Antimicrobial Effects of Thymoquinone on *Entamoeba histolytica* and *Giardia lamblia*

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**ABSTRACT**

**Background:** Parasitic infections are a major difficulty in tropical and subtropical countries. Traditionally medicinal plants have been used in folk medicine to treat parasitic infections and are a valuable source of novel anti-parasitics. **Objective:** In our search for therapeutic alternatives to anti-protozoal chemotherapy, thymoquinone, the active ingredient of Black cumin (*Nigella sativa*) was examined. **Materials and Methods:** Thymoquinone was tested against *Entamoeba histolytica* and *Giardia lamblia* using *in vitro* susceptibility assays and the mortality of the parasites were then obtained using the standard calculations. The compound was also tested for 48 and 72 hours on both parasites. **Results:** The current study indicate that the mortality of TQ showed 85.5%, 91.5% and 96.8% mortality on *E. histolytica* for 25 ppm at 24 hr, 48 and 72 hr, respectively, with IC₅₀ 2×10⁻⁹. On the other hand, this natural compound showed a mortality of 82.83%, 91.76% and 96.62% mortality on *G. lamblia* for 25 ppm at 24 hr, 48 and 72 hr, respectively, with IC₅₀ 4.8×10⁻⁹. Metronidazole powder gave 70.9% mortality at 156 ppm at the same times. **Conclusion:** The current results indicate that TQ is more potent on *E. histolytica* compared to *G. lamblia*. Further pharmacological studies were needed to help in the clinical presentation of thymoquinone.

**Key words:** Prophetic Medicine, *Nigella sativa*, Thymoquinone, *Entamoeba histolytica*, *Giardia lamblia.*

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**INTRODUCTION**

Protozoa are one of the three chief types of parasites that lead to diseases in humans. Protozoan Infections are contagious and can be transmitted via the fecal-oral route.¹,² Blood protozoa can be transmitted through a third source such as insects.³ There are four main groups of protozoa according to how they move. That includes the sarcodina (ameba), mastigophora (flagellates), ciliophora (ciliates) and the Sporozoaa.⁴-⁵ Many parasitic diseases are known to clinicians and public health professionals, which includes trichomoniasis, babesiosis, malaria, toxoplasmosis, leishmaniasis, african sleeping sickness, giardiasis and amoebiasis.⁶-⁷ Giardiasis is transmitted through oral contact and caused by *Giardia lamblia* and known as intestinal protozoan diseases.⁷ Amoebiasis is caused by sarcodina group of protozoa (Such as Entamoeba histolytica) that invade body tissues.⁸⁻⁹ The ailments triggered by protozoan parasite are accountable for substantial mortality and morbidity, distressing more than 500 million of the world population. The epidemiological management of protozoan is insufficient due to complicatedness of vector and reservoir control; while the progress in the development of protozoan vaccine is slow and arduous. Currently, the chemotherapy stays necessary constituent of both clinical management and disease control programmer in prevalent areas. The medications in utilization as anti-protozoan agents were discovered over 60 years and a number of issues limit their usefulness such as: high cost, drug resistance, poor compliance, low effectiveness and deprived safety. Recently, the searches for new drugs against protozoan parasite have been increased and more interest has been put on the traditional medicine, especially herbal remedies.¹⁰⁻¹¹ Seeds’ oil of *Nigella sativa* (Ranunculaceae), is widely used as a food and cure.¹²⁻¹³ Experimental studies confirmed that the plant is respiratory stimulant, diuretic, hypoglycemic, anti-inflammatory, antioxidant, anticancer, antimicrobial and analgesic. Previous phytochemical reports showed that the seed contain alkaloids, tannins, steroids and flavonoids.¹⁴⁻¹⁵ Thymoquinone (TQ), an active ingredient of *Nigella sativa*, has been reported to exhibit anti-oxidant, anti-inflammatory, antimicrobial and anti-tumor activities. The current study was designed to assess the Effects of thymoquinone on *Entamoeba histolytica* and *Giardia lamblia*.

**MATERIALS AND METHODS**

**Parasite isolate**

*Entamoeba histolytica* and *Giardia lamblia* used in all experiments were taken from patient. All positive samples were examined by wet mount preparation. Trophozoites of the two parasites were performed at 37 ± 1°C in RPMI 1640 medium containing 5% of bovine serum. The trophozoites were maintained for the assays and were employed in the log phase of growth. Parasites were counted under the microscope by haemocytometer chamber.

**In vitro susceptibility assays**

*In vitro* susceptibility assays of the current research used the sub-culture method.¹⁶ This is highly stringent and sensitive method for assessing the anti-protozoal effects (gold standard) particularly in *Entamoeba histolytica*, *Giardia intestinalis* and *T. vaginalis*. Five mg from the compound was dissolved in 5% dimethyl sulfoxide (5000 ppm). The concentrates were stored at -20°C for further analysis. Sterile 96-well microtite plate was used for test material, positive control and negative control. The final volume in the wells was 100 μl. Each test included metronidazole pure compound [(1-(2-hydroxyethyl)-2-methyl-5 nitroimidazole], a drug was used as positive control in concentration 312.5 μg/ml, whereas untreated cells were used as a negative controls (culture medium plus trophozoites). Samples were taken for counting at 0, 24, 48, 72, 96, and 120 hours. For counting, the samples
The compound was also tested for 48 and 72 hours on both parasites. The current results indicate that TQ is more potent on *E. histolytica* compared to *G. lamblia*. Metronidazole powder gave 70.9% mortality at 156 ppm at the same times.

*N. sativa* seeds were found to turn render the parasite susceptible to damage by the host and may play a role in the anti-schistosomal potency. The oil prevented most of the hematological and biochemical alterations and noticeably enhanced the antioxidant capability of schistosomiasis mice contrasted to the infected untreated ones. Dietary intake of chloroquine with *N. sativa* was examined previously and found effective in malaria’s mice model of *plasmodium berghei*. N. sativa seed in ethnomedicine and in modern years for the cure of microbial diseases has been used without any known side-effects. Consequently, *N. sativa* can offer a precious medication for microbial ailments. However, supplementary research is needed to assess and discover the specific cellular and biomolecular mechanisms of the antimicrobial effects of TQ, only or in mixture with other drugs.

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### CONFLICT OF INTEREST

Authors declare no conflict of interest.

### ABBREVIATION USED

None.

### REFERENCES

Thymoquinone (TQ) was tested against Entamoeba histolytica and Giardia lamblia using in vitro susceptibility assays. Results indicate that thymoquinone antiprotozoal properties. TQ is more potent on E. histolytica compared to G. lamblia.