Journal of the Egyptian Society of Parasitology, Vol.49, No. 2, August 2019
J. Egypt. Soc. Parasitol. (JESP), 49(2), 2019: 389 – 400

EFFICACY OF *NIGELLA SATIVA* (HABBET EL-BARAKA) AS A TREATMENT FOR CHILDREN AND ADOLESCENTS INFECTED WITH *ENTAMOEBA HISTOLYTICA* COMPLEX: A RANOMIZED CONTROLLED CLINICAL TRIAL

By

SAMAR F. MOHAMED[1], SHAHIRA A. AHMED[2*], MOSLEH ABDEL RAHMAN ISMAIL[1], SAFWAT A. AHMED[3], KHALID SAID HEISSAM[1], And MOHAMED M. EIDA[4]

Department of Family Medicine[1], Department of Medical Parasitology[2], Faculty of Medicine, Suez Canal University, Ismailia, 45122, and Department of Pharmacognosy[3], and Department of Endemic Medicine & Infectious Diseases[4], 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 (*E*co) 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 *E*co 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. *E*co 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. sati*va was safe, cost-effective, herb with potential promising effectiveness against *E*co 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 symptomatology (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 (*E*co). *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 *E*co 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 (Majdalawieh 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. (Majdalawieh 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 *E*co infection. Thus, the effect of *N. sativa* has been evaluated on children and adolescents with *E*co 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 *E*co 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*co infection (Chacín-Bonilla 2011). Only samples with solitary *E*co 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*co 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 28patients/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 31patients/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*co 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.

Variants	Control (n =34) No. (%)	Intervention (n =33) No. (%)	Test value	*p*-value
Male	14 (41.2)	14 (42.4)	0.011^{+}	0.918
Female	20 (58.8)	19 (57.6)		
Age in years: 6-<10	13 (38.2)	17 (51.5)	1.835^{+}	0.4
10- <14	9 (26.5)	9 (27.3)		
14- 18	12 (35.3)	7 (21.2)		
Mean ± SD	11.7 ± 4.7	10 ± 4	453^{++}	0.171
Domestic animals: Yes	30 (88.2)	23 (69.7)	3.482^{+}	0.062
No	4 (11.8)	10 (30.3)		
Source of water: Tap	26 (76.5)	23 (69.7)	0.4188^{+}	0.811
Pump	7 (20.6)	9 (27.3)		
Buying containers	1 (2.9)	1 (3)		
Water quality: Clear	28 (82.4)	28 (84.8)	0.128^{+}	0.938
Turbid	2 (5.9)	2 (6.1)		
Yellow	4 (11.8)	3 (9.1)		

* $p<0.05$; $^{+}$Chi-square test; $^{++}$Mann Whitney U test;

Pre-treatment clinical symptoms of the CoInG: Majority of the CoInG had associated symptoms related to the *E*co 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.

Category	Clinical symptoms	Control (n =34) No. (%)	Intervention (n= 33) No. (%)	Test value*	*p*-value
No symptoms	No	7	2	5.9	0.20
one symptom	Abdominal pain	15 (44.1)	23 (69.7)		
	Diarrhea	1 (2.9)	1 (3)		
	Bloody stool	0 (0)	1 (3)		
Two symptoms or more	Abdominal pain, Diarrhea, Vomiting, Fever	11 (32.4)	6 (18.2)		

*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 *E*co 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 statisti-

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 *E*co 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 *E*co infection in this age category (Mondal *et al,* 2012).

In Egyptian rural areas household wastewater passed directed 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 *E*co 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 *E*co 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 *E*co infection, which abdominal pain was the commonest symptom. The present results agreed others as to the *E*co 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 *E*co 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 *E*co 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

*E*co 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). Ethanolic 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, tocopherols, 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 (Rezaeian 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 anti-oxidant 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 anti-oxidant 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, alphahed 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 *E*co infection.

Further studies of the molecular genotype of *E*co 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

Abamor ES, Allahverdiyev, AM, 2016: A nanotechnology based new approach for chemotherapy of cutaneous leishmaniasis: TIO2 @AG nanoparticles: *Nigella sativa* oil combinations. Exp Parasitol 166:150-63.

Abu El Ezz, NMT, 2005: Effect of *Nigella sativa* and *Allium cepa* oils on *Trichinella spiralis* in experimentally infected rats. J. Egypt. Soc. Parasitol. 35, 3:511-23

Ahmad, A, Husain, A, Mujeeb, M, *et al*, 2013: A review on therapeutic potential of *Nigella sativa*: A miracle herb. Asian Pac. J. Trop. Biomed. 3:337-52.

Ahmed, SA, Guerrero, Flórez, M, Karanis, P, 2018: The impact of water crises and climate changes on the transmission of protozoan parasites in Africa. Pathog. Glob. Hlth. 112:281-93.

Akhtar, M, Riffat, S, 1991: Field trial of *Saussurfa lappa* roots against nematodes and *Nigelia sativa* seeds against cestodes in children. J. Pakist. Med. Assoc. 41:185-7

Akhtar, T, Khan, AG, Ahmed, I, *et al*, 2016: Prevalence of amoebiasis in a model research community and its confirmation using stool antigen ELISA for *Entamoeba histolytica*. Pak. J. Pharm. Sci. 29:1587-90

Ali, M, Eldahab, MA, Mansour, HA, Nigm, A, 2016: *Schistosoma mansoni*: Antiparasitic effects of orally administered *Nigella sativa* oil and/or *Chroococcus turgidus* extract. Acta Biol. Hung. 67:247-60.

American Academy of Pediatrics, 2015: Red Book: 2015 Report of the committee on infectious diseases RED BOOK®, 30th edition. American Academy of Paediatrics, 141 Northwest Point Blvd Elk Grove Village, IL 60007-1019.

Amin, B, Hosseinzadeh, H, 2016: Black cumin (*Nigella sativa*) and its active constituent, thymoquinone: An overview on the analgesic and anti-inflammatory effects. Planta Med. 82:8-16.

Bahrami, F, Haghighi, A, Zamini, G, Khademerfan, M, 2019: Differential detection of *Entamoeba histolytica*, *Entamoeba dispar* and *Entamoeba moshkovskii* in fecal samples using nested multiplex PCR in west of Iran. Epidemiol. Infect. 147:e96-9.

Bordoni, L, Fedeli, D, Nasuti, C, *et al*, 2019: Antioxidant and anti-inflammatory properties of the *Nigella sativa* oil in human pre-adipocytes. Antioxidants 8:E51-6.

CDC, 2017: Amebiasis (*Entamoeba histolytica*). www.cdc.gov/parasites/.

Chacín-Bonilla, L, 2011: Microscopic diagnosis of amebiasis: an obsolete method but necessary in the developing world. Invest. Clin. 52:291-4.

Cociancic, P, Rinaldi, L, Zonta, ML, Navone, GT, 2018: Formalin-ethyl acetate concentration, FLOTAC Pellet and anal swab techniques for the diagnosis of intestinal parasites. Parasitol. Res. 117:3567-73.

Dawson, B, Trapp, R, 2004: Basic and Clinical Biostatistics. In: McGraw-Hill (Ed.) Basic & Clinical Biostatistics, 4th edition. USA.

Diamond, LS, Clark, CG, 1993: A redescription of *Entamoeba histolytica* Schaudinn, 1903 (Emended Walker, 1911) separating it from *Entamoeba dispar* Brumpt, 1925. J. Eukaryot. Microbiol. 40:340-4

Eida, O, El-Shafei, H, Nomeir, Y, El Safhi, M, 2016: *In vivo* and *in vitro* efficacy of *Nigella sativa* aqueous extract on *Blastocystis hominis*. J. Egypt. Soc. Parasitol. 46, 1:27-34.

Emeka, P, Badger-Emeka, L, Eneh, C, Kh-an, T, 2014: Dietary supplementation of chloroquine with *nigella sativa* seed and oil ex-tracts in the treatment of malaria induced in mice with *Plasmodium berghei*. Pharmacogn. Maga. 10: 357-62.

Fonseca, Z, Uribe-Querol, E, Díaz-Godínez, C, *et al,* 2019: Pathogenic *Entamoeba histolytica*, but not *Entamoeba dispar*, induce neutrophil extracellular trap (NET) formation. J. Leukoc. Biol. Epub. ahead of print.

Gilchrist, CA, Petri, SE, Schneider, BN, *et al,* 2016: Role of the gut microbiota of the children in diarrhea due to the protozoan parasite *Entamoeba histolytica*. J. Infect. Dis. 213: 1579-85.

Gillespie, S, Bradbury, RS, 2017: A Survey of intestinal parasites of domestic dogs in Central Queensland. Trop. Med. Infect. Dis. 2:E60-4.

Gonzales, MLM, Dans, LF, Sio-Aguilar, J, 2019: Antiamoebic drugs for treating amoebic colitis. Cochrane Database Syst. Rev. 1: CD006085.

Huang, B, Vetting, MW, Roderick, SL, 2005: The active site of O-acetylserine sul-fhydrylase is the anchor point for bienzyme complex formation with serine acetyltrans- ferase. J. Bacteriol. 187:3201-5.

Ibrahim, SS, El-Matarawy, OM, Ghieth, M A, *et al,* 2015: Copro prevalence and estimated risk of *Entamoeba histolytica* in diarrheic patients at Beni-Suef, Egypt. World J. Microbiol. Biotechnol. 31:385-90.

Johnson-Ajinwo, OR, Ullah, I, Mbye, H, *et al,* 2018: The synthesis and evaluation of thymoquinone analogues as anti-ovarian cancer and antimalarial agents. Bioorg. Med. Chem. Lett. 28:1219-22.

Jr, PWA, Mondal, D, Peterson, KM, *et al,* 2009: Association of malnutrition with amebiasis. Nutr. Rev. 67:S207-15.

Kabbashi, A, Osman, EE, Garbi, MI, *et al,* 2017: The *In vitro* antiprotozoal activities and cytotoxicity of selected Sudanese Medicinal Plants. Int. J. Biomed. Eng. Clin. Sci. 3:6-13.

Karimi, Z, Alizadeh, A, Dolatabadi, J, Dehghan, P, 2019: *Nigella sativa* and its derivatives as food toxicity protectant agents. Adv. Pharm. Bull. 9:22-37.

Khonche, A, Huseini, HF, Gholamian, M, *et al,* 2019: Standardized *Nigella sativa* seed oil ameliorates hepatic steatosis, aminotrans-ferase and lipid levels in non-alcoholic fatty liver disease: A randomized, double-blind and placebo-controlled clinical trial. J. Ethnopharmacol 234:106-11.

Koshak, A, 2019: Prevalence of herbal medicines in patients with chronic allergic dis-orders in Western Saudi Arabia. Saudi Med. J. 40:391-6.

Kotloff, KL, Nataro, JP, Blackwelder, WC, *et al,* 2013: Burden and aetiology of dia-rrhoeal disease in infants and young children in developing countries (the Global Enteric Multicenter Study, GEMS): A prospective, case-control study. Lancet 382:209-22.

Lozano, R, Naghavi, M, Foreman, K, *et al,* 2012: Global and regional mortality from 235 causes of death for 20 age groups in 1990 and 2010: A systematic analysis for the Global Burden of Disease Study 2010. Lancet 380: 2095-128.

Mady, RF, El-Hadidy, W, Elachy, S, 2016: Effect of *Nigella sativa* oil on experimental toxoplasmosis. Parasitol. Res. 115:379-90.

Mahmoudvand, H, Asadi, A, Harandi, MF, *et al,* 2014: The *in vitro* lethal effects of various extracts of *Nigella sativa* seed on hydatid cyst protoscoleces. Iran. J. Basic Med. Sci. 17: 1001-6.

Mahmoudvand, H, Tavakoli, R, Sharififar, F, *et al,* 2015: Leishmanicidal and cytotoxic activities of *Nigella sativa* and its active principle, thymoquinone. Pharm. Biol. 53:1052-7.

Majdalawieh, AF, Fayyad, MW, 2016: Recent advances on the anti-cancer properties of *Nigella sativa*, a widely used food additive. J. Ayurveda Integr. Med. 7:173-80.

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

mediates acquisition and display of human cell membrane proteins and evasion of lysis by human serum. M-Bio. 10:e00068-19.

Mohamed, AM, Metwally, NM, Mahmoud, S S, 2005: Sativa seeds against *Schistosoma mansoni* different stages. Mem. Inst. Oswaldo Cruz. 100:205-11.

Mondal, D, Minak, J, Alam, M, *et al*, 2012: Contribution of enteric infection, altered intestinal barrier function, and maternal malnutrition to infant malnutrition in Bangladesh. Clin. Infect. Dis. 54:185-92.

Nagpal, I, Raj, I, Subbarao, N, Gourinath, S, 2012: Virtual screening, identification and *in vitro* testing of novel inhibitors of O-acetyl-L-serine sulfhydrylase of *Entamoeba histo-lytica*. PLoS One 7:e30305.

Nassef, NAEM, El-Melegy, MA, Beshay, E V, *et al*, 2018: Trypanocidal effects of cis-platin alone and in combination with *Nigella sativa* oil on experimentally infected mice with *Trypanosoma evansi*. Iran. J. Parasitol. 13:89-99.

Okeola, VO, Adaramoye, OA, Nneji, CM, *et al*, 2011: Antimalarial and antioxidant acti-vities of methanolic extract of *Nigella sativa* seeds (black cumin) in mice infected with *Plasmodium yoelli nigeriensis*. Parasitol. Res. 108:1507-12.

Oliveira, FS, Neumann, E, Gomes, M, Caliari, M, 2015: *Entamoeba dispar*: Could it be pathogenic. Trop. Parasitol. 5:9-14.

Pham Duc, P, Nguyen-Viet, H, Hattendorf, J, *et al*, 2011: Risk factors for *Entamoeba histolytica* infection in an agricultural community in Hanam province, Vietnam. Parasit. Vectors 4:102-6.

Rezaeian A, Khouzani, S, 2018: Effect of the *Nigella sativa* nasal spray on the treatment of chronic rhinosinusitis without a nasal polyp. Allergy Rhinol. 9:215265671880005.

Roshdy, MH, Abd El-Kader, NM, Ali-Tammam, M, *et al*, 2017: Molecular diagnosis of *Entamoeba* spp. versus microscopy in the Great Cairo. Acta Parasitol. 62:188-91.

Shirley, DAT, Farr, L, Watanabe, K, Moonah, S, 2018: A review of the global burden, new diagnostics, and current therapeutics for amebiasis. Open forum Infect. Dis. 5:ofy161.

Speich, B, Croll, D, Fürst, T, *et al*, 2016: Effect of sanitation and water treatment on intestinal protozoa infection: A systematic review and meta-analysis. Lancet Infect. Dis. 16: 87-99.

Stauffer, W, Abd-Alla, M, Ravdin, JI, 2006: Prevalence and incidence of *Entamoeba histolytica* infection in South Africa and Egypt. Arch. Med. Res. 37:265-8.

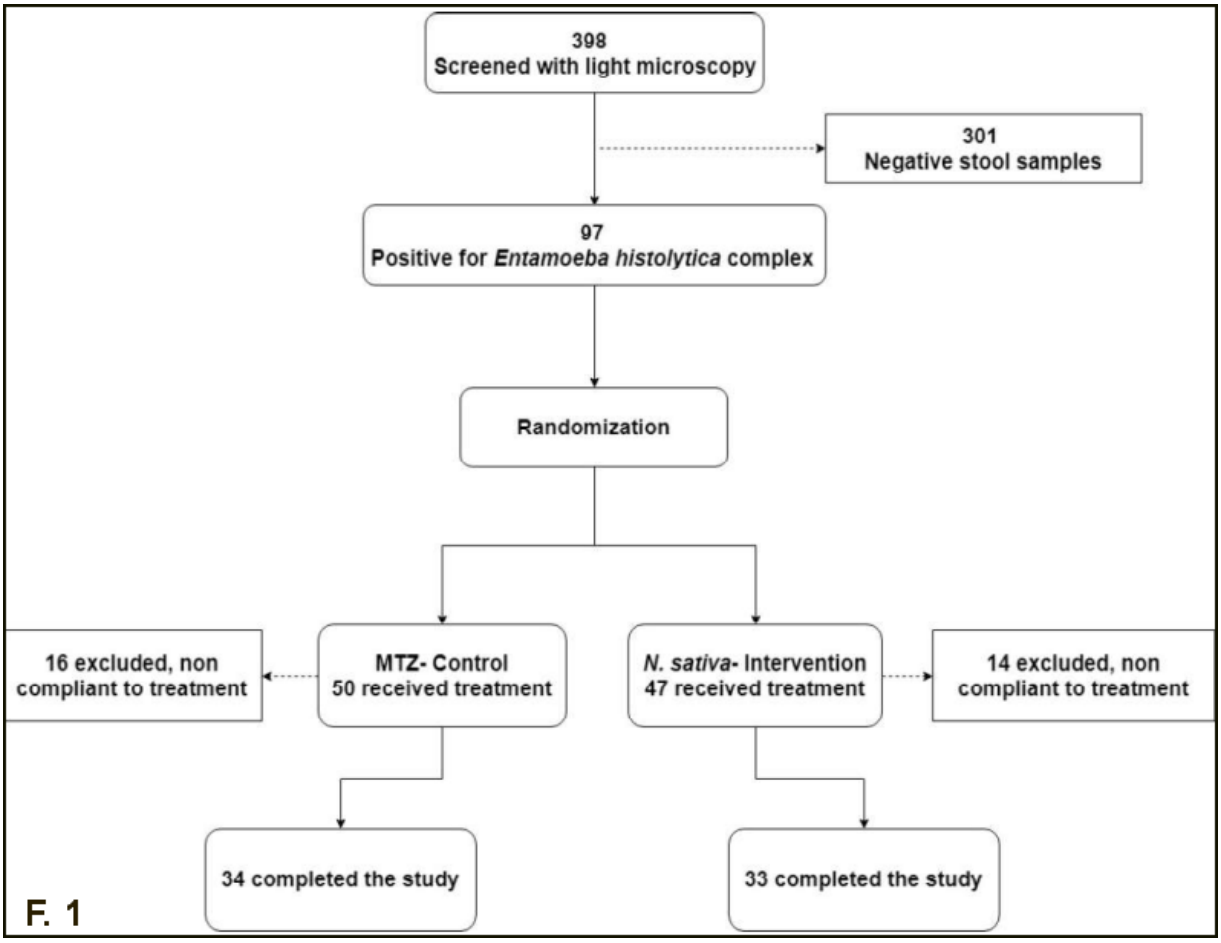
Villamizar, X, Higuera, A, Herrera, G, *et al*, 2019: Molecular and descriptive epidemiology of intestinal protozoan parasites of children and their pets in Cauca, Colombia: A cross-sectional study. BMC Infect. Dis. 19:190-8.

Ximénez, C, Morán, P, Rojas, L, *et al*, 2011: Novelties on amoebiasis: a neglected tropical disease. J. Glob. Infect. Dis 3:166-74.

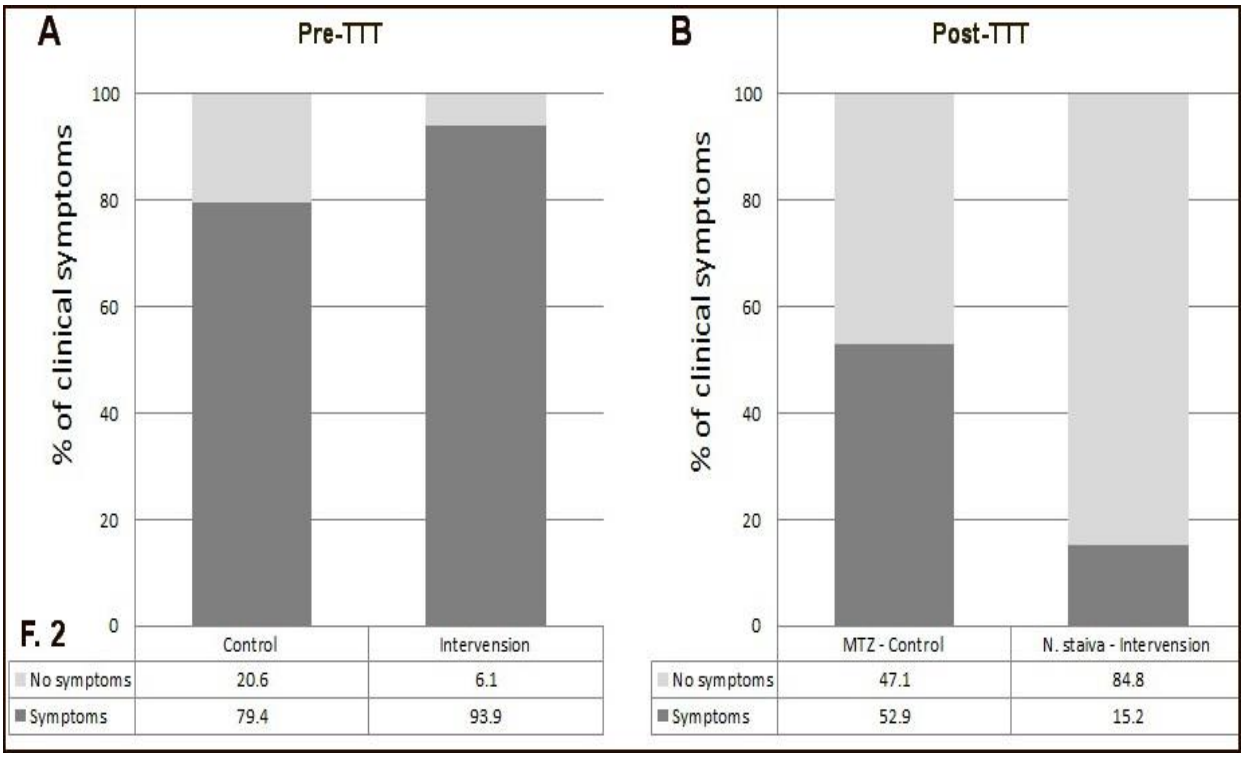
Yimer, M, Zenebe, Y, Mulu, W, *et al*, 2017: Molecular prevalence of *Entamoeba histolytica*/*dispar* infection among patients attending four health centres in northwest Ethiopia. Trop. Doct. 47:11-5.

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)



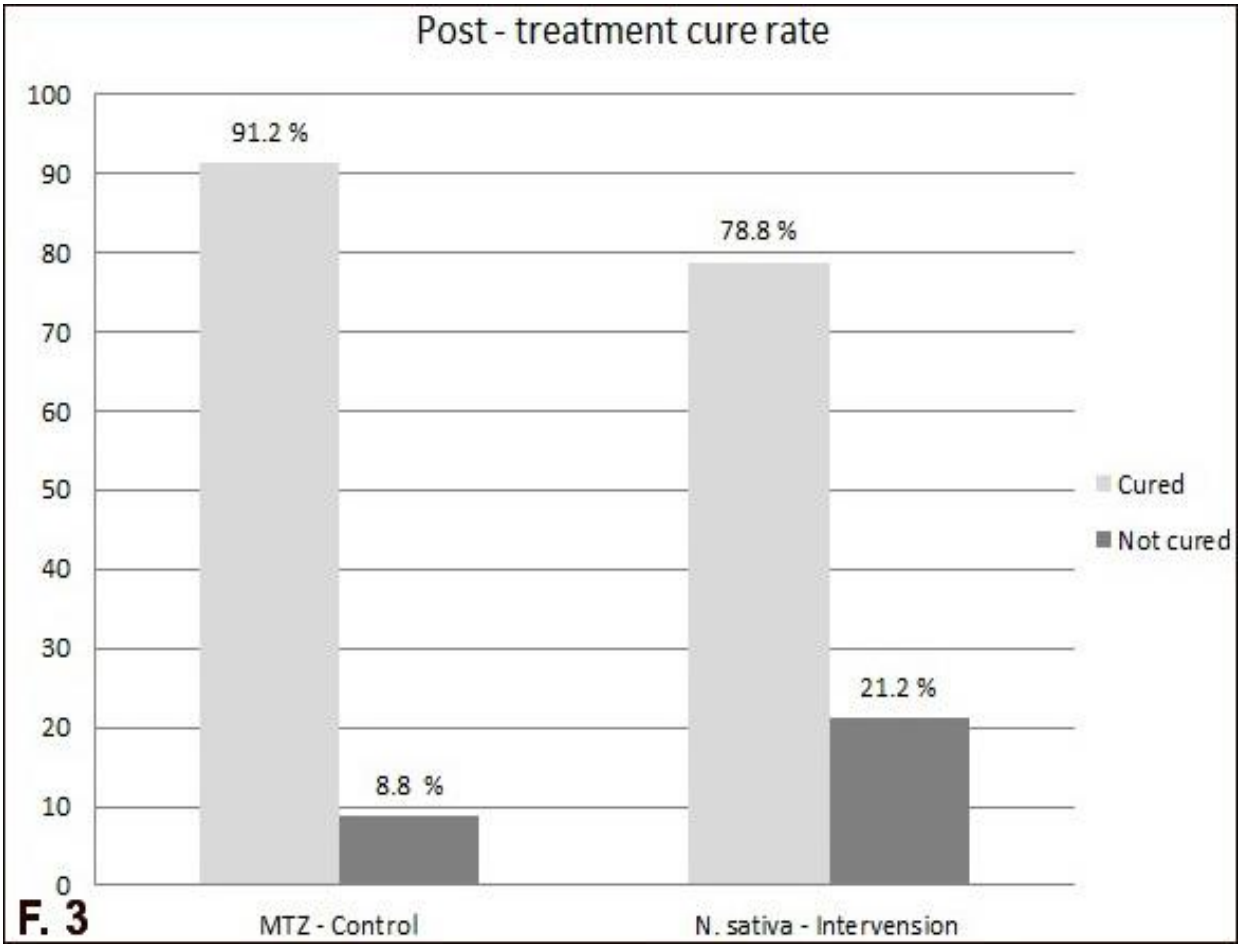
F. 1



F. 2

	Control	Intervension
No symptoms	20.6	6.1
Symptoms	79.4	93.9

	MTZ - Control	N. staiva - Intervension
No symptoms	47.1	84.8
Symptoms	52.9	15.2


Post - treatment cure rate
100
90
80
70
60
50
40
30
20
10
0
91.2 %
8.8 %
78.8 %
21.2 %
Cured
Not cured
MTZ - Control
N. sativa - Intervension
F. 3


Jiji ng
F. 4