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*Original Article*

# 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 $IC_{50}$ $2\times10^{-19}$,. 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 $IC_{50}$ $4.8\times10^{-5}$. Metrondizole 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 Sporozoa.[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] 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 protozoa 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.[13-15] Thymoquinone (TQ), an active ingredient of *Nigella sativa*, has been reported to exhibit anti-oxidant, anti-inflammatory, antimcrobial 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 Gardia lambelia* 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 *Entamoba histolytica*, *Gairdia intestinalis* and *T. vaginalis*. Five mg from the compound was dissolved in 5% dimethyl sulfoxzide (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-hydroxyethl)-2-methyl-5 nitroimidazole], a drug was used as positive control in concentration 312.5 µg/µml, 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

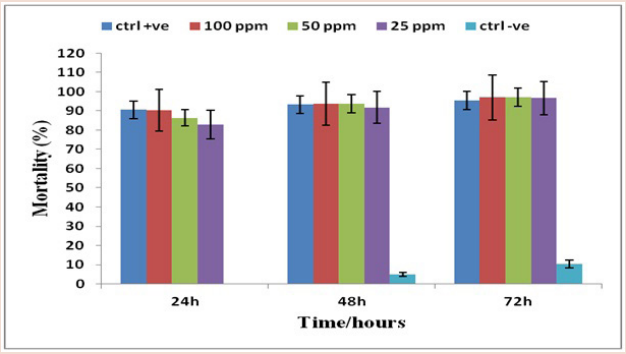


**Figure 1:** *In vitro* activity of Thymoquinone against *Giardia lambelia*; mortality percentage in relation to time

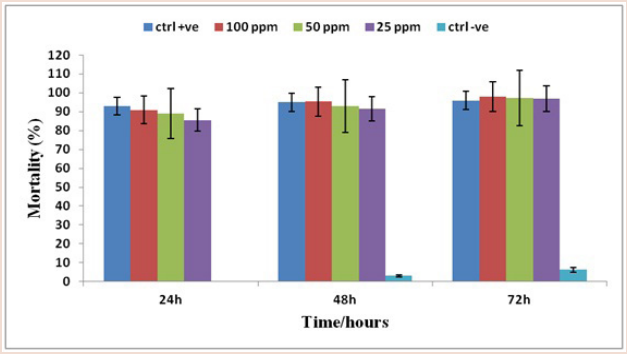


**Figure 2:** *In vitro* activity of Thymoquinone against *Entamoeba histolytica*; mortality percentage in relation to time

were mixed with Trypan blue in equal volumes The final number of parasites was determined with haemocytometer in triplicate. The mortality % of parasite for each extract activity was carried out according to the following formula:

$$\text{Mortality of parasite (\%)} = \frac{\text{Control negative- tested sample with extract} \times 100}{\text{Control negative}}$$

### *Statistical analysis*

All data were presented as means ± S.D. Statistical analysis for all the assays results were done using Microsoft excel program. Student t test was used to determine significant difference between control and plant extracts at level of $P<0.05$.

## RESULTS AND DISCUSSION

Medicinal chemists have discovered an amount of medications which can be utilized against numerous but by far not all endoparasites. A main difficulty is that many of these drugs were synthesized many years ago and some parasites have become resistant to them. The development of novel antiparasitic medications has not been much of precedence for the pharmaceutical industry because many of the parasitic diseases happen in deprived countries where the populations cannot give to pay a elevated price for the drugs. Thus an investment in drug progress against parasitic diseases is a hazardous matter. An alternative to synthetic drugs is the search for anti-parasitic plant extracts or secondary metabolites derived from them.[17-19] The current study was designed to assess the Effects of thymoquinone on *Entamoeba histolytica* and *Giardia lamblia*.

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.[13-15] Thymoquinone (TQ), an active ingredient of *Nigella sativa*, has been reported to exhibit anti-oxidant, anti-inflammatory, antimcrobial and anti-tumor activities.

Thymoquinone was tested on both *Entamoeba histolytica* and *Giardia lamblia* using the range of concentrations 0-200 µg/mL. Figure 1 indicates 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 $IC_{50}$ $2\times10^{-19}$,. 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 $IC_{50}$ $4.8\times10^{-5}$ as shown in Figure 2. 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*. Metrondizole 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.[20-22] Dietary intake of chloroquine with *N. sativa* was examined previously and found effective in malaria's mice model of *plasmodium berghei*.[22,23]

*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.[24] 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.

## ACKNOWLEDGEMENTS

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## CONFLICT OF INTEREST

Authors declare no conflict of interest.

## ABBREVIATION USED

None.

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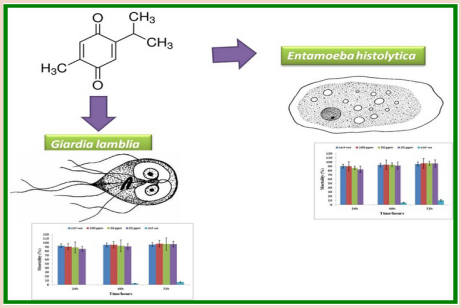
## PICTORIAL ABSTRACT




## SUMMARY


- 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*.


## ABOUT AUTHOR

**Professor Sheikh** Is the first Saudi Neurosurgeon with double specialization holding both Microvascular and Endovascular qualifications. He is the head of Saudi Association of Neurological surgeons vascular/ Endovascular Section. He acted as the program director in several Saudi regions, latest in Madinah. He is the general director of several programs, including Mohamed BinLadin Chair for Scientific Research in Prophetic Medicine, the Hijama-Cupping center, and Madinah Neurosurgery Virtual Academy (MNVA LIVE). Prof Sheikh is known to be passionate for disseminating knowledge. He obtained Master degree in Medical Education from Maastricht University, and is presently a visiting professor of medical education in Takatsuki University, Japan. He is the founder of curriculum "Prophetic medicine" at Taibah University, and is of the founder group of the Pan Arab college of Neurosurgery. He initiated and runs a periodic Micro-surgical laboratory at Madinah. Of the several International responsibilities, he is the Saudi Second Delegate for World Federation of Neurological Surgeons; Member of World Federation of Neurological Surgeons Education and Training committee; the Saudi delegate for the European Society of Neurosurgery and member of its educational committee; and is editor and reviewer in several international and national scientific journals. During his academic career he has achieved 86 publications in international journals, book chapters, book reviews, and scientific reports. He was called upon as invited speaker, Chairperson, and presenter in more than 280 international symposia. Prof Sheikh is an inventor by nature having patented inventions that were appreciated nationally and globally by the reception of several golden awards. Prof Sheikh's ideas and innovations may be followed through his Web Site and YouTube Channel.