Study the Effect of Alcoholic Extract of *Nigella sativa* Seeds on Trichomomas vaginalis In Vitro

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Received in :25/April /2017 , Accepted in : 15 /October/ 2017

Abstract

The present study included experimental effect of Metronidazole drug and Alcoholic extract of *Nigella sativa* seeds on *T. vaginalis* that cultivated on Diamonds' TYM medium. Results showed that the numbers of parasite began increasing during a period after 24-48 hrs then began decreasing after 72-96 hrs, so that 72 hrs from growth considered logarithmic phase of *T. vaginalis* growth. Present results showed poisonous effect of *N. sativa* alcoholic extract that was prepared in laboratory and imported at concentrations (450, 550, 650 and 750) mg/ml on *T. vaginalis* by observing gradual decrease of trophozoite numbers with concentrate increase of extracts during growth periods (24, 48, 72 and 96) hrs.

Different concentrations of Metronidazole were used as chemical therapy and control model, the results of the drug effects were followed-up daily, where current results showed (after 24 hours of concentration added) a high reduction in number and activity of the parasites at all concentrations of the drug were used, especially 150 and 200 µg/ml, the parasites disappeared completely after 96 hours, also the inhibitory concentration$_{50}$ (IC_{50}) of the parasites was 100 µg/ml (after 48 hours incubation).

The inhibitory effect of *N. sativa* alcoholic extract on the number and activity of *T. vaginalis* parasite was noted that the concentrations (450 and 550 mg/ml) were approximated in their effect on parasite growth, while the concentrations 650 and 750 mg/ml were the best in their inhibitory activity for parasite growth after 96 hours of addition. The concentration that causes 50% of death inculture (IC_{50}) was 550 mg/ml (after 72 hours of concentration addition).

Key words: *Trichomonas vaginalis, Nigella sativa*, Treatment, *In vitro*.

https://doi.org/10.30526/30.3.1596

Introduction

Trichomonas vaginalis is a cosmopolitan, it is a parasite that is found in the reproductive tract of both men and women. It lives in the vagina and urethra of women, prostate gland and seminal vesicles of men, its transmission was done through sexual intercourse mainly [1]. Trichomoniasis has a worldwide importance especially in recent years [2], The infection causes a range of symptoms and it also has long-term effects, especially in women and it is associated with a variety of serious complications including preterm labour, low birth weight of newborns, cervical cancer and implicated in amplifying in human immunodeficiency virus (HIV) transmission [3,4,5]. Parent *et al.* [6] referred to *T. vaginalis* causes the infection of 250 million new cases annually throughout the world. Al-Juwary [7] indicated to the biological and poisonous effect of the aqueous extract of *Nerium oleander* leaves and *Melia azedarach* fruits on *T. vaginalis* growing on Diamond's TYM medium, There extracts caused gradual inhibition of trichomonads number with the increase of concentration during different growth periods (24, 48 and 72) hours. AL-mbashaa [8] used crude aqueous extracts (hot and cold) to each *Seidlitzia resmarinus* and *Ziziphus spina- christi* plants and she indicated to the effect of both plants extracts on *T. vaginalis* growing on Diamond's TYM medium and CPLM medium and gave 100% inhibition at concentration of 100 mg/ ml within 24 hours.

The aim of the present study was to focus on the *in vitro* anti parasitic activity of *N. sativa* on the *T. vaginalis* growth.

Materials and Methods

Twenty five samples were collected during august and September /2016 from women those underwent to the Primary Health Care Centers, the samples were collected by speculum and cotton swab by Gynecologist and then added to each sample 2ml of normal saline and were quickly transported to laboratory to make direct smear, the parasite has been diagnosed by its jerky movement flagella motile and undulating membrane among epithelial cells [9]. The stains that mentioned below were used in microscopical examination for diagnosis.

Buffer solution and normal saline

It was prepared according to WHO [10].

Lugol's iodine stain

It was prepared according to WHO [9].

Eosin stain

It was prepared according to Smyth and Barret [11].

Method of staining of *T. vaginalis* by Giemsa stain

T. vaginalis smears were stained according to Manson–Bahar and Bell [12].

Preparation of alcoholic extracts of *N. sativa* seeds

It was prepared according to Harborne [13].

Methode of culturing and detection of *T. vaginalis in vitro*

The samples were cultivated by Diamond's TYM medium [14].

The number of parasites count

The number of generations and generation time was counted by the following two equations [15]:

$$(n = \frac{(\text{Log N} \;\; \text{Log No})}{\text{Log 2}} = \frac{(\text{Log N} \;\; \text{Log No})}{0.301}) \quad \text{Include:}$$

n= Number of generation

N= Number of cells at time (t)

https://doi.org/10.30526/30.3.1596

No= Number of start cells 2.02×10^5 cell/ml.

$$g = \frac{t}{n}$$

Include:

g= Generation time (hours)

t= Incubation period

n= Number of generation.

Evaluation of efficacy of Metronidazole and *N. sativa* extracts on cultured *T. vaginalis* trophozoites.

Metronidazole (MTZ) was produced by Sanofi-aventis company from Egypt under licence of Sanofi-aventis/France (50, 100, 150 and 200) µg/ml and *N. sativa* extract (450, 550, 650 and 750) mg/ml were used. Percentage of growth and inhibition were detected and inhibitory concentration$_{50}$ (IC_{50}) detected also through logarithmic phase according to the following two equations [7]:
(Percentage of growth= 100 - Percentage of inhibition)

Statistical analysis

Statistical program (Minitab) was used for analyzed data by using Duncan's multiple range test, analysis performed probable values less than 0.05 which were considered statistically significant [16].

Results and Discussion

Chemical detection of some active chemical groups in alcoholic extract of *N. sativa* seeds

Present results showed alcoholic extract of *N. sativa* contain some active compounds (Table 1), include flavonoids, glycosides, phenols and resins.

These results agreed with the results of Chakravarty [17], about *N. sativa* contents. Also they agreed with results of Al-Ani [18], about *N. sativa* alcoholic extract contain on flavonoids, glycosides and resins, and not agreed about alkaloids and tannins.

Determination of logaritmic phase of T. vaginalis growth

T. vaginalis numbers were counted by using counting slide of blood cells (hemocytometer) after every 24 hours of growth for four days, thus the log. phase was

$$\text{Percent inhibition of growth \%} = \frac{\text{Mean of parasite numbers in treated group} - \text{Mean of parasite numbers in control group}}{\text{Mean of parasite numbers in control group}} \times 100$$

identified of the parasite developing in Diamond's TYM medium (Figure 1).

T. vaginalis numbers began multiplying and increasing during the period 24-48 hours of growth and then it began decreasing during the period 72-96 hours, so that the period 72 hours was considerd the log. phase of the parasite growth. The table (2) revealed the generations number for *T. vaginalis* in a log. phase after 48 hours pass was 48.28 ± 5.93, also it revealed the generation time after 48 hours was 0.99 ± 0.14.

https://doi.org/10.30526/30.3.1596

This result is an approach to report by Belding [19], where he referred to the log. phase of *T. vaginalis* limited between 24-96 hours of growth *in vitro*, the present results agreed with results of Castro-Garza *et al.* [20], who confirmed that the log. phase of *T. vaginalis* developing up to 72 hours, either Kostara *et al.* [21] pointed out that the log. phase does not exceed 144 hours, and the period determination of the log. phase depends on several factors, including growth medium acidic, primary number of cultivate and temperature.

Effect of Metronidazole drug and N. sativa alcoholic extract (praperation in lab. and import) on T. vaginalis In vitro

Different concentrations were used of the drug as chemical therapy and control model, the results of the drug effect on the parasite the follow-up daily, where present results were shown (after 24 hours of concentration added) a high reduction in number and activity of the parasites at all concentrations of the drug used, especially 150 and 200 µg/ml, the parasites disappeared completely after 96 hours (table 3), also the inhibitory concentration$_{50}$ (IC_{50}) of the parasites was 100 µg/ml (after 48 hours of concentration added).

Table (3) shows the effect of alcohol extract on the number and activity of *T. vaginalis* parasite. It is noted that the concentrations 450 and 550 mg/ml were approach in their effect on parasite growth, while the concentrations 650 and 750 mg/ml were the best in their inhibitory activity for parasite growth after 96 hours of addition. The concentration that causes the death of 50% of *T. vaginalis* parasites (IC_{50}) was 550 mg/ml (after 72 hours of concentration addition).

Table (3) revealed significant statistical differences between some groups treated on the one hand and between them and the control group on the other hand, especially after 72 hours of added concentrations under study.

Metronidazole belongs to the 5-nitroimidazoles group and it is considered first choice for trichomoniasis treatment, this drug is administered orally or intravenously. A bioavailability of 93-100% because metronidazole does not bind to serum proteins and enter the cell and its organelles via facilated diffusion [22,23], then the drug was activated by the reduction of hydrogenosomal ferredoxin via reduction of the 5-nitro group [24,25]. The efficiency of this drug is high, with a cure rate of 85-95% of treated patients and re-infections can be avoided through simultaneous treatment of sexual partners [26]. Chemotherapy commonly using metronidazole causes mild side effects, characterized by the body's defence mechanisms against toxic substances, like nausea, vomiting, diarrhoea, dizziness and headache. More serious side effects, like anorexia, hypersensitivity, leukopenia, palpitation, confusion, encephalopathy, and peripheral neuropathy are clinically rarely observed [27]. The present results don't agree with the results that reported by Ahmed [28], where he indicated to reduction a ratio 100% of *T. vaginalis In vitro* (after 48 hours of MTZ concentrations added) at concentrations of 50 and 100 µg/ml. As the present results do not agree with the results of Sulyman [29], where he stated the percentage of inhibition of MTZ was 100% for some concentrations (1.25%, 2.5%, 0.5% and 10%) used after 48 hours of added and the percentage of inhibition was 100%.

Al-Banea [30] confirmed on importance of the *N. sativa* in the treatment of parasitic infections. It was observed the decrease of *Entamoeba histolytica* parasites number with mice feces after (8, 6 and 5 days) from the administration of the mice with the oil extract by concentrations (0.04, 0.05 and 0.06) ml respectively,

Al-Khuzaay [31] pointed to the importance of the alcoholic extract of the *N. sativa* plant in inhibiting of protoscolices growth of *Echinoccocus granulosus* in vitro. He used concentrations (5%, 10% and 15%). The last concentration (15%) was the best in inhibiting, where it causes a viability low of protoscolices to 15% after 48 hours of concentration

https://doi.org/10.30526/30.3.1596

addition. The effect was attributed to the plant extracts containment on the active compounds (alkaloids, flavonoids and tannins).

The alkaloids, flavonoids and tannins interfere in the chain of proteins necessary metabolites for the survival of the parasite, or it may have the ability to break down the cell wall, stimulating proteins and fats and thus causing kill of the parasite [30].

Conclusions

1-Alcohol extract of *Nigella sativa* seeds which has therapeutic efficiency was close to the efficiency of Metronidazole.
2-The plant extract under study took a period more than Metronidazole in inhibiting the growth of *T. vaginalis* trophozoites *In vitro.*
3-Alcohol extract of *N. sativa* seeds can be used as a natural alternative instead of drugs that used in treatment of *T. vaginalis*, as *N. sativa* seeds are available on the market and easy to obtain.

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https://doi.org/10.30526/30.3.1596

Table (1):Chemical determinat of some active compounds in alcoholic extract of N. sativa seeds.

N	Type of test	Alcoholic extract
1	Alkaloids	--
2	Flavonoids	+
3	Glycosides	+
4	Phenols	+
5	Resins	+
6	Tannins	--

Table (2): Mean of parasites number ($\times 10^5$), Mean of generations number and Mean of generation time ± SD. of control group.

The period (hour)	Number of parasites $\times 10^5$/ml Mean ± SD	Number of generations Mean ± SD	Generation time Mean ± SD
24	4.04 ± 0.96	27.14 ± 6.46	0.92 ± 0.23
48	7.19 ± 0.88	48.28 ± 5.93	0.99 ± 0.14
72	5.55 ± 1.26	37.23 ± 8.42	1.88 ± 0.37
96	4.23 ± 0.73	28.41 ± 4.90	3.46 ± 0.74

https://doi.org/10.30526/30.3.1596

Table (3): Mean ± SD and percentage of growth inhibition of *T. vaginalis* per culture after exposure to various concentration of MTZ and alcoholic *N. sativa* extract in comparison to control.

Incubation Period (hour) / Dosage Treated (µg/ml)		24			48			72			96		
		Mean ± SD	Growth (%)	Inhibition (%)	Mean ± SD	Growth (%)	Inhibition (%)	Mean ± SD	Growth (%)	Inhibition (%)	Mean ± SD	Growth (%)	Inhibition (%)
Control		4.04 0.96 ± E	100	-----	7.19 0.88 ± A	100	-----	5.54 1.25 ± A	100	-----	4.23 0.73 ± A	100	-----
The MTZ (µg/ml)	50	4.12 0.58 ± E	98.02	1.98	2.76 0.66 ± GH	38.39	61.61	1.06 0.51 ± DE	19.14	80.86	0.00 0.00 ± C	0.00	100
	100	5.19 1.08 ± CD	71.54	28.46	3.17 0.35 ± EFGH	44.09	55.91	0.70 0.15 ± EF	12.64	87.36	0.00 0.00 ± C	0.00	100
	150	4.37 1.15 ± DE	91.83	8.16	2.48 0.65 ± H	34.50	65.50	0.14 0.04 ± G	2.53	97.47	0.00 0.00 ± C	0.00	100
	200	3.83 0.80 ± E	94.81	5.19	0.53 0.18 ± I	7.38	92.62	0.00 0.00 ± G	0.00	100	0.00 0.00 ± C	0.00	100
Alcoholic Extract of *N. sativa* seeds prepared in lab. (mg/ml)	450	6.23 0.32 ± AB	45.79	54.20	5.03 0.47 ± BC	69.96	30.04	3.56 0.27 ± B	64.26	35.74	1.93 0.28 ± B	45.63	54.37
	550	5.57 0.89 ± BC	62.12	37.87	4.30 0.44 ± CD	59.81	40.19	2.84 0.36 ± C	51.27	48.73	1.13 0.32 ± C	26.72	73.28
	650	5.11 0.72 ± C	73.51	26.48	3.30 0.62 ± EFG	45.90	54.10	1.45 0.35 ± D	26.18	73.82	0.00 0.00 ± C	0.00	100
	750	4.45 0.62 ± DE	89.85	10.14	3.06 0.38 ± FG	42.56	57.44	0.31 0.15 ± FG	5.60	94.40	0.00 0.00 ± C	0.00	100
Import alcoholic extract of *N. sativa* (mg/ml)	450	7.13 0.90 ± A	23.52	76.48	5.57 1.06 ± B	77.47	22.53	3.74 0.95 ± B	67.51	32.49	1.76 0.33 ± B	41.61	58.39
	550	6.97 1.33 ± A	27.48	72.52	5.12 1.24 ± B	71.22	28.78	3.11 0.56 ± C	56.14	43.86	1.04 0.27 ± C	24.59	75.41
	650	6.25 1.24 ± AB	45.30	54.70	3.89 1.25 ± DE	54.11	45.89	1.35 0.33 ± D	24.37	75.63	0.00 0.00 ± C	0.00	100
	750	5.15 1.31 ± CD	72.53	27.47	2.52 0.61 ± H	35.15	64.95	0.18 0.08 ± FG	3.25	96.75	0.00 0.00 ± C	0.00	100

Similar letters indicate to non-significant differences (P > 0.05) among groups (vertical compare).
Different letters indicate to significant differences (P ≤ 0.05) among groups (vertical compare).

https://doi.org/10.30526/30.3.1596

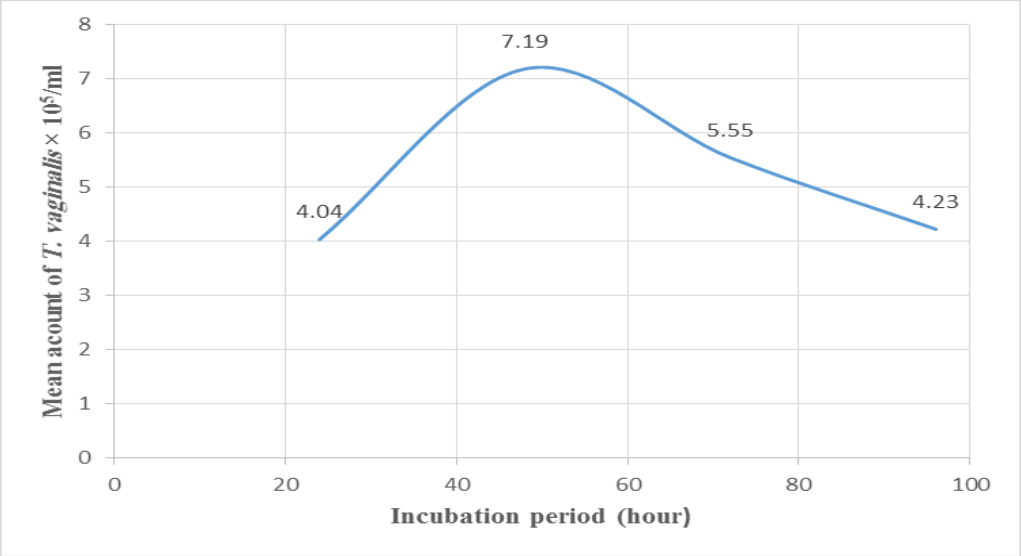

Figure (1): Normal curve of the growth T. vaginalis trophozoites in Diamond's TYM medium.

https://doi.org/10.30526/30.3.1596