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In Vitro Antitrichomonal Effect of *Nigella Sativa* Aqueous Extract and Wheat Germ Agglutinin

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Abstract. Trichomoniasis is one of the most common parasitic sexually transmitted diseases in the world. Metronidazole was known as the most effective drug for human trichomoniasis., however, drug resistance and toxicity appeared. This study was designed to investigate the *in vitro* inhibitory activity of wheat germ agglutinin and *Nigella sativa* aqueous extract on the growth and motility of *Trichomonas vaginalis* in comparison to metronidazole. The inhibitory effect was dose related. Minimal lethal concentration of wheat germ agglutinin was 250µg 250µg/ml in all incubation periods. Minimal lethal concentration of metronidazole was 50µg 50µg/ml after 24 hours. However, lower doses of metronidazole showed a minimal lethal concentration of 25µg 25µg/ml after 48 h and 12µg 12µg/ml after 72 h of incubation; whereas, lower doses of wheat germ agglutinin failed to completely inhibit the parasite growth. Although *N. sativa* aqueous extract had the lowest effect on parasite growth, producing a lethal effect only after 48 h, it still has a remarkable effect. All drugs remarkably inhibited the motility of the trophozoites. The results showed a promising effect of using wheat germ agglutinin and *N. sativa* aqueous extract in treating *T. vaginalis* infection.

Keywords: Trichomoniasis, Wheat germ agglutinin, Nigella sativa, Metronidazole, Herbal medication

Introduction

Trichomoniasis, the disease that caused by the flagellate protozoan *Trichomonas vaginalis* is the sexually transmitted infection with the largest annual incidence, exceeding 170 million cases per year[1]. This is

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more than the incidence rates of gonorrhea, chlamydia, and syphilis combined. Trichomoniasis accounts for 4% to 35% of vaginitis diagnosed in symptomatic women in a primary care setting in the United States[2]. Trichomoniasis is one of the most commonly reported sexually transmitted infections in Saudi Arabia with prevalence rate of (28.1%)[3]. During the year of 2003, the prevalence rate of Trichomoniasis was (0.7%) in 6 cardinal hospitals in Jeddah, city of Saudi Arabia[4].

T. vaginalis colonizes the female and male urogenital tract, and symptoms can vary widely from asymptomatic infections to vaginitis, urethritis, prostatitis[5], low birth weight, preterm delivery, premature rupture of membranes and infertility[6]. Trichomoniasis is now an important health problem in developing countries, as it was found to be associated with increased risk of human immunodeficiency virus type 1 infection[7] and to be involved in cancer of the cervix[8].

Metronidazole has so far been the drug of choice for human trichomoniasis; however, its use can lead to drug resistance[9]. In many cases resistance can be overcome with prolonged therapy and higher doses of metronidazole, but occasionally patients continue to be infected despite these measures[9]. Beside the risk of drug resistance, undesirable side effects such as peripheral neuropathy, headache, dry mouth, metallic taste, glossitis and urticaria caused by lengthy treatment or high doses have also been described[10]. Moreover, there are potential risks of mutagenicity and carcinogenicity[11,12].

Therefore, new antiprotozoal drugs with high effectiveness and low toxicity are urgently required. Medicinal plants used in the treatment of these diseases can be an alternative resource of novel antiprotozoal drugs[13].

Cell surface glycoconjugates of parasites have been postulated to play an important role in a variety of biological functions. Lectins are carbohydrate-binding proteins; a wide range of biological actions is mediated by lectin-glycoprotein interactions, including cellular differentiation, adherence and cytotoxicity to human cells[14]. Many lectins are derived from plant seeds, and some of the best known are components of common human foods such as beans and wheat germ agglutinin (WGA)[15].

The presence of lectin receptors on the surface membrane of *T. vaginalis* has been shown. It was demonstrated that Concanavalin-A and wheat germ agglutinin cause extensive agglutination of *T. vaginalis* isolates[16]. In addition, WGA binding receptors were found in larger quantities in strains having higher pathogenicity[17]. It was suggested that the pathogenicity of *T. vaginalis* depends on a lectin specifically sensitive to N-acetyl-D-glucosamine (GlcNAc)[18]. Moreover, it was demonstrated that *T. vaginalis* has distinct binding sites for concanavalin-A and WGA, indicating the presence of GlcNAc-containing residues in the parasite membrane[19]. Also, one of the main surface polysaccharide in *T. vaginalis* is lipophosphoglycan like molecule (LPG)[20]. *LPG* mutants have reduced adherence and cytotoxicity to human cells and have lost the ability to bind the lectin WGA[21].

Targeting of carbohydrate residues by the use of lectins showed that WGA produce dose related growth inhibition of *Giardia lamblia* trophozoites *in vitro*[22]. Lectins could also inhibit *G. lamblia* excystation as effectively as monoclonal antibodies directed against cyst wall antigens[23]. The combined therapy of nitazoxanide (NTZ) and WGA was shown earlier, and better therapeutic effect against cryptosporidial infection in an experimental study[24]. WGA also interfered with chemoattraction of *Schistosoma mansoni* females to excretory secretory products of males[25].

Nigella sativa (Family: Ranunculacea), commonly known as black seed, black cumin or habatul Barakah, is an annual herbaceous plant growing in Mediterranean countries and it is one of the native plants that are widely distributed in Egypt[26]. It has been used for centuries as a spice, food preservative and curative or medicinal remedy for various ailments, including infectious diseases. It is one of the important medicines of Tibbe Nabawi (Prophetic Medicine) and identified as the curative black cumin in the Holy Bible. The seeds have been considered one of the potential natural sources in folk medicine[27,28].

Crude extracts (aqueous and alcoholic extracts) and essential oil of *N. sativa* were proved to have many therapeutic effects. The *N. sativa* alcoholic extract was found to be as effective as metronidazole in the cure of giardiasis[29]. Moreover, aqueous extract has demonstrated

inhibitory effect against candidiasis[30] and a potential therapeutic effect against *Blastocystis hominis*[31].

Considering the need for new alternatives for trichomoniasis treatment, the therapeutic potential of WGA and the *N. sativa* aquous extract, the present work was carried out to investigate the *in vitro* activity on the growth and motility of *T. vaginalis* in comparison to metronidazole.

Materials and Methods

Parasites and Culture

T. vaginalis was isolated from female patients attending the Obstetrics and Gynecology Clinic at King Abdulaziz University Hospital, Jeddah. The trophozoites were cultured axenically *in vitro* at 37°C in Trypticase-yeast extract maltose (TYM) medium[32], pH 6.0, supplemented with 1 ml heat inactivated horse serum, crystalline penicillin (1,000,000 IU/ml) and streptomycin sulfate (100,000 µg/ml). Isolates were sub-cultured every 24 hours in TYM medium. Trophozoites postinoculation were counted in a Neubauer cell-counter chamber and used to study the effects of WGA and *N. sativa* aqueous extract (NS AE) on growth and motility of *T. vaginalis* trophozoites. The starting concentration of the parasite in culture was adjusted in all tubes to be 2×10^5 trophozoites/ml culture.

Preparation of NS AE and Dilution of Drugs

Aqueous extract of NS AE

N. sativa seeds were purchased from the local market, washed to remove any debris and air dried. Amount of 250 g seeds was boiled in distilled water (1000 ml) for 90 minutes and filtered through muslin. The filtrated water extract was evaporated under reduced pressure and lyophilized to give an aqueous extract[31]. The aqueous extract was dissolved in distilled water. The extract was sterilized by filtration using Acrodisc (Gelman, 0.22 µm size) and then preserved in the deep freezer (–20°C) till it was used. The present study evaluated four doses of NS AE: 500 µg/ml, 1 mg/ml, 5 mg/ml and 10 mg/ml[33].

Wheat germ agglutinin (WGA)

It was obtained from Biomeda corporation, CA, USA and was used in a dose of 20, 50, 100 and 250 µg/ml[34].

Metronidazole

It was supplied as 500 mg tablets (Rhône Poulenc Rorer, France). Tablets were dissolved in distilled water, and then diluted in incubation medium to yield 12µg/ml, 25 µg/ml and 50 µg/ml[35].

Growth inhibition Assay

The effect of NS AE and WGA on the growth of the *T. vaginalis* trophozoites was studied as follows: $2x10^5$ trophozoites were incubated in TYM- medium with drugs in different concentrations: NS AE (500 µg/ml, 1 mg/ml, 5 mg/ml and 10 mg/ml), WGA (20, 50, 100 and 250 µg/ml) and metronidazole (12 µg/ml, 25 µg/ml and 50 µg/ml) for 24, 48, 72 h and 96 h at 37°C. All drugs were tested in duplicates. In addition, controls were included (cultures containing only the parasites) and submitted to the same procedure used for the experimental cultures. Evaluation of the drug efficacy was done by:

1. Counting the number of trophozoites using the haemocytometer (Neubauer cell-counter chamber).

2. Calculation of the percent of inhibition of multiplication according to the equation:

$$\text{Percent inhibition of growth} = \frac{a-b}{a} \times 100$$

Where;

a=Mean number of trophozoites in control tubes and

b= Mean number of trophozoites in test tubes[36].

3. Calculation of the percent of motility of trophozoites which is the ratio of motile to total number of parasites counted per 10 high power field (HPF).

4. The minimal lethal concentration (MLC) of WGA, *N. sativa* (oil and AE) and metronidazole was determined.

Results

The present study was carried out to investigate the *in vitro* activity of WGA and NS AE on the growth and motility of *T. vaginalis*, compared to the standard drug *metronidazole*.

The results showed that the degree of growth inhibition was dependent upon the concentration of WGA, NS AE and *metronidazole* (Fig. 1-6).

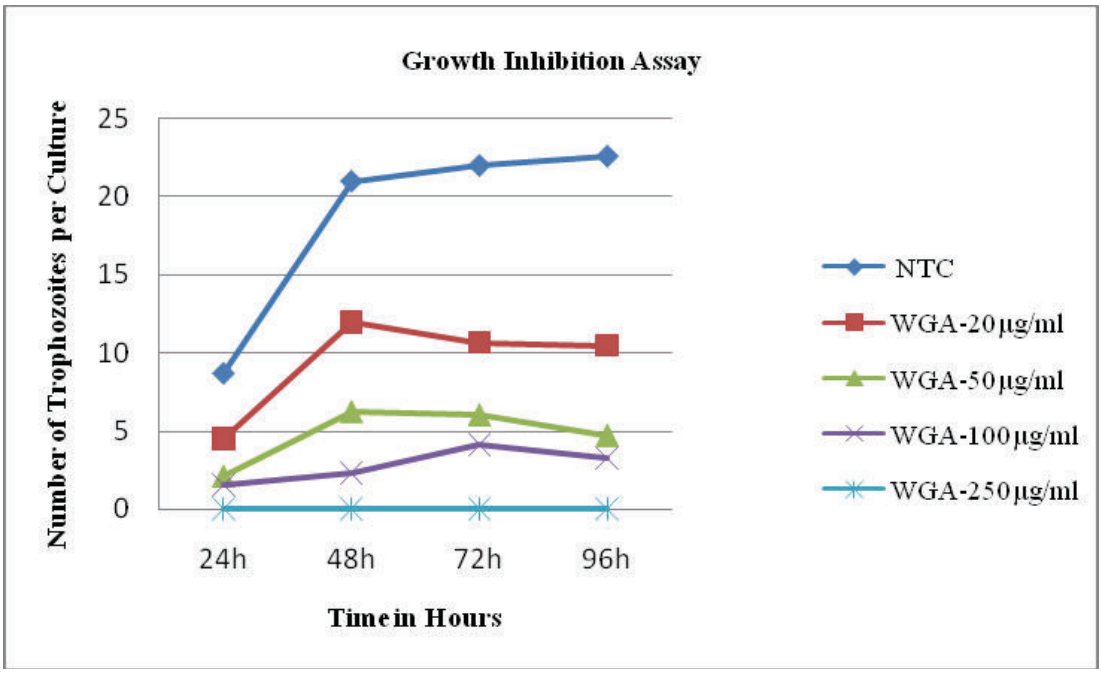

Fig. 1. **Growth curves of *T. vaginalis* in culture (parasite number x10^5) after exposure to various concentrations of WGA in comparison to normal control growth curve.**

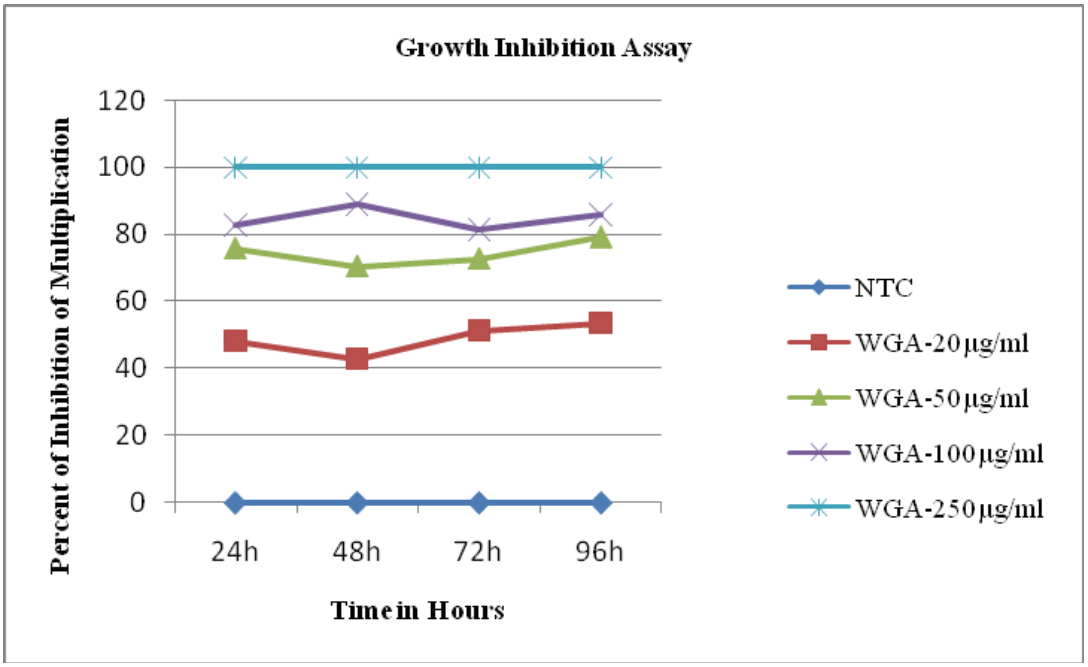

Fig. 2. **An inhibition of a *T. vaginalis* growth by WGA *in vitro*: Trophozoites were grown in the absence (rhomboid) or presence of 20 µg/ml (squares), 50 µg/ml (triangles), 100 µg/ml (x) or 250 µg/ml (*) of WGA, and trophozoites numbers determined at 24 h intervals. The results represent the mean of duplicate determinations.**

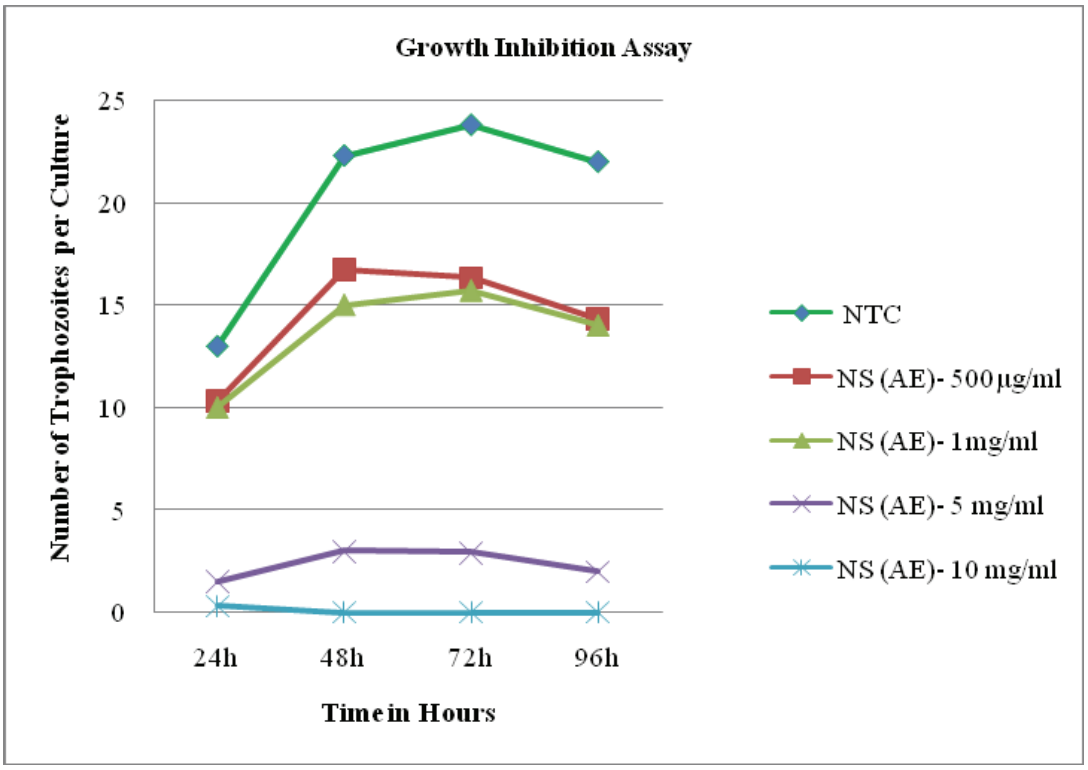

Fig. 3. **Growth curves of *T. vaginalis* in culture (parasite number x10^5) after exposure to various concentrations of NS AE in comparison to normal control growth curve.**

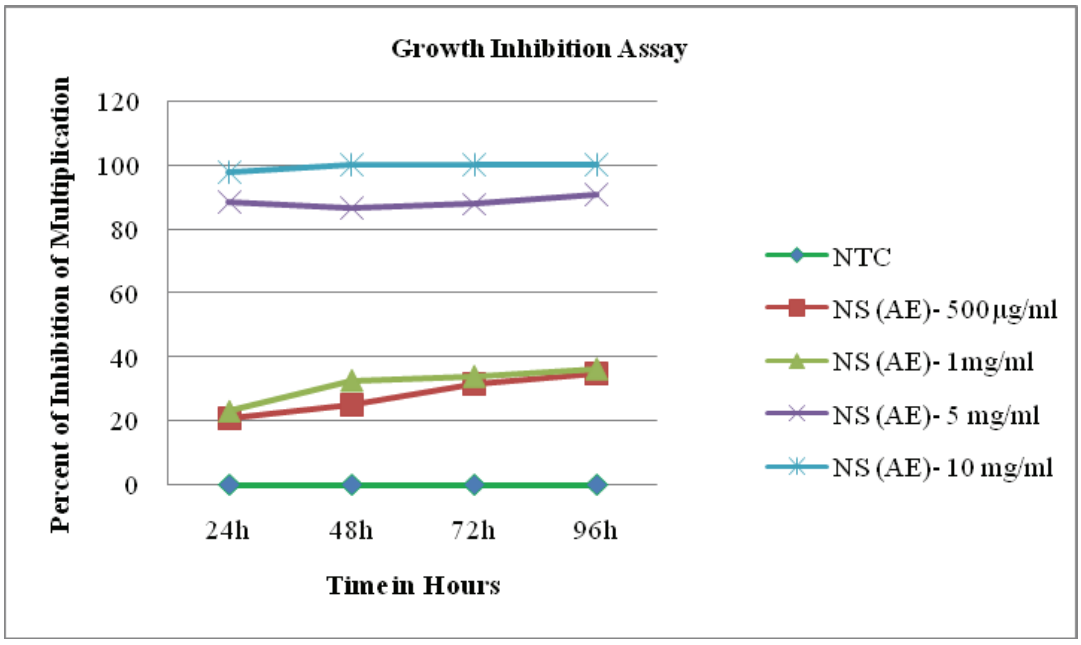

Fig. 4. **An inhibition of *T. vaginalis* growth by NS AE *in vitro*: Trophozoites were grown in the absence (rhomboid) or presence of 500 µg/ml (squares), 1 mg/ml (triangles), 5 mg/ml (x) or 10 mg/ml (*) of NS AE and trophozoites numbers determined at 24 h intervals. The results represent the mean of duplicate determinations.**

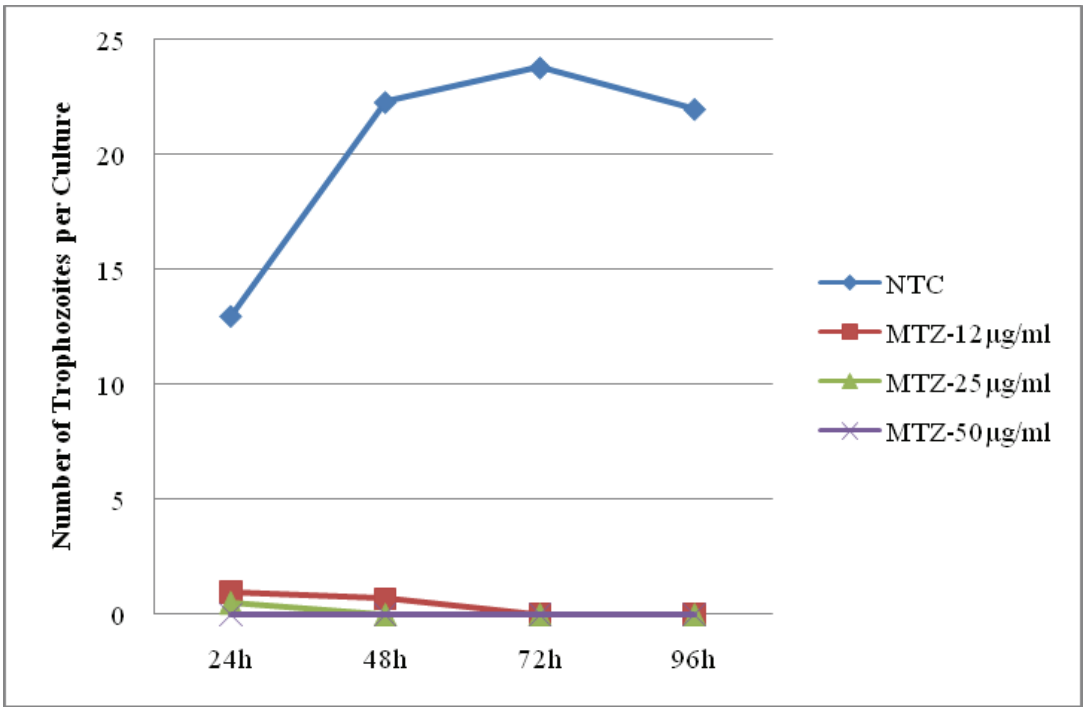

Fig. 5. **Growth curves of *T. vaginalis* in culture (parasite number x10^5) after exposure to various concentrations of MTZ in comparison to normal control growth curve.**

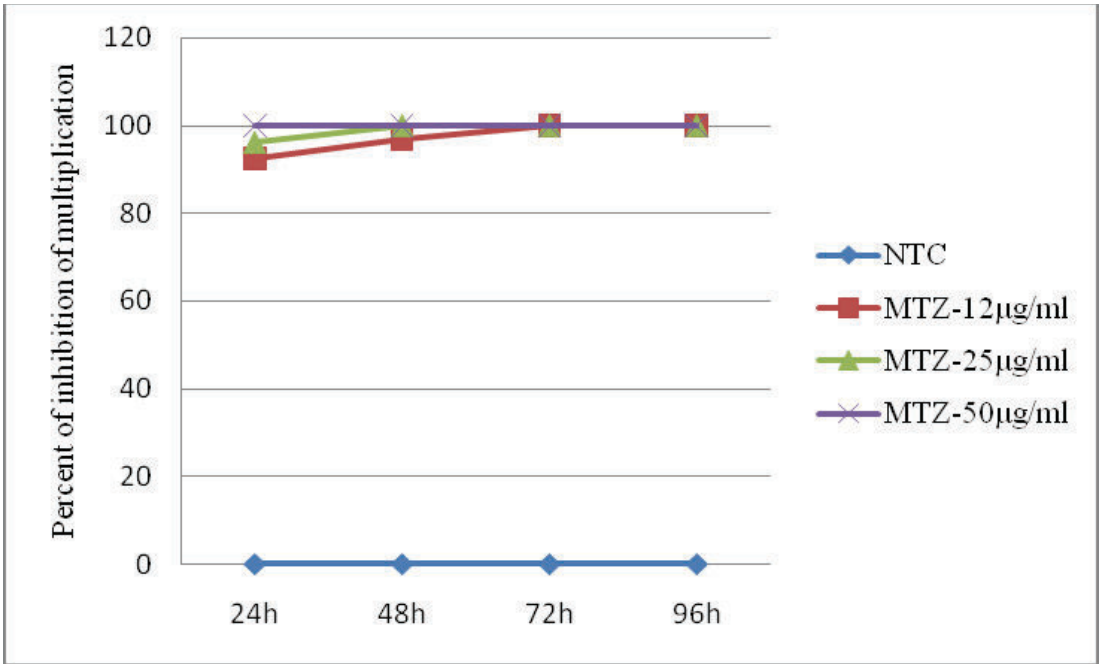

Fig. 6. **An inhibition of *T. vaginalis* growth by metronidazole *in vitro*: Trophozoites were grown in the absence (rhomboid) or presence of 12 µg/ml (squares), 25 µg/ml (triangles), 50 µg/ml (x) of MTZ and trophozoites numbers determined at 24 h intervals. The results represent the mean of duplicate determinations.**

WGA have a remarkable effect on multiplication and motility of the *T. vaginalis*. Minimal lethal concentration of WGA, which caused 100% inhibition of growth of the trophozoite, was 250 µg/ml in all incubation periods (Table 1), similar action obtained with metronidazole 50 µg/ml (Table 2).

Table 1. Mean count and percentage of growth inhibition of *T. vaginalis* per culture (parasite number x10^5) after exposure to various concentrations of WGA in comparison to normal control.

	Duration of Treatment (Hours)							
	24 h		48 h		72 h		96 h	
Dosage of Treatment	Mean	%	Mean	%	Mean	%	Mean	%
NTC	**8.7**	0	**21**	0	**22**	0	**22.6**	0
WGA 20 µg/ml	4.5	48.27	12	42.85	10.7	51.36	10.5	53.53
50 µg/ml	2.1	75.86	6.2	70.48	6.0	72.72	4.7	79.20
100 µg/ml	1.5	82.76	2.3	89.04	4.1	81.36	3.2	85.84
250 µg/ml	0	100	0	100	0	100	0	100

NTC = Non Treated Culture Control.
WGA = wheat germ agglutinin

Table 2. Mean count and percentage of growth inhibition of *T. vaginalis* per culture (parasite number x 10^5) after exposure to various concentrations of NS AE in comparison to normal control.

	Duration of Treatment (Hours)							
	24 h		48 h		72 h		96 h	
Dosage of treatment	Mean	%	Mean	%	Mean	%	Mean	%
NTC	13	0	22.3	0	23.8	0	22.0	0
NS AE 500 µg/ml	10.3	20.76	16.7	25.11	16.3	31.5	14.3	35
1 mg/ml	10	23.07	15	32.73	15.7	34.03	14.0	36.36
5 mg/ml	1.5	88.46	3.0	86.55	3.5	85.29	2	90.90
10 mg/ml	0.3	97.69	0	100	0	100	0	100

NTC = Non Treated Culture Control
NS AE = Nigella sativa aqueous extract.

However, lower doses of WGA failed to completely inhibit the parasite growth. However, it only showed growth reduction by 42.85-89.04% in all incubation periods (Table 1), compared to complete inhibition of parasite growth obtained with lower doses of *metronidazole* with MLC of 25 µg/ml and 12 µg/ml obtained after 48 and 72 hours of incubation, respectively (Table 3).

NS AE produced a lethal effect only after 48 hours using a dose of 10 mg/ml (Table 3). When used in a dose of 5 mg/ml, it showed growth reduction by 90.9% after 96 hours (Table 2).

Table 3. Mean count and percentage of growth inhibition of *T. vaginalis* per culture (parasite number x10^5) after exposure to various concentrations of metronidazole in comparison to normal control.

Duration of Treatment (Hours)								
	24h		48h		72h		96h	
Dosage of Treatment	Mean	%	Mean	%	Mean	%	Mean	%
NTC	13	0	22.3	0	23.8	0	22.0	0
MTZ 12 µg/ml	1	92.30	0.7	96.86	0	100	0	100
25 µg/ml	0.5	96.15	0	100	0	100	0	100
50 µg/ml	0	100	0	100	0	100	0	100

NTC = Non Treated Culture Control.
MTZ = Metronidazole

Lower dose of *metronidazole* (12 µg/ml) showed complete inhibition of growth after 72 hours (Table 3). In contrast, lower doses of *N. sativa* failed to completely inhibit the parasite growth, but only it showed growth reduction by 20.76 - 90.9% in case of NS AE (Table 2).

Both of WGA, NS AE and metronidazole were able to inhibit the motility of the parasite with increasing percent of immotile trophozoites in proportion to concentration and incubation time (Tables 4, 5 & 6).

Table 4. Percent of motility of *T. vaginalis* in TYM culture medium after exposure to various concentrations of WGA, in comparison to normal control.

Percent of Motility				
Groups	24 h	48 h	72 h	96 h
NTC	98%	85%	50%	Non motile
WGA-20 µg/ml	98%	80%	30%	Non motile
WGA-50 µg/ml	80	70	20	Non motile
WGA-100 µg/ml	30	10	Non motile	Non motile
WGA-250 µg/ml	No organism	No organism	No organism	No organism

NTC = Non Treated Culture Control
WGA = Wheat germ agglutinin

Table 5. Percent of motility of *T. vaginalis* in TYM culture medium after exposure to various concentrations of NS AE in comparison to normal control.

Percent of Motility				
Groups	24h	48h	72h	96h
NTC	95	70	25	Non motile
NS AE- 500 µg/ml	93	15	5	Non motile
NS AE- 1 mg/ml	91	10	Non motile	Non motile
NS AE- 5 mg/ml	16	Non motile	Non motile	Non motile
NS AE- 10 mg/ml	Non motile	No organism	No organism	No organism

NTC = Non Treated Culture Control
NS AE = Nigella sativa aqueous extract

Table 6. Percent of motility of *T. vaginalis* in TYM culture medium after exposure to various concentrations of metronidazole in comparison to normal control.

	Percent of Motility			
Groups	**24 h**	**48 h**	**72 h**	**96 h**
NTC	98%	85%	50%	Non motile
MTZ 12 µg/ml	50	Non motile	No organism	No organism
25 µg/ml	10	No organism	No organism	No organism
50 µg/ml	No organism	No organism	No organism	No organism

NTC = Non Treated Culture Control
MTZ = Metronidazole

Discussion

T. vaginalis is the most common parasitic sexually transmitted infection in the world[37].

Treatment is relied on metronidazole. Although, there are some problems related to resistance and toxicity. Thus, the search for new alternative treatments for trichomoniasis is necessary, such as natural products[38].

Lectins (agglutinins) are naturally occurring proteins recognized by their ability to bind, with high specificity, to glycosylated residues on parasite membrane. Although the anti-infective role of lectins has long been suspected, the perceived non-specificity of lectin binding, a lack of awareness within the medical community of which food contains lectin activity and subsequent discovery of antibodies, limited interest in these molecules for at least 2-3 decades[39]. *Triticum vulgaris* agglutinin (wheat germ, WGA) is one of the most popular applied lectins that have been frequently used as an investigative tool in glycobiology.

Many trials were done to target carbohydrate residues on the surface membrane of protozoa by the use of lectins[34,23]. To investigate the functional role that such lectin receptors may play in the process of infection, the present work tested the influence of WGA as an exogenous lectin on the growth of *T. vaginalis in vitro*. To our knowledge, this study is the first report with regards to *in vitro* effects of WGA against *T. vaginalis*.

Some authors have studied the antiparasite properties of WGA against *G. lamblia* cysts[23] *Cryptosporidium*[24] and *S. mansoni*[25]. WGA activity on *in vitro* protozoan proliferation has been reported in previous studies[22,40].

The present study reported a concentration 250 µg/ml of WGA as a lethal concentration to *T. vaginalis in vitro*. In contrast, previous studies dealing with WGA activity on *in vitro G. lamblia* trophozoites proliferation did not report complete inhibition of parasite growth. This may be due to the lower concentration (100 µg/ml) they used. However, this concentration of 100 µg/ml WGA inhibited the growth of *Giardia* trophozoites by 80%[22] and 56.7%[40] after 72 h incubation. This is consistent with the 81.36% inhibition of *T. vaginalis* growth encountered in the present study, by applying the same dose at the same incubation period.

There may be several possible mechanisms by which WGA could inhibit *T. vaginalis* growth which could be the same as suggested by some investigators[22], it was stated three possible mechanisms by which WGA could inhibit *Giardia* growth *in vitro*; (1) the lectin could be cytotoxic to the parasite, as it is to a number of mammalian cell lines, (2) WGA could agglutinate trophozoites and, in so doing, prevent them from multiplying, (3) WGA interferes with the function of surface glycoproteins involved in *Giardia* attachment, as is the case with other cell types.

This growth inhibition by WGA could be explained on the basis of specificity of WGA for N-acetyl-D-glucosamine (GlcNAc)-containing residues in the *T. vaginalis* membrane[19] as suggested by others[41], in case of cryptosporidial infection. It has been suggested that the known lectin induced changes in parasite biology may be sufficient to alter the balance between the immune system and the parasite[42].

N. sativa dried whole seeds are used as flavoring agent to some foods. They are very popular spice for some special baked products. They are also used as carminative and diuretic. Several beneficial pharmacological effects have been attributed to various crude and purified components of blackseeds, including antihistaminergic, antihypertensive, hypoglycemic, antimicrobial, mast cell stabilizing and anti-inflammatory activities**.** These include immune stimulation[43], anti-inflammatory[44] anti-tumor[45], and anti-oxidant[46]. Most of these biological activities have been attributed to thymoquinone, the main active constituent of the volatile oil extracted from the seeds[47].

The use of *N. sativa* against protozoal infections has been tested by several researchers. The *N. sativa* alcoholic extract was found to be as

effective as metronidazole in the cure of giardiasis[29]. Moreover, aqueous extract has demonstrated inhibitory effect against *B. hominis*[31]**.** It is been demonstrated that strong biocidal anti-malarial activities of different extracts of *N. sativa* seeds against *Plasmodium berghei*[48]. The active components of black seed were studied against nematodes and cestodes[49]. It has been reported that essential oil from the seeds of *N. sativa* showed antimicrobial and antihelmintic activities [50]. Also, the anti-schistosomicidal properties of aqueous extract of *N. sativa* seeds has been reported[51].

Although, NS AE gave the lowest effect on parasite growth recorded in this study, it still had a remarkable effect. The higher activity of metronidazole at certain concentrations may be due to the fact that *N. sativa* extract was a crude extract in comparison to the raised activity of the purified metronidazole.

The present results hold the perspective for the utilization of WGA and *N. sativa* as an anti-trichomonal agent. Furthermore, beside the direct *in vitro* anti-trichomonal effect of WGA and NS AE found in this study and their potency as antiparasitic found by other authors. Both were proved to have an immunmodulatory effect. *N. sativa* has a prominent stimulatory effect on CD4 positive T-cells and macrophages causing an immunomodulatory effect both in humans and animals[52]. Furthermore, it is been indicated that the aqueous extract of *N. sativa* seeds exhibits an inhibitory effect on nitric oxide production by murine macrophages[53]. It was proved that WGA can cause redistribution of mucosal T-cells, and an apparent increase of CD4 and CD8 T lymphocytes was observed[54]. Also, WGA was found to induced IL-12 and IF-∞ production which suggested that WGA is involved in the early pro-inflammatory response[55]. However, their exact mechanism of action on the individual components of the immune system needs to be deeply investigated. Understanding of such mechanisms will put a great impact on the management of many infectious as well as immunological disorders.

It could be concluded that WGA is as efficient as *metronidazole* on *T. vaginalis in vitro* with the added advantage of being a natural product. Although, NS AE was less effective in comparison to metronidazole, its therapeutic potential, it may not be discarded. Even so, the present results hold the perspective for the finding of new therapeutic alternative to trichomoniasis treatment. New and efficient natural products inhibiting

the growth of *T. vaginalis* trophozoites without side effects may be very useful in the treatment of the infection. Further experimental and clinical investigations are needed to evaluate and standardize the doses of these natural products.

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تأثير المستخلص المائى لبذور الحبة السوداء والقمح الجرثومي الملزن على الطفيل المسبب لداء الدوييات الشعرية

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المستخلص. يعتبر داء الدوييات الشعرية واحد من أكثر الأمراض الطفيلية المنقولة جنسيًا في العالم. ويعتبر عقار المترونديزول بأنه أكثر العقاثير فعالية لعلاج داء الدوييات الشعرية، وقد أظهر الطفيل مقاومة ضده، بالإضافة الى سميته. أجريت هذه الدراسة بهدف التحقيق من التأثير المثبط المخبري لكل من القمح الجرثومي الملزن، والمستخلص المائى لبذور الحبة السوداء على نمو وحركة طفيل الدوييات الشعرية مقارنة بالتأثير المثبط لعقار المترونديزول. ووجد أن التأثير المثبط ذو صلة بالجرعة. كما وجد أن التركيز الأدنى المميت للقمح الجرثومي الملزن كان ٢٥٠ ميكروجرام/مل في جميع فترات الحضانة. والتركيز الأدنى المميت للميترونديزول هو ٥٠ ميكروجرام/مل بعد ٢٤ ساعة. غير أن جرعات أقل من الميترونديزول أظهرت تركيزاً أدنى مميت عند ٢٥ ميكروجرام/مل بعد ٤٨ ساعة، و١٢ ميكروجرام/مل بعد ٧٢ ساعة من الحضانة، بينما جرعات أقل من القمح الجرثومي الملزن فشلت في المنع الكامل لنمو الطفيل، ولم تظهر أثراً مميتاً على نمو الطفيل إلا بعد ٤٨ ساعة. كما أن جميع العقاقير ثبطتت وبشكل ملحوظ حركة الطفيل. وأظهر استخدام القمح الجرثومي الملزن، وبذور الحبة السوداء نتائج واعدة في علاج داء الدوييات الشعرية، مما يشير إلى أهمية العلاجات العشبية الطبيعية.