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Brief Original Article

Evaluation of the effect of some medicinal plants on cultured *Trichomonas Vaginalis*

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Abstract

Introduction: *Trichomoniasis* is a worldwide sexually transmitted disease caused by *Trichomonas vaginalis.* It inflicts severe complications to the human genitourinary system. The devastating negative effects and the emergence of resistance to common medication impose the search for safer and effective alternatives. This research aimed to investigate the effect of the *Allium sativum*, *Nigella sativa* crude extracts (NsCE) and the combination between their most effective doses with metronidazole.

Methodology: Vaginal swabs were obtained from symptomatic patients, and cultured on Diamond's medium. Assessment of various concentrations of these herbs at different follow-up periods was done by counting the number of dead *T. vaginalis* trophozoites using the hemocytometer and trypan blue staining. Transmission electron microscope study was done.

Results: NsCE 9 mg/mL yielded the highest lethal effect on *T. vaginalis* trophozoites after 72 hours, compared with metronidazole. Combination of NsCE 9 mg/mL and metronidazole 50 µg/mL gave the best result. Additionally, Tomex90 µg/mL, represents a tolerable effect after 72 hours, but metronidazole 100 µg/mL still has higher effect. These results were confirmed by the ultrastructural changes observed in *T. vaginalis* trophozoites, signifying severe damage of nucleus and cytoplasm with large vacuolization and cell membrane defects.

Conclusions: NsCE is a promising anti-*Trichomonas* especially its combination with metronidazole which showed a high synergistic effect.

Key words: *Trichomonas vaginalis*; *Allium sativum*; *Nigella sativa*.

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Introduction

Trichomoniasisis a common worldwide sexually transmitted disease caused by the flagellated protozoan *Trichomonas vaginalis* (*T. vaginalis*) [1]. It can be considered as a re-emerging infectious disease [2]. The prevalence of trichomoniasis estimated between 2012 and 2016 by WHO, is 110.4 million cases [3]. *Trichomonas vaginalis* affects the urogenital tract of male and female especially at childbearing period [4]. The disease stages vary from an asymptomatic carrier state to obvious vaginitis [5]. *T. vaginalis* infection is associated with cervicitis, urethritis and serious complications as pelvic inflammatory disease (PID) [1], infertility [6] cervical cancer and HIV transmission. In men, trichomoniasis causes urethritis, complicated with epididymitis, prostatitis and infertility. In addition, *T. vaginalis* may be associated with cancer prostate [7]. Although, there are different methods for diagnosis of this infection, as staining techniques, immunochromatography and nucleic acid amplification [8], the gold standard method for diagnosis of *T. vaginalis* is the culture on Diamond's medium [5].

The traditional drug for trichomoniasis is the metronidazole (MTZ) or tinidazole. It is an antibiotic and a member of the 5-nitroimidazole family [9]. Yet, it has many side effects [10] particularly when higher doses are needed in the steadily increased resistant cases. [11]. Metronidazole causes nausea, dizziness, induce hypersensitivity reactions and dermatological symptoms [12], in addition to its teratogenic and carcinogenic effects on fetus [13]. Currently, no alternative safe therapy is approved to overcome the serious side effects of the traditional drugs or treat the refractory cases of trichomoniasis. These facts emphasize the need for other safe effective treatment. Natural products are an attractive resource [2].

Allium sativum (Garlic) is a popular vegetable which is used as a spice and food additive [14] and in modern medicine [15]. Thiosulfinates, sulphur-containing amino acids, and allicin are responsible for the therapeutic benefit of garlic [16]. These sulphur compounds have antimicrobial, anticancer, antioxidant, anti-inflammatory, cardio protective, antidiabetic and immunomodulatory activities [17]. They also produce

glutathione which has antioxidant activity [18]. Garlic strengthens the activity of immune cells through the bioactive properties of allicin [19]. Moreover, it has an antiprotozoal effect [20]. *Nigella sativa* (*N. sativ*a) is an ancient annual plant. It has therapeutic effects against infections caused by hepatitis C virus, and *Helicobacter pylori* [21].

Bearing in mind the need for an alternative safe antitrichomonal therapy, this study was planned to evaluate the different concentrations of garlic, the crude alcoholic extract of *Nigella sativa* and the combination between their highest effective doses and metronidazole as an anti-Trichomonas therapy at 24, 48,72 hours. Assessment of *T. vaginalis* trophozoites viability was done by trypan blue method. The results were confirmed by studying the ultrastructural changes of *T. vaginalis* trophozoites using Transmission Electron Microscope (TEM).

Methodology

Parasite Culture

Vaginal swabs were taken from 90 females with suspected trichomoniasis based on signs and symptoms. Wet mount examination of vaginal swab was done within 2 hours [22] or the samples were preserved in amies transport medium for 24-48 hours [23]. From 90 examined samples, only15 positive samples were found for study. The parasites were axenically grown on modified trypticase-yeast extract-maltose (TYM) Diamond's medium of pH: 6.2 [24] and Broth medium [25] supplemented with fetal bovine serum, antibiotic mixture of penicillin and streptomycin at 37 °C. Maintenance of *T. vaginalis* stocks was done by sub-culturing [26]. Examination of these subcultures was done on Diamond's medium ensuring the viability of *T. vaginalis* trophozoites by an inverted microscope.

Preparation of Drugs and Herbs

MTZ was obtained in the form of tablets 250 mg (Flagyl, Sanofi-Aventis, Egypt). Tablets were crushed and dissolved in distilled water, then diluted in the culture medium to yield the concentrations of 25 µg/mL, 50 µg/mL and 100 µg/mL [27]. Garlic was obtained as 200 mg (Tomex, Sekem) then 30, 60 and 90 µg/mL were prepared [27]. *N. sativa* crude extract (NsCE) was prepared from air dried seed, the grounded seeds were soaked in aqueous methanol 85% (1/10-w/v) then filtered and the plant residue re-extracted with 50% methanol. Removal of methanol by rotatory evaporator below 40% was used [28].

Experimental design

T. vaginalis trophozoites were cultured on Broth and Diamond's media to decide the suitable media for this study. Cultures treated with different concentrations of MTZ were tested and the highest effective dose was used as a positive control. *T. vaginalis* trophozoites were incubated (5×10^4cells /tube) with each herb as duplicate for 24, 48 and 72 hours. Both the MTZ treated culture and the control cultures of the parasites (containing the parasite only), were submitted to the same procedure used for the extracts cultures. The combination groups were done by adding half the dose of MTZ and the highest effective dose of the used herbs.

Ethical approval

This experimental random sample study was performed at Medical Parasitology, Gynecology and Obstetrics Departments and Scientific and Medical Research Centre (ZSMRC), Faculty of Medicine, Zagazig University. Written informed consents were taken from all patients before sample collection. The study approval was obtained from the Institutional Review Board (IRB) Unit, Faculty of Medicine, Zagazig University (IRB#:4290/14-1-2018).

Antitrichomonal assessment

a) Inverted microscope was used to examine each tube after addition of the drug or herb in 24 hours, 48 hours and 72 hours.
b) The haemocytometer and trypan blue staining were used to count the number of dead trophozoites in each tube after 24 hours, 48 hours, and 72 hours [29].
c) Transmission electron microscope (TEM) was used to detect the ultrastructural changes of the parasite in the culture. Trophozoites were fixed with glutaraldehyde and cacodylate buffer. Then the samples were prepared to be examined in a JOEL1200EXII electron microscope [30].

Statistical analysis

The collected data were computerized and statistically analysed using SPSS program (Statistical Package for Social Science) version 25.0 and the ANOVA.

Results

Comparing the modified Diamond's with Broth media, it was better concerning the survival of *T. vaginalis* trophozoites. Each concentration of MTZ showed very highly significant difference ($P < 0.001$) at

different follow-up periods, whereas, there was an insignificant difference between different concentrations of metronidazole at the follow-up period except at 72 hours, Met 100 µg/mL showed the highest mean number of dead *T. vginalis* trophozoites, so it was used as the positive control (Table 1).

There was a statistically significant difference between all Tomex concentrations and the positive control after 24and 72 hours as well as between Tomex 90µg/mL and the positive control at 48 hours. Also, there were statistical significant differences between 24, 48 and 72 hours' readings for Tomex 30 µg/mL, highly statistically significant differences for Tomex 60 µg/mL and highly statistically significant difference at Tomex 90 µg/mL, which represent the best result in all Tomex concentrations after 72 hours, yet metronidazole 100 µg/mL still has higher effect. Also, there were a statistically significant difference between the different used concentrations of alcoholic *N. sativa* crude extract (NsCE): 3 mg/mL, 6 mg/mL and 9 mg/mL, with the positive control at all follow up periods. The NsCE 9 mg/mL gave the best results represented by the highest mean count of dead *T. vaginalis* trophozoites after 72 hours (Table 2).

Regarding the combination between the highest effective dose of each herb and MTZ 50 µg/mL, the combination between NSCE 9 mg/mL and Met50 µg/mL gave the highest mean number of dead *T. vaginalis* trophozoites at different follow up periods (Table 3).

For demonstrating the trophozoite viability, trypan blue was used (Figure 1a and b). In TEM, the untreated cultured *T. vaginalis* trophozoites at 72 hours, showed a clear nucleus, well organized cytoplasm and well preserved cell membrane (Figure 2a). In contrast, the MTZ treated culture demonstrated an integral nucleus, centripetal displacement of organelles and a large vacuole although, the cell membrane, cytosome and the kinetoplast were intact (Figure 2a and b). The Tomex treated culture showed a homogenized nucleus with condensed chromatin, minimal cytoplasmic degenerative changes and distorted, plugged cell membrane (Figure2c and d).

Figure 1. showing **A**: Unstained wet mount of *T. vaginalis*trophozoites (×40). **B:** Trypan blue of live (yellow arrow) and dead (red arrow) *T. vaginalis*trophozoites (×40).

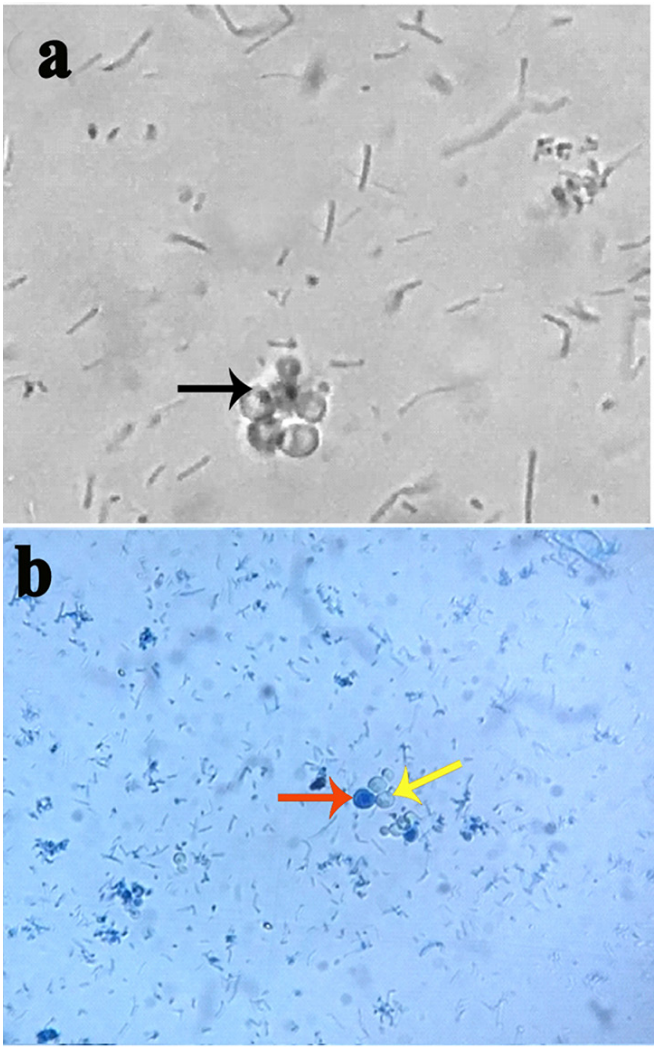

Table 1. Comparison between Diamond and Broth media and different concentrations of Metronidazole according to the mean number of dead *T. Vaginalis* trophozoites.

Group	24hours		48hours		72hours		P^ value
	Mean	SD	Mean	SD	Mean	SD	
Diamond media	20.00	5.00	22.00	2.00	40.00	2.00	0.01*
Broth media	102.67	45.80	283.33	126.42	328.67	146.68	< 0.001**
P $ value	0.04*		0.02*		0.03*		
Met 25 µg/mL	5.00 [a]	1.00	18.00 [a]	7.00	40.00 [a]	14.00	< 0.001**
Met 50 µg/mL	5.00 [a]	3.00	19.00 [a]	4.00	45.00 [a]	9.00	< 0.001**
Met 100 µg/mL	6.00 [a]	2.00	24.00 [a]	8.00	76.67 [b]	25.50	< 0.001**
P# value	0.81 NS		0.52 NS		0.04*		

Diamond media: negative control; Met 100µg/mL: positive control; SD: Standard deviation; P$: Independent t test; P#: ANOVA test; P^: Repeated measure ANOVA test; NS: Non significant (P > 0.05); *: Significant (P < 0.05); **: Highly significant (P < 0.01); Groups with different letters are statistically significant (P < 0.05).

Table 2. The effect of different concentrations of Tomex and *N. sativa* crude extract (NsCE) on the mean count of dead *T. vaginalis* trophozoites.

Group	24 hours		48 hours		72 hours		P^ value
	Mean	SD	Mean	SD	Mean	SD	
Diamond media	20.00[a,3]	5.00	22.00[a,3]	2.00	40.00 [a,3]	2.00	0.01*
Met 100	6.00 [b,4]	2.00	24.00 [a,3]	8.00	76.67 [b,3]	25.50	< 0.001**
Tomex30 µg/mL,	12.00 [a]	2.00	25.00 [a]	9.00	37.00 [a]	10.00	0.01*
Tomex60 µg/mL	15.00[a]	2.00	27.00[a]	2.00	39.00[a]	7.00	0.002**
Tomex 90 µg/mL	15.00[a]	2.00	37.00[b]	3.00	47.00 [a]	9.00	< 0.001**
P [$]value	0.002**		0.04*		0.02*		
NsCE 3 mg/mL	46.00 [1]	4.00	69.00 [1]	6.00	103 [1]	18.00	< 0.001**
NsCE6mg/mL	50.00 [1]	3.00	74.00 [1]	22.00	182.33 [2]	62.50	< 0.001**
NsCE9mg/mL	104.00 [2]	52.0	136.00 [2]	68.51	194.33 [2]	98.01	0.004**
P [#] value	0.004**		0.009**		0.02*		

Diamond media: negative control; Met 100µg/mL =positive control; SD: Standard deviation; P^: Repeated measure ANOVA test; P[$]: ANOVA test comparing Diamond media; Met 100 and different Tomex concentration; P[#]: ANOVA test comparing Diamond media; Met 100 and different NsCE concentrations; NS: Non significant (P > 0.05); *: Significant (P < 0.05); **: Highly significant (P < 0.01); Groups with different letters or numbers are statistically significant (P < 0.05).

Figure 2.TEM of cultured *T. vaginalis* trophozoites at 72 hours. **a:** Untreated culture showing (A) observed nucleus, (B) well organized cytoplasm (C) well preserved cell membrane. **b:** Treated culture with MTZ showing (A) observed nucleus, (B) centripetal displacement of organelles and a large vacuole. (C)intact cell membrane (D) cytosome. (E) the kinetoplast. **cand d:** Treated culture with Tomex showing (A) nucleus is not homogenized containing chromatin condensation, (B) minimal cytoplasmic degenerative changes in (c) distorted cell membrane (black arrow) and plugged (black arrow in d). **e** and **f:** Treated culture with NsCE showing (A) nucleus, (B) massive cytoplasmic degenerative changes and vacuolization (c) thickening of parts of cell membrane with protrusion (black arrow) and distortion (yellow arrow) (D) remnant of axostyle. Fig (e) showed an extreme disfigurement.

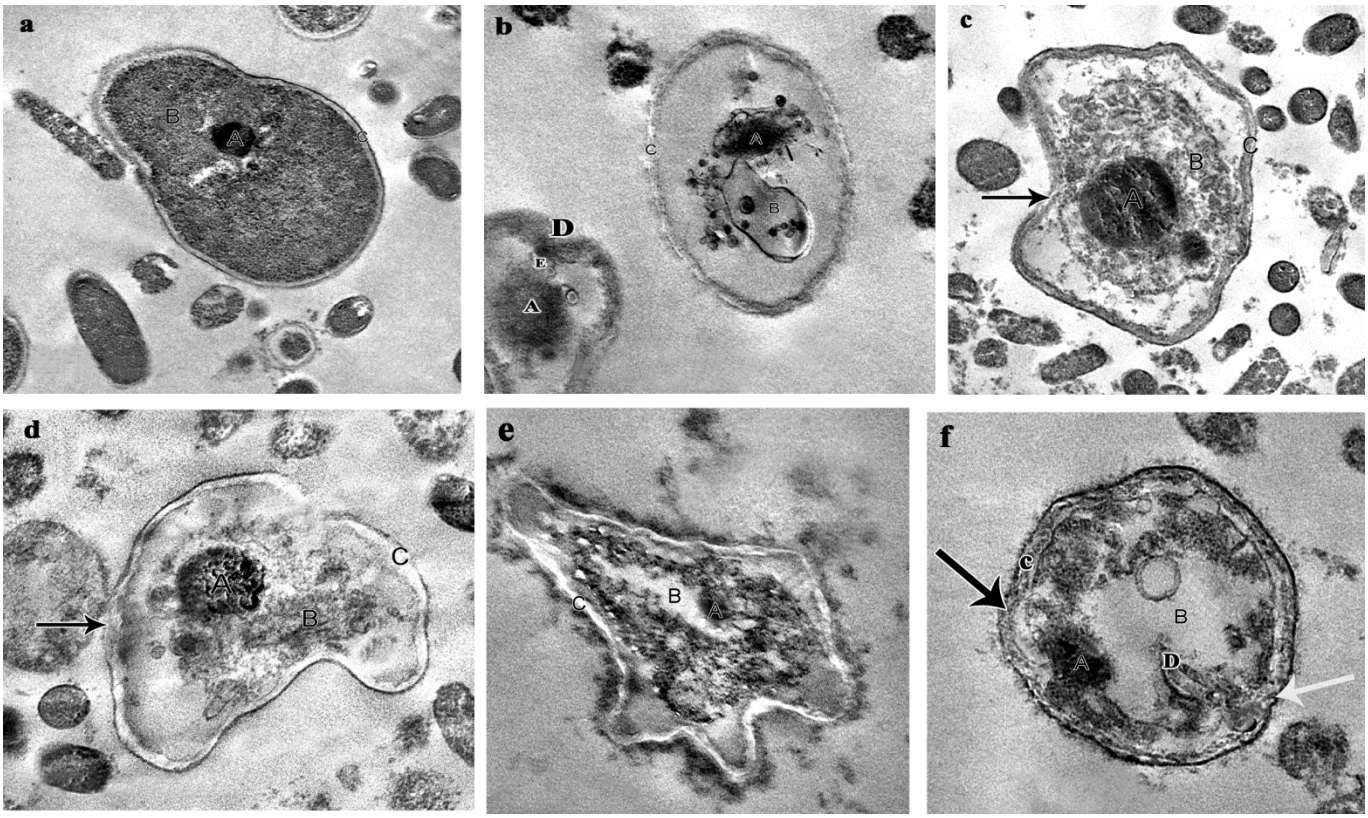

Table 3. The mean number of dead *T. vaginalis* trophozoites after incubation with the combination between the highest effective doses of the used herbs and Metronidazole 50 µg/mL.

Group	24 hours		48 hours		72 hours		P^ value
	Mean	SD	Mean	SD	Mean	SD	
Met+Tomex 90µg/mL	40.00 [a]	5.00	57.00 [a]	7.00	109.33 [a]	14.012	< 0.001**
MTZ+NSCE 9 µg/mL	15.00 [b]	8.00	157.00 [b]	82.51	760.67 [b]	400.020	< 0.001**
Diamond media	20.00[b]	5.00	22.00[c]	2.00	40.00 [c]	2.00	0.01*
MTZ 100 µg/mL	6.00 [c]	2.00	24.00 [c]	8.00	76.67 [d]	25.50	< 0.001**
P[$]value	< 0.001**		< 0.001**		< 0.001**		

Diamond media: negative control; Met 100µg/mL: positive control; SD: Standard deviation; P^: Repeated measure ANOVA test; P[$]: ANOVA test; NS: Non significant (P > 0.05); *: Significant (P < 0.05); **: Highly significant (P < 0.001); Groups with different letters are statistically significant (P < 0.05).

The NsCE treated culture (Figure 2e and f) signified an observed nucleus with massive cytoplasmic degenerative changes and vacuolization, thickened parts of cell membrane with protrusion and distortion in addition to the remnant of axostyle. Summarizing all, *T. vaginalis* trophozoite had a salient disfigurement (Figure 2 e).

Discussion

The present study is an *in vitro* trial to investigate the efficacy of different concentrations of commercially available garlic tablet (Tomex®) and NsCE, the combination between their highest effective doses with metronidazole against *T. vaginalis* infection at 24, 48,72 hours follow up periods.

Comparison between modified Diamond's and Broth media was done to get the most suitable medium for the growth of *T. vaginalis*. It was found that the mean number of dead trophozoites on modified Diamond's medium was less than Broth medium with statistical significant difference at all follow up periods. Therefore we settled on using the modified Diamond's medium. Our results agree with Gelbart *et al*. [31] who proved that modified Diamond's medium is recommended as the medium of choice for *T. vaginalis*.

Observing the effect of different Tomex concentrations (30 µg/mL, 60 µg/mL and 90 µg/mL) on cultured *T. vaginalis*. The concentration of 90 µg/mL has the highest mean number of dead trophozoites after 72 hours (47.00 ± 9.00) but MTZ 100 µg/mL still has higher effect (76.67 ± 25.50). Our results are compatible with Ahmed [32] and Ibrahim [27] who established that garlic is as efficient as metronidazole on reduction of trophozoites multiplication and motility. Furthermore, Alyasari *et al*. [33] stated that the growth rate, viability and motility of *T. vaginalis* were completely inhibited on using the aqueous garlic extract. The difference in results may be due to the use of commercial Tomex in the present study, but they used the aqueous extract (Table 2). The effect of Tomex is due to allicin production [34] that leads to disruption of the normal physiological functions of the parasite [35].

Concerning the different concentrations of *N.sativa*, crude extract, The NsCE 9 mg/mL gave the highest mean count of dead *T. vaginalis* trophozoites (194.33 ± 98.01) after 72 hours compared with the positive control (Table 2). These results agree with Tonkal [36] who found that NsCE has a remarkable inhibitory effect on the growth of *T. vaginalis* trophozoite. Also Mahmoud *et al*. [28] and Al-Ammash [37] pointed out that NsCE had an *in vitro* antitrichomanal effect. As the adhesion process plays a crucial role in the pathogenesis of *Trichomonas, N. sativa* has an anti-adhesion effect for *T. vaginalis* to human epithelial cells [38].

Comparing between the effect of metronidazole 100 µg/mL alone and its combination with the uppermost effective concentration of each herb (Tomex 90 µg/mL and NsCE 9 mg/mL) on the mean number of dead *T. vaginalis* trophozoites (Table 3), the combination between metronidazole and NsCE showed the best antitrichomonal effect (760.67 ± 400.020) paving the way towards a new era of treatment. The combination between drug and herb was also, practiced by Mady *et al*. [39] who emphasized that the combination between pyrimethamine and NSO against murine toxoplasmosis, was better than the herb alone. Also the combination done by Nassef *et al*. [40] between cisplatin, a cytotoxic drug, and NSO showed an anti-*Trypanosome evansi*. The explanation for these results could be that the herbs showed a synergistic effect when combined with drugs, as evidenced by Boullata [41] who highlighted that the co-administered herbs altered the drug concentration owing to the modification of intestinal and hepatic metabolizing enzymes.

Concerning the TEM study, it was found that the ultrastructure of the untreated *T. vaginalis* trophozoites (control group) confirmed an intact cell membrane, one nucleus, hydrogenosomes and few vacuoles. These findings are in accordance with Costamagna and Figueroa [42] who studied the ultra-structure of *T. vaginalis* in liquid cultures. The *T. vaginalis*

trophozoites treated with metronidazole after 72 hours were swollen loosing much of the material within cytoplasm when compared to the non-treated group. The remaining part of the organelles (nucleus, some vesicles and few hydrogenosomes) seemed as a large vacuole with centripetal displacement (Figure 2b). These results are in agreement with Oxberry *et al*. [43] who established the loss of the cytoplasmic material, vacuolization and disruption of cytoplasmic membrane after the usage of metronidazole. Tomex treated culture after 72 hours in our research, *T. vaginalis* trophozoites lost their normal morphology with nuclear condensation, disorganization of internal organelles, abnormal vacuolization and cell membrane distortion (Figure 2c and d). This damage in the morphology may affect the virulence and pathogenesis of the trophozoites. *T. vaginalis* trophozoites treated with NsCE after 72 hours showed severe cell damage, destroyed nucleus, large vacuolization within cytoplasm and cell membrane defects that may influence the virulence of the parasite (Figure 2e and and f).

Conclusions

NsCE is promising as an anti-*Trichomonas* particularly its combination with MTZ that showed anactive synergistic effect. The Diamond's medium was superior to Broth medium for *T. vaginalis* culture.

References

1. Poole DN, McClell RS (2013) Global epidemiology of *Trichomonas vaginalis.* Sex Transm Infect BMJ 89: 418-422.
2. Setzer M, Byler K, Ogungbe I, Setzer W (2017) Natural products as new treatment options for Trichomoniasis: A molecular docking investigation. Sci Pharm85:5.
3. Rowley J, Hoorn SV, Korenromp E, Low N, Unemo M, Abu-Raddad LJ, Chico RM, Smolak A, Newman L, Gottlieb S, Thwin SS, Broutet N, Taylor MM (2019) Chlamydia, gonorrhoea, trichomoniasis and syphilis: global prevalence and incidence estimates, 2016. Bull World Health Organ 97: 548–562.
4. Paz-Bailey G, Sternberg M, Puren AG, Steele L, Lewis AD (2010) Determinants of HIV type 1 shedding from genital ulcers among men in South Africa. Clin Infect Dis50: 1060-1067.
5. Hodiwala Bhesania A, Narayankhedkar A (2016) Trichomoniasis-A review. Int J Curr Microbiol App *S*ci5: 731-741.
6. *T*sevat DG, Wiesenfeld HC, Parks C, Peipert JF (2017) Sexually transmitted diseases and infertility. Am J Obstet Gynecol 216: 1-9.
7. Sutcliffe S, Neace C, Magnuson NS, Reeves R, Alderete JF (2012) Trichomonosis, a common curable STI, and prostate carcinogenesis: a proposed molecular mechanism. PLoSPathog8: e1002801.
8. Chalamilla G, Mbwana J, Mhalu F, Mmari E, Majigo M, Swai A, urassa W, Sandstro E (2006) Patterns of sexually transmitted infections in adolescents and youth in Dares Salaam, Tanzania. BMC Infect Dis 6: 1-8.
9. Muzny CA, Schwebke JR (2013) The clinical spectrum of *Trichomonas vaginalis* infection and challenges to management. Sex Transm Infect 89:423–425.
10. Ali V, Nozaki T (2007) Current therapeutics, their problems, and sulfur-containing-amino-acid metabolism as a novel target against infections by "amitochondriate" protozoan parasites. Clin Microbiol Rev 20: 164-187.
11. Howe K, Kissinger PJ (2017) Single-dose compared with multidose metronidazole for the treatment of trichomoniasis in women: a meta-analysis. Sex Transm Dis 44:30-35.
12. Schwebke JR, Barrientes FJ (2006) Prevalence of *T. vaginalis* isolates with resistance to metronidazole and tinidazole. Antimicrob Agents Chemother50: 4209-4242.
13. Calzada F, Yépez-mulia L, Tapia-contreras A (2007) Effect of Mexican medicinal plant used to treat trichomoniasis. J Ethnopharmacol113: 248-251.
14. Singh TU, Kumar D, Tandan SK, Mishra SK (2009) Inhibitory effect of essential oils of *Allium sativum* and *Piper longum* on spontaneous muscular activity of liver fluke, *Fasciolagigantica*. Exp Parasitol 123:302-308.
15. Martins N, Petropoulos S, Ferreira ICFR (2016) Chemical composition and bioactive compounds of garlic (*Allium sativum* L.) as affected by pre- and post-harvest conditions: A review. Food Chem 211: 41-50.
16. Hornickova J, Kubec R, Cejpek K, Velíšek J, Ovesná J, Stavelikova H (2010) Profiles of S-alkylcysteine sulfoxides in various garlic genotypes. Czech J of Food Sci 28: 298-308.
17. Lanzotti V, Scala F, Bonanomi G (2014) Compounds from Allium species with cytotoxic and antimicrobial activity. Phytochemistry Rev 13: 769-791.
18. Banerjee SK, Mukherjee PK, Maulik SK (2003) Garlic as an antioxidant: The good, the bad and the ugly. Phytotherapy Res 17: 97–106.
19. Alorainy MS (2011) Evaluation of antimicrobial activity of garlic (*Allium sativum*) against *E. coli* O 157:H 7. JAVS 4: 149-157.
20. Ayrle H, Mevissen M, Kaske M, Nathues H, Gruetzner N, Melzig M, Walkenhorst M (2016) Medicinal plants-prophylactic and therapeutic options for gastrointestinal and respiratory diseases in calves and piglets? A systematic review. BMC Vet Res 12:89.
21. Tavakkoli A, Mahdian V, Razavi BM, Hosseinzadeh H J (2017) Review on clinical trials of black seed (*Nigella sativa*) and its active constituent, thymoquinone. Pharmacopuncture 20:179-193.
22. Patil MJ, Nagamoti JM, Metgud SC (2012) Diagnosis of *Trichomonas vaginalis* from vaginal specimens by wet mount microscopy, in pouch TV culture system, and PCR. JGID 4: 22-25.
23. Washington DC (1896) Transport medium for specimens in public health bacteriology. Public Health Rep 74: 431-438.
24. Diamond LS (1957) The establishment of various Trichomonads of animals and man in axenic cultures. J Parasitol43: 488.
25. Rayner CF (1968) Comparison of culture media for the growth of *Trichomonas vaginalis*. Br J Vener Dis44: 63-66.
26. Diamond, LS, Bartgis, IL (1962) Axenic cultivation of *Trichomonas tenax*, the oral flagellate of man I. Establishment of cultures. J Protozool 9: 442-444.

27. Ibrahim AN (2013) Comparison of *in vitro* activity of metronidazole and garlic-based product (Tomex®) on *Trichomonas vaginalis*. J Parasitol Res 112: 2063–2067.
28. Mahmoud MA, EFA, Aminou HA, Hashem HA (2016) Are the fatty acids responsible for the higher effect of oil and alcoholic extract of *Nigella sativa* over its aqueous extract on *Trichomonas vaginalis* trophozoites? J Parasit Dis 40: 22-31.
29. Arbabi M, Delavari M, Fakhrieh Kashan, Z, Taghizadeh M, Hooshyar H (2016) Ginger (*Zingiber officinale*) induces apoptosis in *Trichomonas vaginalis in vitro*. IJRM 14: 691-698.
30. Cedillo-Rivera R, Chavez B, Gonzalez-Robles A, Tapia A, Yepez- Mulia L (2002) *In vitro* effect of nitazoxanide against *Entamoeba histolytica*, *Giardia intestinalis* and *Trichomonas vaginalis* trophozoites. J Eukaryot Microbiol 49: 201-208.
31. Gelbart SM, Thomason JL, Osypowski PJ, Kellett AV, James JA, Broekhuizen (1990) Growth of *Trichomonas vaginalis* in commercial culture media. J Clin Microbiol 28: 962-964.
32. Ahmed SA (2010) *In vitro* effects of aqueous extracts of garlic *(Allium sativum)* and onion *(Allium cepa)* on *Trichomonas vaginalis.* PUJ 3:45-54.
33. Alyasari HF, Al-Khafaji JKT, Al-Masoudi HK (2018) Inhibitory effects of garlic extract on uropathogenic *Escherichia coli*; *Proteus mirabilis* and *Trichomonas vaginalis* isolated from urogenital tract cases. Research J Pharm and Tech 11: 1071-1077.
34. Ross ZM, O'Gara EA, Hill DJ, Sleightholme HV, Maslin DJ (2001) Antimicrobial properties of garlic oil against human enteric bacteria: evaluation of methodologies and comparisons with garlic oil sulfides and garlic powder. Appl Environ Microbiol67: 475-480.
35. Masamha B, Gadzirayi CT, Mukutirwa I (2010) Efficacy of *Allium sativum* (garlic) in controlling nematode parasites in sheep. Inter J Appl Res Vet Med 8: 161-169.
36. Tonkal AMD (2009) *In Vitro* antitrichomonal effect of *Nigella sativa* aqueous extract and wheat germ agglutinin. JKAU Med Sci16:17-34
37. Al-Ammash M (2017) Study the effect of alcoholic extract of *Nigella sativa* seeds on *Trichomomas vaginalis in vitro*. IHJPAS 30: 10-18.
38. Shaaban MT, El Silk SE, Tayel MA (2011) Efficiency of some plant extracts, carbohydrates and inorganic salts as anti-adhesion agents against the adhesion of Staphylococcus strains to HEp-2 cells. Life Sci J 8:1172-1182
39. Mady RF, El-Hadidy W, Elachy S (2016) Effect of *Nigella sativa* oil on experimental toxoplasmosis. Parasitol Res 115:379-390.
40. Nassef NAE, El-Melegy MA, Beshay EV, Al-Sharaky DR, Al-Attar TM (2018) Trypanocidal effects of cisplatin alone and in combination with *Nigella sativa* oil on experimentally infected mice with *Trypanosoma evansi*. Iran J Parasitol13:89-99.
41. Boullata J (2005) Natural health product interaction with medication. Nutr Clin Pract 20: 33-51.
42. Costamagna SR, Figueroa MP (2001) On the ultrastructure of *Trichomonas vaginalis*: cytoskeleton, endocytosis and hydrogenosomes. Parasitología al día, 25: 100-108.
43. Oxberry ME, Thompson RCA, Reynoldson JA (1994) Evaluation of the effects of albendazole and metronidazole on the ultrastructure of *Giardia duodenalis, Trichomonas vaginalis* and *Spironucleus muris* using transmission electron microscopy. Int J Parasitol 24:695-703.

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