Efficacy of *Nigella sativa* (Habbet El-Baraka) as a Treatment for Children and Adolescents Infected with *Entamoeba histolytica* Complex: A Randomized Controlled Clinical Trial

By

SAMAR F. MOHAMED¹, SHAHIRA A. AHMED², MOSLEH ABDEL RAHMAN ISMAIL¹, SAFWAT A. AHMED³, KHALID SAID HEISSAM¹,

And MOHAMED M. EIDA⁴

Department of Family Medicine¹, Department of Medical Parasitology², Faculty of Medicine, Suez Canal University, Ismailia, 45122, and Department of Pharmacognosy³, and Department of Endemic Medicine & Infectious Diseases⁴, Faculty of Medicine, Suez Canal University, Egypt (*Correspondence: shahira_ahmed@med.suez.edu.eg; Tel: 00201096237668*).

Abstract

Amoebiasis is caused by *Entamoeba histolytica* infection. In African countries amoebiasis is endemic and one of the top 10 causative agents of diarrhea in children. The common diagnostic method for *Entamoeba* spp. in Egypt is microscopic observation and identification of cysts/trophozoites in fecal samples. It is known that metronidazole (MTZ) is the choice of treatment for amoebiasis. However, drug resistance and side effects are considered to be disadvantages. In this study children and adolescents infected with *E. histolytica* complex (Eco) have been divided into two groups. As a control treatment, one group received MTZ capsules. The other group (intervention) received herbal medicinal capsules of *Nigella sativa* (N. sativa) powder to test for its effectiveness against Eco infection and compare its results with the control group. Socio-demographic data, cure rate, recession of symptoms and residual side effects of treatment were evaluated for the patients. In the intervention group, *N. sativa* showed a cure rate of 78.8% compared to 91.2% in MTZ control group with no significant difference. Eco infected patients complained about diarrhea, bloody stool and abdominal pain with significant reduction in *N. sativa*-control group symptoms (from 93.9 to 15.2 %) compared to MTZ. Treatment with MTZ caused several symptomatic residual side effects of metallic taste, anorexia, abdominal pain and diarrhea. After *N. sativa* treatment, however, no residual symptoms were noted, an indicator of its safety. *N. sativa* was safe, cost-effective, herb with potential promising effectiveness against *Eco* infection.

Keywords: Egyptian patients, *Nigella sativa*, *Entamoeba histolytica* Complex

Introduction

Amoebiasis is a parasitic infection caused by *Entamoeba histolytica* (*E. histolytica*) enteric protozoan parasite. It is one of the most significant enteropathogen worldwide and a leading cause of death after malaria and schistosomiasis, regardless of symptomaticatology (Lozano *et al*, 2012; Shirley *et al*, 2018). The disease kills 40,000-100,000 deaths worldwide each year, resulting in 30–50 million cases of colitis, amoebic liver abscess and amoebic dysentery (Lozano *et al*, 2012). All of its death could return to the invasive *E. histolytica*, however, its prevalence is overestimated. The microscopic morphological similarity to the non-pathogenic *Entamoeba dispar* (*E. dispar*) is the reason why the two species overlap (Diamond and Clark, 1993). About 10% of the world’s population is infected with *E. histolytica* complex (Eco). *E. histolytica* accounts for only 10%, but the remaining 90% return to *E. dispar* infection (Yimer *et al*, 2017).

Amoebiasis prevalence varies globally. It increased disproportionately in developing countries (tropics and subtropics) due to the poor sanitation and socioeconomic status (Fonseca *et al*, 2019).

In the developed countries, amoebiasis is generally seen in migrants from and travelling to endemic areas. However, it is endemic in the developing parts of the Central and...
South America, Asia, and Africa (Shirley et al., 2018). Across the Sub-Saharan Africa and South Asia, *E. histolytica* was one of the top ten causative agents of moderate to severe diarrhea in children under the age of five. It causes the highest hazard ratio for death in the second year of life (Kotloff et al., 2013).

Egypt led the African countries to report protozoa in their resources. *Entamoeba* spp. took fourth place among other protozoa reports analyzed (Ahmed et al., 2018). There are high rates of asymptomatic *E. histolytica* infection in the Egyptian fecal samples reaching up to 21% (Stauffer et al., 2006). In Cairo, a study using nested PCR reported a prevalence of 10.3% *E. histolytica* and 8.7% *E. dispar* in 194 cases diagnosed microscopically positive to *Eco* infection (Roshdy et al., 2017). On microscopic observation of cysts/trophozoites, Egyptian Physicians commonly prescribe systematic treatment for the amoebiasis.

Several anti-amoebic drugs are currently available. The most common drugs are derivatives of 5-nitromidazole (metronidazole and tinidazole). Other non-imidazoles drugs (nitazoxanide, paramomycin and niridazole) have also been effective against *E. histolytica*. However, these anti-amoebic drugs are less effective against the cyst than the trophozoite of *E. histolytica*. The most commonly reported side effects of these drugs were (nausea, vomiting, diarrhea, metallic taste, hypersensitivity, dizziness and anorexia). The ability of metronidazole (MTZ) to cross placental barrier also limited its use (Nagpal et al., 2012; Gonzales et al., 2019). Appearance of *E. histolytica*-drug resistance to MTZ was indicated by decreasing uptake and altering the metabolic pathway of pyruvate-oxidizing (Huang et al., 2005). Therefore, there is a need for other drugs that are more effective with lesser adverse effects.

The naturally herbal agents have been shown to possess therapeutic potential for several medical conditions. The use of herbal medicine is more prevalent in Africa and Asia indicating peoples’ perception of its safety and therapeutic efficacy (Majdalawi and Fayyad, 2016).

*Nigella sativa* (*N. sativa*) is a flowering plant, native to Middle East and Southern Europe, South and Southwest Asia, North-ern Africa particularly the Mediterranean Countries. Since ancient times, *N. sativa* has been used as a treatment for several diseases in its oil and/or extract form. The plant is used for asthma, bronchitis, diabetes, hypertension, gastrointestinal disturbances, inflammation, headache, cough, eczema, fever and dizziness (Amin and Hosseinzadeh, 2016). Its crude extract was used as appetite stimulants, bronchodilators, liver tonics, analgesics...etc. (Majdalawi and Fayyad, 2016). The oil of *N. sativa* has the anti-inflammatory, anti-oxidant, immunomodulatory, and the anti-cancer activities (Bordoni et al., 2019).

In Egypt, *N. sativa* is well known under the Arabic name of (Habbet El-Baraka or Habbah Al-Sauda). The seeds of *N. sativa* have been used as a spice and additive in bread, cookies, and other Egyptian dishes. It contains compounds, such as fixed oil (22-38%), volatile oil (0.40-1.5%), carbo- hydrates (25-40%), proteins (21-31%), vitamins (1-4%), minerals (3.7-7%), alkaloids (0.01%) and saponins (0.013%), Amin and Hosseinzadeh (2016) added that it could contribute to its biological properties. However, the biological activity of the seeds was associated with its content of thymoquinone (Bordoni et al., 2019).

As the anti-parasitic action, *N. sativa* has been reported to be effective against many parasites. Its effective potential against protozoa were demonstrated as anti-Leishmania (Mahmoudvand et al., 2015; Abamor and Allahverdiyev 2016), anti-Toxoplasma (Mady et al., 2016), anti-Plasmodium (Okeola et al., 2011; Emeka et al., 2014; JohnsonAjinwo et al, 2018), anti-Trypanosoma (Nassef et al, 2018), and anti-Blastocystis (Eida et al, 2016). It has been effective as well against several helminthes such as the anti-Schistosoma species (Ali et al, 2016), anti-
Trichinella (Abu El Ezz, 2005), and anti-hydatidosis (Mahmoudvand et al, 2014).

Most of these studies focused on in-vivo and in-vitro efficacy of the N. sativa. There are very few clinical trials but none of them tested N. sativa against Eco infection. Thus, the effect of N. sativa has been evaluated on children and adolescents with Eco infection irrespective to the present genotype.

Materials and Methods

Ethical consideration: The study was approved by the Faculty of Medicine, Suez Canal University’s ethical review committee for ethical clearance. All participants and/or their kin have given written informed consent for participation. Information of medical history sheets was explained in local language (Arabic). In all cases, the patients can withdraw their consent at any time, resulting in the prompt disposal of their derived fecal material and discontinuation of their intervention.

This study was double-blinded randomized control clinical trial conducted on young children and adolescents (ChAd) living in El-Mahsama Village, the rural west periphery of Ismailia governorate, Egypt. In the family practice center of El-Mahsama, investigators identified the criteria of eligibility. Eligible ChAd were between 6-18 years of age whose parents had given informed written consent and their stool analysis was positive for Eco infection (cysts/trophozoite). Both genders were eligible for this study.

The randomization sequence was performed by enclosing assignments that were numbered sequentially using opaque, sealed envelopes. Investigators were ensured that the envelopes were opaque when held to the light, and opened sequentially only after the name of participant and other details were written on the appropriate envelope.

Nigella sativa seeds were purchased from Egyptian herbal stores. Approximately 1000 grams of seeds were roasted in conventional oven at 180°C for 15 minutes with occasional stirring. After roasting, seeds were left to cool then grinded. Powdered N. sativa were packed in hard gelatin capsules. Each capsule contained 40 mg of N. sativa powder.

The participants were screened, selected, randomized and intervened (Fig. 1). The selected ChAd was randomly assigned to one of the two treatment groups (control and intervention). Control group received 40 mg/kg/24hrs of MTZ capsules and intervention group received capsule 40 mg/kg/24hrs of N. sativa powder (Akhtar and Riffat, 1991). Total dose for each participant was calculated according to his/her body weight. The dose was divided into 3/day for each ChAd in both groups and maintained orally for 10 days. Formulation of supplemented drugs was identical in shape, size, weight, texture, and packing. The supplemented drugs were packed in Faculty of Pharmacy, Department of Pharmacognosy.

During the follow up period, research staff administered the treatment to ChAd parents containing the respective supplements with instructions on how to administer the treatment for the remaining days.

Compliance with the supplementation regimen was determined weekly. Kin of ChAd was also subjected to a questionnaire that included demographic data, amoebiasis related clinical symptoms, associated risk factors, past history of comorbid chronic diseases, current medications, post-treatment residual symptoms for clinical improvement and reported side effects after treatment. The physician of the study ensured the quality of the data collection.

Parasitological examination: Before intervention, ChAd was provided with clean labeled plastic containers attached with applicator stick for fecal sample collection and transfer. Instructions were given regarding the collection, transfer and quantity of stool sample required. In case of urine/water contamination or small size amount, the stool specimens were refused. Stool specimens were transferred to the parasitology laboratory in El-Mahsama’s family practice center for the further processing. Blinded laborato-
ry personnel received and handled the specimens using technique of formalin-ethyl acetate to concentrate fecal samples (Cociancic et al., 2018). After several washes, the sediments were microscopically examined with wet mount and iodine stain to remove ethyl acetate. Iron-hematoxylin stain was used to identify protozoa cysts and trophozoites at 1000x and to confirm positive and negative E. coli infection (Chacín-Bonilla, 2011). Only samples with solitary E. coli infection were selected and included in this study.

After intervention, ChAd was requested to provide spoon-sized fresh stool samples on three consecutive days after the end of the treatment. The fecal samples were then re-examined (the same procedure as previously mentioned before intervention). Data was carefully documented as plus (positive) or minus (negative) recording according to the result of microscopy examination.

Planned outcome: Primary outcome measure was to investigate effectiveness of the N. sativa capsules on E. coli eradication. Secondary outcome measure was to observe improvement treatment’s clinical symptoms and reported side effects.

Exclusion criteria: ChAd was excluded from the study when they had history of conclusive seizures, hypersensitivity to MTZ or other Nitroimidazole derivatives. Exclusion included participants, who didn’t provide stool sample and participants with fecal samples contaminated with mixed infection of other parasites. Also, excluded were pregnant and breastfeeding females.

Statistical analysis: Data were collected based on the used N. sativa against cestodes in a field trial. The mean of groups was used as a substitute for calculating sample size equation (Akhtar and Riffat, 1991). Sample size was calculated to be 28 patients/group according to the formula of Dawson and Trapp (2004). Assuming a failure in of both groups’ cure rate was 10%, the final sample size would be 31 patients/group IBM SPSS Statistics V23.0 (IBM Corp., Armonk, NY, USA) was used. Comparison of variables between control and intervention groups (CoInG) was done using the chi-square test and Mann-Whitney U test.

Results
The ChAd was recruited and follow-up completed between 2015-2016. A total of 67 participants with positive E. coli stool were included in the final analysis (Fig. 1). The conventional MTZ treatment was assigned to 34 participants in control group, whereas, 33 participants were assigned to N. sativa treatment in the intervention group.

The socio-demographic characteristics of ChAd in both CoInG regarding gender, age, dwelling of domestic animal and water facilities were presented (Tab. 1). Age ranged from 6 to 18 years with mean of (11.7±4.7) years in the control group and (10±4) years in the intervention group. Patients were females (58.8%), and (57.6%) respectively in control and intervention groups. Majority of CoInG had domestic animals and about three quarters of them had facility to clear water supply (tap water).

The details are given in tables (1 & 2) and figures (1, 2, 3 & 4).
Table 1: Comparative socio-demographic data of both control and intervention groups.

<table>
<thead>
<tr>
<th>Variants</th>
<th>Control (n =34)</th>
<th>Intervention (n =33)</th>
<th>Test value</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>14 (41.2)</td>
<td>14 (42.4)</td>
<td>0.011*</td>
<td>0.918</td>
</tr>
<tr>
<td>Female</td>
<td>20 (58.8)</td>
<td>19 (57.6)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age in years: 6-&lt;10</td>
<td>13 (38.2)</td>
<td>17 (51.5)</td>
<td>1.835*</td>
<td>0.4</td>
</tr>
<tr>
<td>10-&lt;14</td>
<td>9 (26.5)</td>
<td>9 (27.3)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>14-18</td>
<td>12 (35.3)</td>
<td>7 (21.2)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>11.7 ± 4.7</td>
<td>10 ± 4</td>
<td>453**</td>
<td>0.171</td>
</tr>
<tr>
<td>Domestic animals: Yes</td>
<td>30 (88.2)</td>
<td>23 (69.7)</td>
<td>3.482*</td>
<td>0.062</td>
</tr>
<tr>
<td>No</td>
<td>4 (11.8)</td>
<td>10 (30.3)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Source of water: Tap</td>
<td>26 (76.5)</td>
<td>23 (69.7)</td>
<td>0.4188*</td>
<td>0.811</td>
</tr>
<tr>
<td>Pump</td>
<td>7 (20.6)</td>
<td>9 (27.3)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Buying containers</td>
<td>1 (2.9)</td>
<td>1 (3)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Water quality: Clear</td>
<td>28 (82.4)</td>
<td>28 (84.8)</td>
<td>0.128</td>
<td>0.938</td>
</tr>
<tr>
<td>Yellow</td>
<td>4 (11.8)</td>
<td>3 (9.1)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* p<0.05; *Chi-square test; **Mann Whitney U test;

Pre-treatment clinical symptoms of the CoInG: Majority of the CoInG had associated symptoms related to the Eco infection, whereas the other infected patients had no symptoms at all. Medical history taken has excluded symptoms of other diseases. Most of the CoInG had symptoms (diarrhea, bloody stool and abdominal pain) with in the control group 79.4% and intervention group 93.9%. The asymptomatic patients were the lowest percentages, with 20.6% in the control group and 6.1% in the intervention group.

In CoInG’s symptomatic patients, abdominal pain was the commonest symptom with (44.1%) in control group & (69.7%) in the intervention group. In CoInG, diarrhea and bloody stools represented a small proportion of (2.9%) and (3%) respectively (Tab. 2). The difference in the clinical symptoms between the CoInG was not significant (P=0.150) (Fig. 2a).

Table 2: Frequency of pre-treatment clinical symptoms in both symptomatic patients groups.

<table>
<thead>
<tr>
<th>Category</th>
<th>Clinical symptoms</th>
<th>Control (n=34)</th>
<th>Intervention (n=33)</th>
<th>Test value</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>No symptoms</td>
<td>No</td>
<td>7</td>
<td>2</td>
<td>5.9</td>
<td>0.20</td>
</tr>
<tr>
<td>one symptom</td>
<td>Abdominal pain</td>
<td>15 (44.1)</td>
<td>23 (69.7)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Diarrhea</td>
<td>1 (2.9)</td>
<td>1 (3)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Bloody stool</td>
<td>0 (0)</td>
<td>1 (3)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>two symptoms or more</td>
<td>Abdominal pain, Diarrhea, Vomiting, Fever</td>
<td>11 (32.4)</td>
<td>6 (18.2)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Chi square test, statistically significant at p <0.05

Post-treatment cure rate of both CoInG: The cure rate and the eradication of cysts/trophozoites was estimated by stool microscopic examination of wet mount and iron-hematoxylin stained fecal samples obtained on three consecutive days post-treatment. The Eco eradication cure rate was higher in the MTZ-control group (91.2%) compared to N. sativa- intervention group (78.8%), but the difference was statistically not significant (Fig. 3).

Post-treatment of clinical symptoms of the CoInG: Clinical symptoms in N. sativa- intervention group compared to controls with significant difference (P=0.001). Improvement was noted by the decline in the patients’ clinical symptoms previously mentioned in section 2 with 15.2% in the MTZ-controls and 84.8% in N. sativa- intervention group (Fig. 2b).

The N. sativa intervention group didn’t complain about any side effects, however the MTZ-control group complained about metallic taste and anorexia with high statis-
cally significant difference \((P = 0.000)\).

The frequently side effects were metallic taste in 11/34 (32.35%) followed by anorexia in 6/34 (17.6%) of MTZ- controls. Five patients developed diarrhea and abdominal pain as side effects of MTZ although; they were asymptomatic at pre-treatment phase.

Number needed to be treated (NNR): In CoInG, the NNR indicates that there are three patients need to be treated with \(N. sativa\) capsules 40mg/kg/24hrs/10 days to prevent adverse outcome of \(Eco\) infection. Considering the evaluated factors (cure rate, clinical symptoms improvement and residual side effects), \(N. sativa\) was an effective drug.

**Discussion**

Amoebiasis is one of the most common infections in humans’ worldwide (Shirley et al, 2018). Developing countries are rich environment of risk factors that could facilitate infection with this disease (Jr et al, 2009; Speich et al, 2016).

In this study, ChAd came from rural areas, and domestic animals were breaded almost in every house. Young ChAd was the most affected group and even lesser age was observed in other studies. By two years of age, near to 80% of infants living in slum urban areas of Bangladesh, had been found infected with \(E. histolytica\) (Gilchrist et al, 2016). The malnutrition could be the reason of raising the \(Eco\) infection in this age category (Mondal et al, 2012).

In Egyptian rural areas household wastewater passed directly into irrigation channels and human and animal excreta are intensely used as fertilizer in agriculture, contaminating water supplies and increasing the unsanitary conditions. They live in the poor sanitation, poor education, overcrowding and poverty, and more vulnerable to get infected with \(Eco\) which is transmitted via the fecal-oral route (CDC, 2017). The presence of domestic animals and direct contact with them could increase the risk of infection (Pham Duc et al, 2011). Amoebiasis can also be easily transmitted from person to person (Akhtar et al, 2016). The rural areas facilitate the \(Eco\) infection development and endemicity (Pham Duc et al, 2011; Akhtar et al, 2016; Gillespie and Bradbury, 2017; Villamizar et al, 2019).

In this study, asymptomatic and symptomatic CoInG were associated with \(Eco\) infection, which abdominal pain was the commonest symptom. The present results agreed others as to the \(Eco\) exposure between the asymptomatic and symptomatic individuals (Shirley et al, 2018; Miller et al, 2019; Bahrami et al, 2019; Fonseca et al, 2019).

\(E. histolytica\) is commonly associated with clinical symptoms; but \(E. dispar\) is commonly believed to be a non-virulent species (Ximénez et al, 2011; Oliveira et al, 2015). At Beni-Suef, Egypt, patients with \(E. histolytica\) infection were all associated with mucoid and bloody stool making these symptoms predictors for this protozoan species (Ibrahim et al, 2015).

In Egypt, diagnosis of Entamoeba species depends on the laboratory microscopic detection and the identification of cysts/ trophozoite in the human feces. Other tests to distinguish species are not available due to practical and financial limitations. A status requires the treatment of symptomatic individuals regardless of the underlying causative species. For this study results further molecular differentiation between \(Eco\) species will be required to explain the real association of symptomatology and the main causative species.

Indiscriminate use of MTZ derivatives has been reported to increase the minimum inhibitory concentration against \(E. histolytica\) with possibility of resistance to the drug (Nagpal et al, 2012). Drug unsuitability, delayed diagnosis and continued morbidity (Gonzales et al, 2019) make it imperative to investigate other substances for treatment.

In this study, \(N. sativa\) powder capsules were used as MTZ-alternative treatment herbal agent against \(Eco\) infection. Capsules of \(N. sativa\) powder reached a cure rate of 78.8% compared to 91.2% of the MTZ. Although MTZ produced higher cure rate of
Eco infection compared to N. sativa, the statistical difference was not significant. N. sativa extract (ethanolic/ aqueous) and oil were used in many studies (single/combined) and reported higher efficacy as anti/protozoa and anti-helminthic.

Crude extract of N. sativa showed potent in vitro activity against trophozoites of E. histolytica and G. lamblia with verified safety evidence for use (Kabbashi et al. 2017). N. sativa oil combined with silver doped titanium dioxide nanoparticles had significant anti-leishmanial potential with demonstrated killing effects on the Leishmania tropica amastigote (Abamor and Allahverdiyev, 2016). Another combination of the N. sativa oil and cisplatin showed trypanocidal effect against Trypanosoma evansi preserving vital organs functions and architecture (Nassef et al, 2018). As anti-Toxoplasma, the combined formula of N. sativa oil and pyrimethamine resulted in a significant increase in mice survival rate and a decrease in parasite density (Mady et al. 2016). Aqueous extract of N. sativa had inhibitory effect of Blastocystis hominis in vitro and prevented pathological changes in vivo (Eida et al, 2016). Ethanol extract and oil of N. sativa have also proven to be the promising anti-helminthic (Schistosoma species and hydatid cyst) substances (Mahmoudvand et al, 2014; Ali et al, 2016).

The inferiority of this study results with N. sativa could be explained by the use of seed powder rather than extract or oil form. In Arabic countries N. sativa seeds powder is usually used as flavoring additive due to its known bioactive compounds (i.e. thymoquinone, tocopherol, β-carotene, vitamin A and C...etc.) in the seed (Bordoni et al, 2019). It is also known for its safety and its lack of toxicity (Karimi et al, 2019). Cost and availability in developing countries are considered to be important factors. N. sativa seeds are present for the affordability of Egyptians in almost all spice dealers shops with cheap cost.

In this study, improvement in the symptoms of patients treated with N. sativa was noted with a significant decrease in the percentage of the clinical symptoms from 93.9 to 15.2 %. However, the MTZ group showed lesser response (symptoms decreased from 79.4 to 52.9%). Since N. sativa has not yet been tested with parasitic infection in humans, it would be difficult to compare current study results with others. Nevertheless, N. sativa in other diseases showed symptomatic improvements in patients with chronic allergic disorders (Koshak, 2019), hepatic steatosis (Kho-nche et al, 2019) and chronic rhinosinusitis (Rezaelian and Amoushahi Khouzani, 2018). It has also been reported that the experimental effect of N. sativa decreases the pathological insult of intestine, liver, spleen and vital organs of mice infected with B. hominis, Toxoplasma and Trypanosoma (Eida et al, 2016; Mady et al, 2016; Nassef et al, 2018).

Various pharmacological activities of N. sativa have been reported on various studies verifying its therapeutic efficacy against diverse human diseases (Ahmad et al, 2013). N. sativa modulates the action of antioxidant enzymes in different organs to act as reactive oxygen species (scavengers). Thymoquinone, the main bioactive component of N. sativa can remove and inhibit various toxic compounds of food products (Karimi et al, 2019). As an anti – parasitic, N. sativa methanolic extract improved the antioxidant status in red blood cells due to effect of Plasmodium yoelli (Okeola et al, 2011). N. sativa seeds have caused oxidative stress to the adult worms of Schistosoma by decreasing the activity of its anti-oxidant enzymes, making the parasite vulnerable to host damage (Mohamed et al, 2005).

The associated unpleasant adverse effects of MTZ conventional doses (headaches, metallic taste, and loss of appetite, nausea, dizziness and vomiting) were known (Gonzales et al, 2019). The unpleasant adverse effects affect the compliance of the patient’s treatment, which usually discontinues the opti-
mum dose, resulting in no complete eradication of parasitic stages and resistance development of parasites.

However, N. sativa treated group did not complain about any adverse effects. Several articles have shown N. sativa lack of toxicity (Karimi et al, 2019) with the safety of its ethanolic extract in the Vero cell line using MTT-assay (Kabbashi et al, 2017).

Different forms of the N. sativa and its other components (thymoquinone, alphaned erin..., etc.) can be combined with other existing pharmaco-therapeutic agents to effectively treat various infectious diseases and to overcome the appearance of resistance problems.

Conclusion

Nigella sativa (= the black seed) powder proved to be effective in eradicating the Entamoeba histolytica complex infection. The patients cure was achieved through retraction of the symptoms with no side effects of N. sativa.

Using N. sativa extracts in a safe form to human would be beneficial for achieving full cure rate from Eco infection.

Further studies of the molecular genotype of Eco species would be necessary to realize its association with symptomatology of patients. N. sativa should be used as a supplement in daily diet to ensure protection from parasitosis and inhibit side effects of food toxins.

Acknowledgements

The authors would like to introduce their great thanks to Professor Dr. Panagiotis Karanis, Medical Faculty, University of Cologne, Cologne, Germany for his kind critical review of this study.

Conflict of interest: The authors have neither conflict of interest nor received funds

References


Miller, HW, Suleiman, RL, Ralston, KS, 2019: Trogocytosis by Entamoeba histolytica


Explanation of figures

Fig. 1: Profile of the trial
Fig. 2: Clinical symptoms of CoInG pre and post-treatment (TTT)
Fig. 3: Post-treatment percentage of cure rate in CoInG.
Fig. 4: Nigella sativa (Black Seeds/Black Cumin Seeds) Family Ranunculaceae (adopted from Google)
399

Screened with light microscopy

Positive for *Entamoeba histolytica* complex

Randomization

16 excluded, non compliant to treatment

MTZ - Control
50 received treatment

*N. saizia* - Intervention
47 received treatment

14 excluded, non compliant to treatment

34 completed the study

33 completed the study

A Pre-TTT

<table>
<thead>
<tr>
<th>% of clinical symptoms</th>
<th>Control</th>
<th>Intervention</th>
</tr>
</thead>
<tbody>
<tr>
<td>No symptoms</td>
<td>20.6</td>
<td>6.1</td>
</tr>
<tr>
<td>Symptoms</td>
<td>79.4</td>
<td>93.9</td>
</tr>
</tbody>
</table>

B Post-TTT

<table>
<thead>
<tr>
<th>% of clinical symptoms</th>
<th>MTZ - Control</th>
<th><em>N. saizia</em> - Intervention</th>
</tr>
</thead>
<tbody>
<tr>
<td>No symptoms</td>
<td>47.1</td>
<td>84.8</td>
</tr>
<tr>
<td>Symptoms</td>
<td>52.9</td>
<td>15.2</td>
</tr>
</tbody>
</table>