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# Evaluating the in vitro Efficacy of *Artemisia absinthium* Alcoholic Extract in Neutralizing *Toxocara spp*. Eggs

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This study was conducted in the department of Parasitology, College of Veterinary Medicine, University of Baghdad. Microscopically, Adult worms of *Toxocara* spp appeared milky white with a coiled posterior end notable and from the erect tail, adult male was shorter than the female, with a finger-like projection. Eggs of *T. cati* in different stages development were incubated with *Artemisia absinthium* extract in different concentrations and time period. *Artemisia absinthium* alcoholic extract the leaves dried were detached and powdered through electrical grinder and the adult parasites were kept in phosphate-buffered saline (PBS). The process of extracting *Artemisia absinthium* and dilution in distilled water at concentrations of 0.3, 0.6, 1.2 and 2.4 mg/ml and negative control group with PBS. Microscopic investigations of the eggs treated with *Artemisia absinthium* extract demonstrated restricted larval growth at doses of 2.4 mg/ml for 21 days but uninhibited development larvae in groups 0.3, 0.6, and 1.2 mg/ml. A significant difference ($P \leq 0.05$) was found between the positive control groups (Albendazole) and the other *Artemisia absinthium* groups.

**Keywords**: *Artemisia absinthium*, Albendazole, Cats, *Invitro*, *Toxocara cati*, Zoonotic Parasite.

## INTRODUCTION

*Artemisia absinthium has a wide spectrum of pharmacological activities, and the Artemisia genus consists of about 500 varieties distributed worldwide* (Bora and Sharma, 2010). *A. absinthium* has been proven to have an anthelmintic effect against organisms like *Haemonchus, Trichinella spiralis, Trichostrongylus colubriformis, and Ascaris suu* it secreted substances able of controlling with the parasite infection (Szopa *et al.*, 2020). These plants have been usually used as an empirical treatment because of their antiparasitic effect and *has* medicinal plant sources and numerous biological actions, including antioxidant, anticarcinogenic, and antidiabetic (Magryś *et al.*, 2021). Aromatic oils are found in all *Artemisia* species and numerous are employed as culinary herbs, hallucinogens, flavorings, vermifuges, and medicines *(*Sy *et al.*, 2001*)*. Toxocariasis leftovers are problematic worldwide as they cause systemic zoonotic infection in paratenic hosts like ruminants, persons, rabbits, poultry and rodents (Hade *et al.*, 2018). Numerous paratenic hosts with humans can be infected with *Toxocara spp* eggs, infectious larvae can be generated by two capacities to release proteins (MUC-120) that support it to pass through the intestinal wall and then enter the journey and expand many tissues, including the lungs and liver (Strube *et al.*, 2013; Hade, 2020). Infected water and food may be transferred by zoonotic agents, with extensive minor linkages to animal secretions (Hadi and Faraj, 2014). The gastrointestinal helminths in the Ascaridia group can affect some clinical signs and make the eggs an environmental factor (Faraj *et al.*, 2019). Humans are essential for effective preventative strategies, since there are no realistic means for reducing environmental egg levels, the most critical approach is to prevent initial contamination and long-term preventive treatment programs aim to reduce *Toxocara spp* eggs output throughout puppyhood utilizing a multidose schedule (Overgaauw and van Knapen, 2013). Many workers in Iraq used the ELISA test as an accurate technique for serodiagnosis of a large number of parasites in man and animals (Lamy and Kawan, 2022). However, this study aimed to morphological study and investigate the efficiency of Artemisia absinthium alcoholic extract against eggs of *T. cati* when compare to positive control groups (Albendazole) in vitro.

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## MATERIALS AND METHODS

***Fecal samples collection*:** Fecal samples were collected randomly freshly after defecation from a private veterinary clinic in Baghdad city and microscopically examined by direct and flotation with NaCl solution by traditional method procedure in the laboratory of parasitology/ Veterinary Medicine/ University of Baghdad (Kareem and Kawan, 2020).

***Direct wet smear method*:** A direct fecal sample smear method was useful to distinguish the eggs of the *T. cati* by putting a slight quantity of feces on a slide and mixing it fully with one to two drops of distilled water with sticks mounted and a coverslip. They exanimated under a light microscope at 10x and 40x (Alani *et al.*, 2021; Rashid *et al.*, 2022).

***Flotation using saturation with Nacl solution*:** A 50-mL saturated sodium chloride solution was utilized, along with one to two grams of fecal material. The liquid was filtered and divided into two test tubes, with a coverslip placed on the surface of each test tube after 10 minutes. The coverslips were detached horizontally, placed on the glass slides, and observed under microscopic 10x (Rashid *et al*. 2022).

***Preparing Artemisia absinthium extract*:** The stems and leaves of the *Artemisia* plant were obtained from the producing company (Ahmet Arifoglu) in Turkey, as this variety of *Artemisia absinthium* species in northern Turkey. The scientific taxonomy of the *Artemisia absinthium* used in the study was conducted in the Department of Biology/ College of Science/ University of Baghdad. The dry leaves were detached and powdered through an electrical grinder. A quantity of 50g of the powder to a sterile Erlenmeyer and di-ethyl ether at 30-35°C for seven hours was added until the alcohol was suspended. Then, the mix was mobilized by a shaker for 48 hours, and the mixture was filtrated. The obtained fluid was to vaporize di-ethyl ether. The extracted was left to stand at room temperature overnight exposed to the alcohol evaporated (Zenebe *et al*., 2017).

***Collection of the Adult worms*:** Adults of *T. cati* were collected directly from cat feces and the adult parasites were kept in phosphate-buffered saline (PBS) (Tariq *et al*., 2009). In an appropriate container, mix 800 ml of distilled water with 8g of sodium chloride, 0.2g of potassium chloride, and 1.44g of sodium phosphate dibasic. Finally, 0.245g of potassium phosphate monobasic were added to the solution (Islary *et al.*, 2024). *T. cati* eggs were obtained by newly collecting and crushing an adult female and sieving. McMaster method, the obtained egg was diluted and adjusted to a concentration of 500 eggs/ml.

***In vitro trial*:** The suspensions were centrifuged for 60 seconds at 300 RPM. Sediment was held and then re-suspended. The collected egg was diluted and adjusted to 500 eggs per milliliter. Extract and dilute *Artemisia absinthium* in distilled water at concentrations of 0.3, 0.6, 1.2, and 2.4 mg/ml. In all trials, a test tube containing 450µl of *Artemisia absinthium* alcoholic extract with altered concentrations was introduced to 50µl of egg-rich sediment containing 500 unembryonated eggs. The tubes were incubated at 28°C for 24 hours, seven, fourteen, and twenty-one days. Albendazole positive group at 0.25 mg/ml concentration compared to medications after being dissolved in 0.5% dimethyl sulphoxide (DMSO) (Tariq *et al.*, 2009).

***Statistical analysis*:** The statistical analysis of the results by using the SAS (Statistical Analysis System-version20.1), using two-way ANOVA and Least significant differences (LSD) post hoc trial was achieved to assess significant differences among means of the groups (Cary, 2012)

## RESULTS

The result of *Artemisia absinthium* ovicidal activity revealed that no inhibition occurred on larvae development inside the eggs following exposure to 0.3, 0.6, 1.2, and 2.4 mg/ml for 24 hours and 7th day in comparison to the positive control, which is 84.6% at 7$^{th}$ day (Table 1). However, the ovicidal efficacy of *Artemisia absinthium* extract was obtained at concentrations of 2.4 mg/ml with treatment times 14$^{th}$ and 21$^{th}$ is 30.4% and 74.8%, respectively (Table 2).

***Efficacy of Artemisia absinthium at 0.3, 0.6 and 1.2 mg/ml concentration*:** No inhibition was observed on embryonic larval development at 0.3, 0.6 and 1.2 mg/ml *Artemisia absinthium* concentration at 24hrs and 7$^{th}$ day after treatment during invitro screening study. Developed larvae inside *T. cati* eggs treated in vitro with *Artemisia* alcoholic extract were observed from 14$^{th}$ and 21$^{th}$ day Fig. 1.

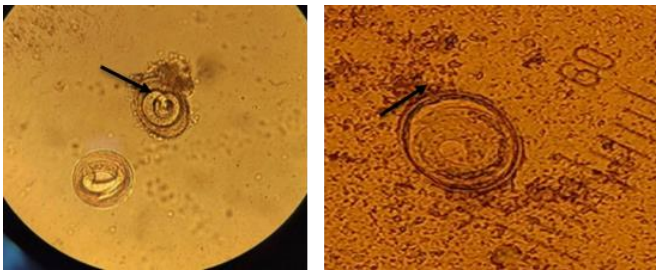

**Figure 1. A and B. Development larvae inside *Toxocara* spp eggs after treatment invitro with *Artemisia absinthium* at concentration 0.3, 0.6 and 1.2 mg/ml 21$^{th}$ day (40x).**

***Efficacy of Artemisia absinthium at 2.4 mg/ml concentration*:** The results of study documented uninhibited development larvae inside *T. cati* eggs at 24 hours and 7th days. *Artemisia absinthium* alcoholic extract at 2.4 mg/mL concentration can inhibited larvae inside the eggs at 14$^{th}$ and 21$^{th}$ days Fig. 2.

***Efficacy of Albendazole at 0.25 mg/ml concentration*:** Invitro the efficacy of albendazole at concentration 0.25 mg/ml in compared with *Artemisia absinthium* alcoholic

**Table 1. Effect of *Artemisia absinthium* alcoholic extract and Albendazole on *T. cati eggs* invitro after 24h and 7th day.**

| Treatment | Concentrations | Number of eggs before treatment | Days after Treatment | | | |
|---|---|---|---|---|---|---|
| | | | 24hr dead eggs | % | 7th days dead eggs | % |
| *Artemisia* | | 500 | 0±0.00c | 0 | 0±0.00c | 0 |
| *absinthium* | G1=0.3mg/ml | 500 | 0±0.00c | 0 | 0±0.00c | 0 |
| | G2=0.6mg/ml | 500 | 0±0.00c | 0 | 0±0.00c | 0 |
| | G3=1.2mg/ml | 500 | 0±0.00c | 0 | 0±0.00c | 0 |
| | G4=2.4mg/ml | | | | | |
| Albendazole | G5=0.25mg/ml | 500 | 0±0.00c | 0 | 423±0.00a | 84.6 |
| Control | G6=PBS | 500 | 0±0.00c | 0 | 0±0.00c | 0 |

**Table 2. Effect of *Artemisia absinthium* alcoholic extract and Albendazole on *T. cati* eggs invitro after 14th and 21th days.**

| Treatment | Concentrations | Number of eggs before treatment | Days after Treatment | | | |
|---|---|---|---|---|---|---|
| | | | 14th days dead eggs | % | 21th days dead eggs | % |
| *Artemisia* | G1=0.3mg/ml | 500 | 0±0.00c | 0 | 0±0.00c | 0 |
| *absinthium* | G2=0.6mg/ml | 500 | 0±0.00c | 0 | 0±0.00c | 0 |
| | G3=1.2mg/ml | 500 | 0±0.00c | 0 | 0±0.00c | 0 |
| | G4=2.4mg/ml | 500 | 152±0.00b | 30.4 | 374±0.00a | 74.8 |
| Albendazole | G5=0.25mg/ml | 500 | 500±0.00a | 100 | 500±0.00a | 100 |
| Control | G6=PBS | 500 | 0±0.00c | 0 | 0±0.00c | 0 |

extract revealed uninhibited larvae development inside eggs of at 24 hours and 7th days. While showed inhibited larvae development at 14th days Fig. 3.

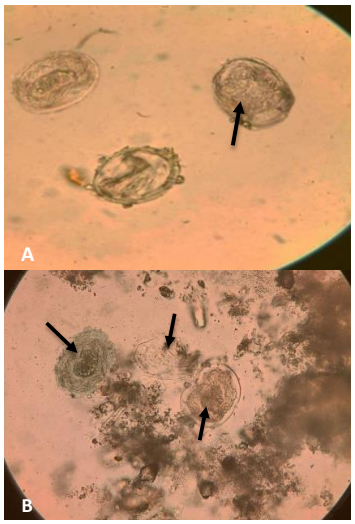


**Figure 2. A and B. *Toxocara* spp eggs treatment invitro with *Artemisia absinthium* at concentration 2.4 mg/ml showed inhibit larvae development inside the eggs for 21th days (40x).**

***Morphology of T. cati*:** Isolated adult *Toxocara spp* parasite from cats appeared grossly in fresh sample as large round worms, milky white in color. The adult female was larger than male, the average length range were (6-8 cm). Adult male was shortly than female average range were (4-6 cm).

***Microscopically***: Adult female of *Toxocara spp* appeared milky white in color with coiled posterior end notable and from the erect tail. While the adult male was shortly than female, with finger like projection. Female and male anterior end of *toxocara spp* appeared microscopically with cephalic alae arrow like tappear end of female and finger like projection end of male. Microscopically *Toxocara spp* eggs appeared by direct and flotation methods under 40x round to ovoid in shape, have thick pitted wall with total mean size ranging (65-75 µm). *Toxocara spp* eggs appeared by direct and flotation methods under 40x round to ovoid in shape, have thick pitted wall with total mean size ranging (65-75 µm) Fig. 4.

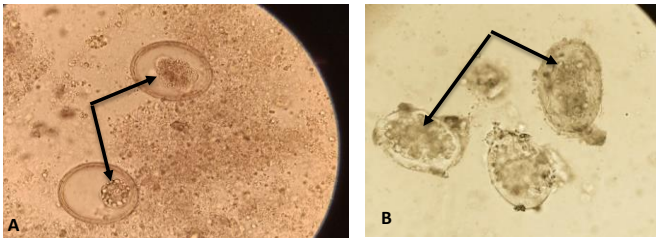


**Figure 3. Toxocara spp eggs treatment invitro with Albendazole at concentration 0.25 mg/ml. A. Partial inhibition development of larvae inside the eggs at 12th day. B. *Complete* inhibition development larvae inside the eggs at 14th day (40x).**

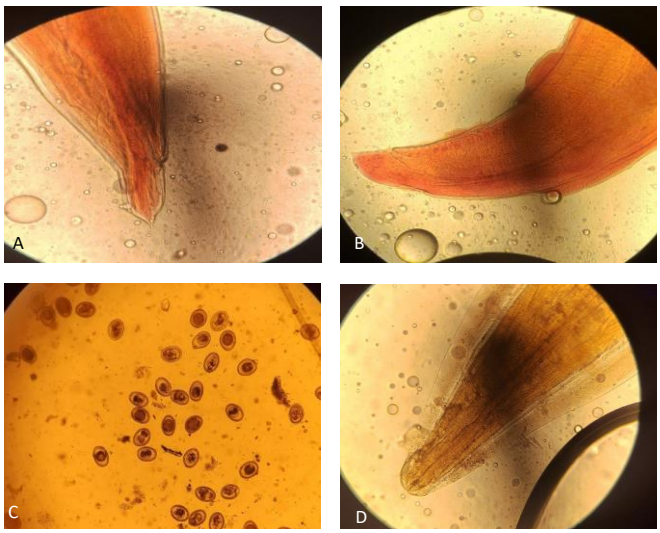


**Figure 4. A. Posterior end of *Toxocara spp* male with finger-like project (40x). B. Posterior end of *Toxocara spp* female (40x). C. Beginning of unembryonated egg of *Toxocara spp* (10x). D. Cervical alae in the anterior end of *Toxocara spp* (40x).**

## DISCUSSION

Artemisia acts as an anti-parasitic efficiency, where this herb was used in eggs to decrease the stool of sheep after treatment and management by *Artemisia absinthium* extract (Iqbal *et al.*, 2004; Tariq *et al.*, 2009). In the present investigation, the results of *Artemisia absinthium* extract concentrations 0.3 and 0.6mg/ml corresponded with the previous study, which indicated no inhibition of larval development (Yildiz *et al.*, 2011). *Artemisia* has essential oils that possess an antiparasitic efficiency. The major workings of essential oils in *Artemisia* release were reported as camphor and 1.8-cineole, representing an antiparasitic efficiency (Kordali et al., 2005; Yae *et al.*, 2006). In previous study, *Artemisia* agonist oocyte of *Eimeria tenella*, the results were revealed that the percentage of the parasite in groups treated with alcoholic and aquatic extracts reduced by 75% and effect of *Artemisia* is the carrier proteins in mitochondrial and endoplasmic reticulum disturbance in the process of development because it affects the cell wall is inhibitory Sarcoplasmic-Endoplasmic Reticulum Calcium ATPase-Serca (Al-Salhi *et al.*, 2015). *A. absinthium* extract for reduced larvae of *Trichinella spiralis* in mice muscle at dose 300 and 600mg/kg b.w (Caner *et al.*, 2008). *Artemisia* extracts have a direct influence on the survival of adult nematodes both invitro and invivo, inhibiting their motility (Tariq *et al.*, 2009). In vitro, the extract Artemisia absinthium was used against adult worms of Hymenolepis nana at concentrations one and 5 mg/ml induced worm paralysis, death and ultrastructural alterations (Beshay, 2018). However, there are currently no investigations on the efficacy of *Artemisia* absinthium extract on the amount of larval suppression inside the eggs of the *Toxocara spp* parasite in vitro. This plant extract may be a selection in the managing of intestinal parasitic diseases in the future. The morphology study showed the results within the range of previous study, showing adult parasites are roundworms measuring (6.5 to 10 cm) long for the females. In comparison, the adult males appeared (4 to 6 cm), and microscopically, the results were by Overgaauw *et al*. (2009) who recorded almost similar characteristic features. Microscopically, *Toxocara spp* eggs within the range of Fillaux and Magnaval (2013) showed the eggs round in form, and the size was measured (65-75 µm).

***Conclusion***: Albendazole at a dosage of 0.25 mg/ml was more effective than *Artemisia absinthium* alcoholic extract in inhibiting larval development at 14 days. Extracted *Artemisia absinthium* can be used over concentration than in the current study or combined with other plants to high effect helminthes. Clinically, cats are important reservoirs of zoonotic parasites. *T. cati* infection represents a notifiable parasitic disease, and due to a lack of adequate data, added research on epidemiology and clinical pathology aspects of *T. cati* infection in Iraq is required.

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***Ethical Approval*:** The Committee of Animal Ethics, Research of Scientific Deanship, College of Veterinary Medicine, University of Baghdad, approved the experimental procedures of this study.

***Conflict of interest*:** The authors state that there is not conflict of notice.

***Ethical consideration*:** Not applicable.

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