T. canis* which primarily infect dogs with high zoonotic potential and these parasite belonging to the family Toxocaridae and the genus Toxocara (Rashid *et al.,* 2022). The mature parasites inhabit within the small intestine of their definitive host (dogs), where females release eggs with feces into the external environment. These eggs can resist harsh conditions. Dogs become infected by consuming contaminated food that contains either eggs (which contain L2) or larvae (L2) encysted in the paratenic hosts tissues such as mice, birds and earthworms (Maqbool *et al.,* 1998; Despommier, 2003; Hosin, 2008). Furthermore, puppies get up Toxocara larvae either through vertical lactation via infected milk or through transmission across the placenta from an infected mother. (Salazar *et al.,* 2020; Jesudoss *et al.,* 2021). In human, infection occurs following ingestion of eggs, the larvae released and travel to the major organs, such as liver, lungs, or brain causing damage while inducing inflammatory responses, this form of infection is known as visceral larva migrans. Otherwise, the larvae may migrate to the eye, where they can occasionally cause unilateral blindness; this type of infection is called ocular larva migrans (Magnaval *et al.,* 2001; Öge *et al.,* 2014; Kong *et al.,* 2016). Flotation technique is the most dependable laboratory method for confirming *T. canis* eggs in clinically suspected cases because it gets more eggs than other techniques (Magnaval *et al.,* 2001; Dryden *et al.,* 2005).

On the other hand, the benzimidazole carbamates group of anthelmintic medications is the most widely utilized for treating toxocariasis (Pawlowski, 2001). However, because to their exceedingly poor solubility, their low tissue bioavailability, these medications thus require

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relatively high doses to be administered over prolonged periods (Hrckova and Velebny, 2001). Furthermore, drug resistance could result by using these medications to treat different helminth infections (Geerts and Gryseels, 2000). Therefore, there is an urgent need for novel medications to treat helminthic diseases like the application of crude medicinal herbs has a beneficial influence on people as well as animals due to their anthelmintic efficacy without causing any negative effects. Recently, there has been an explosion of interest about discovering medicinal herbal that could potentially employed as novel anti-parasitic as a result of the increasing contraindications to the use of synthetic medications (Muhammed, 2015; Jasim *et al.*, 2023). In veterinary medicine, natural treatments derived from plant extracts are anticipated to take the place of traditional parasite control techniques. Many plants can be employed as anti-parasitic agents like onions (*Allium cepa*) have been demonstrated in numerous investigations that they have nematocidal and trematocidal properties (Orengo *et al.,* 2016). The objective of this research was to evalute the influence of *A. cepa* ethanol extract against *T. canis* eggs *in vitro*, in compare to negative control groups (Water) and positive control group (Drontal® Plus).

## MATERIALS AND METHODS

### Study area

The study was conducted in the Parasitology Laboratory at the College of Veterinary Medicine, Al-Muthanna University, during the period from January, 2023 to the end of July, 2023.

### *Toxocara canis* eggs preparation

Shortly after the stray dogs' deceases, 46 fully developed female *T. canis* were removed from the intestines. The identification of the adult female *T. canis* worms was based on their morphological characteristics as described by Soulsby (1982). In order to get rid of the tissue debris, the adult female worms had been washed with phosphate-buffered saline (PBS). Subsequently, the female worms' uterus was dissected, eggs of *T. canis* had been eliminated from the adult worms, washed many times in PBS and then centrifuged at 3,000 rpm to 5 minutes. Then discarding the supernatant, the precipitate eggs were put into an opaque glass filled with normal saline to be kept at 40°C until needed again (El-Sayed, 2017).

### McMaster's technique for counting eggs

The number of eggs that had been collected from the uteri of adult worms was calculated employing the McMaster technique, as described by Taylor, (2016) and Jasim and Al-Amery, (2023).

### Preparation of onions (*Allium cepa*) ethanol extract

The fresh onions (*Allium cepa*) bulbs had been bought from the herbal market in Muthanna province, Iraq. The onions were cut into slices, then left to dry in the shade for a week. After that, they were mechanically powdered using electric blender, then packed in in a glass container and maintained in a dry, cool environment. One hundred gram of dry onion powder and 400 ml of 99.5% ethanol were mixed together and gently shaken with a magnetic stirrer for an hour in order to obtain the ethanol extract. The product solution was mixed again and filtered through Whatman cellulose filter paper after being left at room temperature for 72 hours. Above a sand bath heated to 80°C, the solvent evaporated. In order to avoid the dry extract adhering to the container walls, the final step of the drying process was carried out in an oven heated to 40°C. Following, a maroon paste was obtained from the extraction process of *A. cepa* was placed in a sterile container of glass and stored at 4°C for subsequent use (Abhijeet *et al.,* 2012; Akintobi *et al.,* 2013; Orengo *et al.,* 2016).

### Onions (*Allium cepa*) extract: An experimental study on eggs of *T. canis in vitro*

After dissolving *A. cepa* extracts in the water bath at 40°C with vigorous agitation for 15 minutes, various concentrations (5, 10, 20, 30, 40 and 50 mg/ml) prepared. Unembryonated *T. canis* eggs were exposed *in vitro* to *A. cepa* extracts at different concentrations and for different period of time (24 and 48 hrs). A total of 50ìl of rich sediment (500) unembryonated eggs was added into an eppendroff tube containing 450 µl of *A. cepa* extract with different concentration. The eppendroff tubes were put in an incubator adjusted to 28°C for periods of 24 and 48 hours. After the incubation period, the extract solution's supernatant was removed with a pipette and eggs are washed several times with PBS. After that, each tube's eggs were transported into new eppendroff tubes containing 750 µl tap water (dechlorinate) and they have been incubating for two weeks at 28°C. At the same time, 50 µl of egg rich sediment was incubated at 28°C without exposure to the extract as a negative group (Orengo *et al.,* 2016; El-Sayed, 2017). In order to make Drontal® Plus (5 mg/ml), 25 mg of the drug was dissolved in 5 ml of distilled water containing 5% DMSO as the positive control. The presence of larvae inside eggs, developing cells, or dead eggs was used to investigate the influence of *A. cepa* extracts on the *T. canis* egg embryogenesis. Ovicidal activity had been calculated as described by Jasim and Al-Amery, (2023).

## RESULTS AND DISCUSSION

The experience was accomplished to evaluate the influence of *A. cepa* extract against *T. canis* eggs development through different concentrations (5, 10, 20, 30 and 50 mg/ml) for different periods (24 and 48 hours), in compare to negative control groups (Water) *in vitro*. According to the results of microscopic investigations of *T. canis* eggs subjected to different *A. cepa* extract doses and intervals *in vitro*, revealed that eggs are sensitive to *A. cepa* extract at different concentrations and their activities are concentration and time dependent, as shown in Fig 1. The highest efficacy, reaching 100% was recorded with concentrations of 30 and 50 mg/ml after 24 and 48 hours,

respectively. But, the eggs exposed to 5 and 10 mg/ml of *A. cepa* extract after 24 and 48 hours demonstrated ovicidal activity by 88.4, 93.9, 94.3 and 98.6%, respectively, in compared with Drontal® Plus (praziquantel/pyrantel pamoate/febantel; 5 mg/ml) and negative control group were 100 and 11.8 % respectively, as showed in Table 1, 2. Also, Table 1 and 2 show that the extract's efficacy at concentrations of 20 mg/ml for 24 and 48 hours is 98.8 and 100%, respectively.

Because there is currently no approved vaccination for any parasitic disease and the growing contraindications associated with synthetic medications stemming from their adverse effects, or the increase of resistance to parasites, it is crucial to require alternative anti-parasitic pharmaceuticals (Thakur *et al.,* 2022; Barua *et al.,* 2023). Consequently, there has been significant recent interest in identifying plants with biological properties that can utilized as new anti-parasitic drugs without causing harmful side effects (Wink, 2012). Moreover, successful natural product research requires careful selection of herbs based on diverse criteria, such as chemotaxonomic data, field observation and information from conventional medicine (Queiroz *et al.,* 2009).

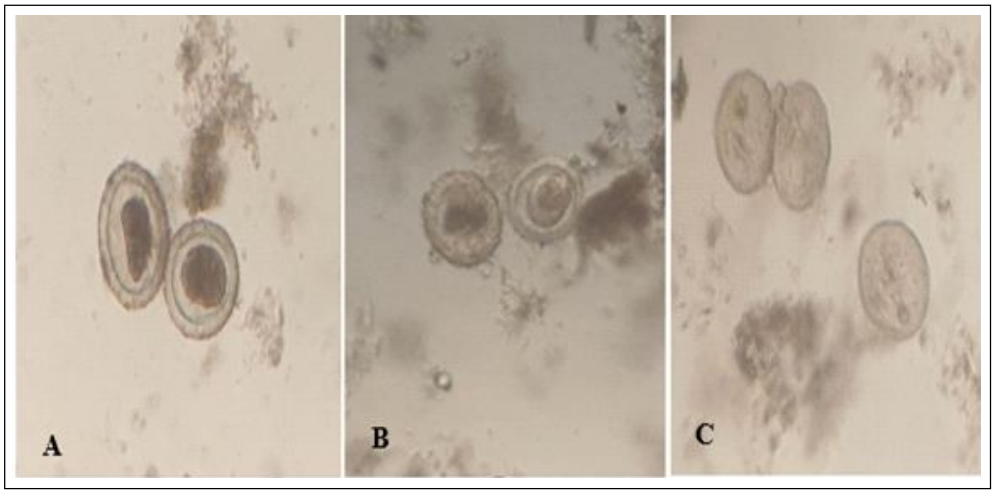


**Fig 1:** A- A viable *T. canis* eggs, (B; C) Eggs with lysed embryo (dead eggs) after exposure to extracts of *A. cepa* and Drontal® Plus.

**Table 1:** Results of exposure *T. canis* eggs to *A. cepa* extract for 24 hrs. *in vitro*.

| Concentration of drug and ginger extract (mg/ml) | No. of examine eggs | After 14 day | | | | | |
|---|---|---|---|---|---|---|---|
| | | Expose *T. canis* eggs to *A. cepa* for 24 hrs. | | | | | |
| | | Eggs contain larval | % | Growth eggs | % | Dead eggs | % |
| 5 | 413 | 12± 0.57b | 3.03 | 35±1.73b | 4.08 | 365.33±1.855d | 88.4 |
| 10 | 418.33 | 4.66±0.88c | 1.06 | 16.66±2.96c | 4.43 | 395±2.51b | 94.36 |
| 20 | 424 | 0.66 ±0.33d | 0.13 | 3.33±0.33d | 0.73 | 420±3.46a | 99.05 |
| 25 | 409.33 | 0±0.00e | 0 | 0±0.00e | 0 | 409±2.08c | 100 |
| 50 | 421.33 | 0±0.00e | 0 | 0±0.00e | 0 | 421.33±2.33a | 100 |
| 5 Drontal Plus positive control | 423 | 0±0.00d | 0 | 0±0.00e | 0 | 423.66±3.48a | 100 |
| Distal water negative control | 435.33 | 330±8.96a | 75.66 | 60±2.64a | 13.73 | 45.66±4.37e | 0.433 |

**Table 2:** Results of exposure *T. canis* eggs to *A. cepa* extract for 48 hrs. *in vitro*.

| Concentration (mg/ml) | No. of examine eggs | After 14 day | | | | | |
|---|---|---|---|---|---|---|---|
| | | Expose *T. canis* eggs to *A. cepa* for 48 hrs. | | | | | |
| | | Eggs contain larval | % | Growth eggs | % | Dead eggs | % |
| 5 | 426.33 | 5±0.57b | 1.13 | 16.66±2.96b | 4.33 | 402.66±3.28d | 94.43 |
| 10 | 432.33 | 0.66±0.33c | 0.13 | 3±1.15c | 0.633 | 428.6±0.21a | 99.13 |
| 20 | 421.333 | 0.33±0.33c | 0 | 0±0.00e | 0 | 421.33±2.33b | 100 |
| 25 | 429.33 | 0±0.00d | 0 | 0±0.00e | 0 | 426±2.64a | 100 |
| 50 | 412.66 | 0±0.00d | 0 | 0±0.00e | 0 | 412.66±1.45c | 100 |
| 5 Drontal plus positive control | 423.66 | 0±0.00d | 0 | 0±0.00e | 0 | 423.66±3.48b | 100 |
| Distal water negative control | 435.666 | 330±8.96a | 75.66 | 60±2.64a | 13.73 | 45.66.66±4.05e | 10.43 |

This study was designed to evaluate the influence of *A. cepa* extract on the viability and embryogenesis of *T. canis* eggs due to their significant role in the transmission of human toxocariasis. The findings of ovicidal activity for *A. cepa* ethanol extract against *T. canis* eggs at different concentrations (5, 10, 20, 30 and 50 mg/ml) for varying periods (24 and 48 hrs.), were agreement with the obtained results by Orengo *et al.* (2016), which employed the *A. cepa* ethanol extract of  as an anti-parasite. It was demonstrated that 100% of *T. canis* eggs were suppressed by *A. cepa* ethanol extract at doses of 10,000-1,250 ug/ml. Furthermore, our investigation findings indicated that the efficiency of *A. cepa* ethanol extract increases with duration of exposure and concentration. Several studies have suggested that the biological and medical functions of *A. cepa* can be attributed to its phytochemical constituents. The anthelmintic properties of *A. cepa* extract are primarily due to sulfur compounds like Allyl propyl disulfide and Allicin, as well as flavonoids such as Quercetin (Githiori *et al.,* 2006; Chakraborty *et al.,* 2022). Allicin exhibits its antimicrobial activity primarily by rapidly and completely inhibiting the synthesis of DNA, RNA and proteins (Feldberg *et al*., 1988; Etewa and Abaza, 2011; Al-Zayyadi, 2020). Moreover, onion-derived sulfur compounds particularly diallyl trisulfide (DTS), inhibited the growth the parasites and damage to the cell membrane of parasites, resulting in reduced viability and destruction (Krstin *et al.,* 2018). Besides, Gomes *et al.* (2016) Zingiber officinale contains phytochemical constituents (saponin) is act on destabilization of the cell membrane and lead to increased permeability, which causes embryonic lysis. Onion extract contains modest amounts of the same components (saponin) that may be responsible for *T. canis* egg destruction. On the other hand, the results of the current study regarding Drontal®Plus (praziquantel/pyrantel pamoate/febante) effectiveness against *T. canis* eggs *in vitro* were consistent with the findings of Orengo *et al*. (2016), who revealed that eggs of *T. canis* are sensitive to Vermic TotalTM (praziquantel/pyrantel pamoate/febante) *in vitro*, which has the same composition as Drontal® Plus but different concentrations. Also, the drug's effectiveness is dependent on time and concentration. Drontal® Plus is a widely used veterinary dewormer designed to treat various intestinal parasites in dogs. While, it effectively targets adult parasites such as tapeworms, roundworms, hookworms and whipworms, but, its ability to kill parasite eggs is not clear (Yadav *et al.,* 2007).

## CONCLUSION

Based on the data, the results indicated that eggs of *T. canis* treated with *A. cepa* extract *in vitro* are sensitive to the extract at the different concentrations and times *in vitro* and that their activities are time- and concentration-dependent.

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### Disclaimers

The views and conclusions expressed in this article are solely those of the authors and do not necessarily represent the views of their affiliated institutions. The authors are responsible for the accuracy and completeness of the information provided, but do not accept any liability for any direct or indirect losses resulting from the use of this content.

### Informed consent

All procedures for experiments were approved by the Committee of Experimental Animal care.

### Conflict of interest

The researchers have not declared any conflicts of interest.

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