ISSN NO. 2320-5407

Journal homepage: http://www.journalijar.com

INTERNATIONAL JOURNAL OF ADVANCED RESEARCH

RESEARCH ARTICLE

Assessment of in vitro anti-*Trichomonas vaginalis* activity of deer musk

Abeer Fathy Badawy, Noha Abdelfattah Elleboudy, Hend Mohamed Hussein
Parasitology Department, Faculty of Medicine, Ain-Shams University, Abbasya, Cairo, Egypt

Manuscript Info

Manuscript History:

Received: 25 June 2014
Final Accepted: 26 July 2014
Published Online: August 2014

Key words:
Trichomonas vaginalis, Trichomoniasis, deer musk

Corresponding Author

Abeer Fathy Badawy

Abstract

Trichomoniasis is a sexually transmitted disease afflicting many women worldwide. The disease is usually not life-threatening, but has been associated with the development many serious health problems. The development of resistant *Trichomonas vaginalis* strains necessitates the development of other nontraditional medications. Studies on deer musk showed its anti-bacterial and anti-fungal affects. This study was carried out to evaluate in vitro effects of different concentrations of musk 10, 15, 20, 25 and 30% (vol/vol) for 24, 48, 72and 96 hours at 37 °C on multiplication, viability and motility of *Trichomonas vaginalis* also morphological changes using scanning and transmission electron microscopy were reported and optical density of treated cultures were assayed. The minimal inhibitory concentrations of musk were 10% at 96h, 15% at 72h, 20% and 25% at 48h and 30% at 24h. The calibration curve of musk studied concentrations was found to be linear ($R^2 = 0.942$) indicating large positive association between the concentration of musk and the optical density of the culture.Musk is a safe natural product having the privilege of being anti *Trichomonas vaginalis* as well as antifungal and antibacterial properties so it provides a promising source for new drugs development.

Introduction

Trichomoniasis; caused by *Trichomonas vaginalis* flagellated protozoan parasite is the most common non-viral sexually transmitted disease (STD) (WHO, 2001) with an estimated 250 million cases worldwide (Calzada et al., 2007), yet little attention is paid to trichomoniasis (Soper, 2004). Trichomanas vaginalis is more prevalent in females than in males with no definite reason (Bhunu and Mushayabasa, 2011) .The clinical presentation shows a wide spectrum as it varies from asymptomatic infection to severe health problems as pelvic inflammatory disease, infertility, premature rapture of membranes, low-birth-weight infants, pre-term delivery, abortion and high infant mortality (Cudmore and Garber, 2010; Scopel et al., 2013) also acquiring infection at delivery may predispose to maternal postpartum sepsis (Sebitloane et al., 2011). It is also implicated in cervical cancer (Viikki et al., 2000) and studies links trichomoniasis to amplifying human immunodeficiency virus (HIV) and enhancing its transmission by two-folds (McClelland et al., 2007; and Sharafi et al., 2013; Sherrard et al., 2014) as co-infection facilitated by local inflammation and microscopic breaches in mucosal barriers as well as degradation of a secretory leukocyte protease inhibitor, which normally protects cells from HIV infection (Gehrig and Efferth ,2009).

Approved drugs of treatment of *Trichomonas vaginalis* infection are 5-nitroimidazoles, with metronidazole being the drug of first choice, which is also used for treatment of other microorganism as Entamoeba, Giardia, and anaerobic bacteria (Rabiee et al., 2012). Considering the limited drugs in protozoal chemotherapy with nearly similar mechanisms of action when resistant strains develop there will be no available drugs to use. Also the association of metronidazole treatment with numerous side effects prompted the need to identify new alternative drugs to improve the current chemotherapy of *Trichomonas vaginalis* infection (Gehrig and Efferth, 2009). The search for new drugs is both cost and time consuming, while the development and progress of vaccination against

Trichomonas vaginalis is still ongoing, natural products showed the potential source of new antiprotozoal drugs with high activity, low toxicity and lower price (Calzada et al., 2007).

The word Musk is derived from the ancient Indian word Muskáh meaning testicles (Homes, 1999). musk is also known as Moschus and Shexiang is a natural dried substance with a special fragrance, black to brown in color, obtained from a male musk deer of the genus Moschus glandular secretion (Ye et al., 2011) which is located in a small sac caused by infolding of the skin near the preputial orifice. Musk was extensively used not only as a fragrance but also for medicinal purposes since ancient times (Takaoka, 2007). It has been recommended in Traditional Chinese Medicine and in the Japanese pharmacopoeia to induce cardiovascular stimulation, anti-inflammatory medication and androgenic hormonal therapy (Thevis et al., 2013). Also in resuscitation and refreshment, promoting blood flow and clearing channels, detumescence and alleviating pain (Cheng et al., 2011).

Musk is formed of several compounds, the main compound which causes the odour is muscone also lipid constituents and numerous steroidal components were characterized (Thevis et al., 2013).The antimicrobial activity of Musk was studied on fungi and bacteria by Saddiq, 2004; 2008; 2011; Saddiq El-Eliany, 2009 and 2014 but still not on protozoa parasites.

The common methods used to assess post drug effect on *Trichomonas vaginalis* mainly focuses on motility and viability of the trophozoite. Usage of a standard spectrophotometer to measure optical density (OD) is the most common method used to assess microbial growth, cell concentration and biomass present in the suspension. When light passes through a suspension two mechanisms contributes significantly to attenuation of the light intensity; absorbance and light scattering (Myers et al., 2013). As the amount of light scattering is closely related to the dry weight of micro-organisms it is used to monitor the concentration of pure cultures (Sutton, 2011). This study was carried out to evaluate in vitro effect of different concentrations of deer Musk on *Trichomonas vaginalis* motility, viability, multiplication and to detect post drug trophozoite changes using scanning and transmission electron microscope and to detect optical density changes of studied parasite culture after drug exposure.

Materials and methods

Trichomonas vaginalis protozoan was isolated from female patients attending the outpatient clinic, Gynecology and Obstetrics Hospital, Ain Shams University. An informed consent was taken from all patients before taking vaginal samples. The study was approved by the Research Ethics Committee, Faculty of Medicine, Ain Shams University in compliance with the relevant laws and guidelines in accordance with the ethical standards of the Declaration of Helsinki.

The trophozoites were cultured axenically in vitro at 37 ℃ in trypticase yeast extract–maltose (TYM) medium ((Diamond, 1957), 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 subcultured every 48 h in TYM medium and maintained in Parasitology Diagnostic and Research Unit, Faculty of Medicine, Ain Shams University.

Musk was supplied as an oily solution commercially available at Abd-El Samad El Korashy Official stores preserved in natural environmental circumstances at temperature 25 – 28 C°. Trichomonas vaginalis trophozoites were incubated in 5 ml TYM medium containing musk solution in different concentrations 10, 15, 20, 25 and 30% (vol/vol) for 24, 48, 72and 96 hours (h) at 37 ℃.

Metronidazole was supplied as 250-mg tablets (Flagyl, Sanofi-Aventis, Egypt). Tablets were dissolved in distilled water. Trichomonas vaginalis trophozoites were incubated in 5 ml TYM medium containing metronidazole in different concentrations (20,30 and 40 µg/ml) for 24, 48, 72and 96 h at 37 ℃ according to Ali (2007). In addition, one control group was included in the study with, cultures containing only the parasites.

Assessment of multiplication and motility of the trophozoites were done using inverted microscopy daily examination of the culture tubes and the percent of trophozoites motility was calculated microscopically by eye lens (×10) as the ratio of motile trophozoites to total number of parasites counted (Ali, 2007). While, Neubauer cell counting chamber was used to count the number of trophozoites and the multiplication inhibition percent was calculated (Palmas et al., 1984) using this equation: Percent inhibition of growth= a-b/a x 100. Where a = mean number of trophozoites in control tubes and b = mean number of trophozoites in test tube of each group. The viability of trophozoites was tested using trypan blue stain. The minimal inhibitory concentration (MIC) was the lowest concentration of the drug in which no motile cells were detected. To check whether MIC reflects the effectiveness of the drug in killing the cells, re-cultivation of the parasite on fresh medium, and examination for the presence of motile parasites after 5 and 10 days of re-cultivation was done. If the parasites didn't resume growth, this concentration is referred to as minimal lethal concentration (MLC) (Meingasser and Thurner, 1979; Meri et al., 2000 and Cedillo-Rivera et al., 2002).

Transmission and scanning electron microscopic (TEM & SEM) pictures were done at Theodor Bilharz Research Institute, Giza to follow up the effect of the drugs on the ultra- structure of the parasites after 24 hours incubation

period using 20% as a musk concentration The trophozoites were fixed with 2.5% (vol/vol) cold glutaraldehyde and processed for examination with Philips Electron Microscope 208 S with set of cell image analysis and video system (Rose et al., 2011 and Cheon et al., 2013).

Optical density of the culture tubes were measured by a Behring ELISA Photometer (Behringwerke, Marburg,Germany) in the Biochemistry Department Faculty of Medicine Ain Shams University, indicating the parasite concentration as greater scatter of the visible light indicates more parasites presence. The parasite growth after incubation with musk in concentration of 10, 15, 20, 25 and 30% (vol/vol) for 24 h at 37 ℃ were compared with respect to the control media of culture with drug before incubation a wavelength of 492nm. The calibration curve was constructed by plotting concentration of musk studied against the OD at 492 nm. Each experiment was repeated three times with triplicates of each concentration for statistical analysis.

Statistical analysis

Quantitative data are presented as mean ± standard deviation (SD), percent of growth inhibition, percent of viability and percent of motility. Variables were compared using Mann–Whitney U test. A significance level of $P < 0.05$ was used in all tests. All statistical procedures were carried out using SPSS version 17 for Windows (SPSS Inc, Chicago, IL, USA).

Results:

The effect of different concentrations of musk and metronidazole on Trichomonas vaginalis trophozoites count (Table 1) and motility (Table 2) are shown

Table 1 Effect of different concentrations of musk on the in vitro growth of Trichomonas vaginalis trophozoites (10^6) for different incubation periods

Study Groups		After24h		After 48h		After 72h		After 96h	
		Mean±SD (Range)	% of growth inhibition	Mean±SD (Range)	% of growth inhibition	Mean±SD (Range)	% of growth inhibition	Mean±SD (Range)	% of growth inhibition
Conrol Groups	Parasite control	1.92±0.10 (1.82-2.02)	0%	3.20±0.21 (2.99-3.41)	0 %	12.5±0.12 (12.38-12.62)	0%	6.15±0.12 (6.03-6.27)	0%
	Metronidazole, 20µg	0.5±0.01* (0.49-0.51)	74%	0.31±0.30* (0.01-0.61)	90.3%	0.13±0.06* (0.07-0.19)	99 %	.00*	100%
	Metronidazole, 30µg	0.34±0.09*• (0.25-0.43)	82%	0.11±0.06*• (0.05-0.17)	96.6%	.00*	100%	.00*	100%
	Metronidazole, 40µg	.00*	100%	.00*	100%	.00*	100%	.00*	100%
Musk	Musk, 10%	1.09±0. 08• (1.01-1.17)	43.2%	0.71±0.03*• (0.68-0.74)	77.8%	0.48±0.15*• (0.33-0.63)	96.2%	.00*	100%
	Musk, 15 %	0.97±0. 06*• (0.91-1.03)	49.5%	0.19±0.04*• (0.15-0.23)	94%	0.08±0.05*• (0.03-0.13)	99.4%	.00*	100%
	Musk, 20%	0.5±0.01*• (0.49-0.51)	74%	.00*•	100%	.00*•	100%	.00*	100%

	Musk, 25%	0.05±0.01*• (0.04-0.06)	97%	.00*•	100%	.00*•	100%	.00*	100%
	Musk,30%	.00*•	100%	.00*•	100%	.00*•	100%	.00*	100%

*p<0.05, statistically significant difference in comparison to non-treated parasite control in the same time interval; ** p<0.05, statistically significant difference in comparison to Metronidazole 20µg in the same time interval;

•= No statistically significant difference (p>0.05) in comparison to Metronidazole control 20µg in the same time interval; SD standard deviation

Table 2 Percentage of viability and motility of *Trichomonas vaginalis* trophozoites in culture after exposure to Musk at different incubation periods

Dosage of Treatment	After 24 h (%)		After 48 h (%)		After 72 h (%)		After 96 h(%)	
	Viability %	Motility %	Viability %	Motility %	Viability %	Motility %	Viability %	Motility %
Parasite Control	100%	98%	100%	76.2%	100%	21%	100%	9.6%
Metronidazole, 20µg	36%	33.2%	9.7%	7.3%	1%	0.5%	0%	0%
Metronidazole, 30µg	8%	9%	3.4%	4.2%	0%	0%	0%	0%
Metronidazole, 40µg	0%	0%	0%	0%	0%	0%	0%	0%
Musk, 10%	56.8%	49%	22.2%	10%	3.8%	0.5%	0%	0%
Musk, 15%	50.5%	22.8%	6%	3.5%	0%	0%	0%	0%
Musk, 20%	26%	19.2%	0%	0%	0%	0%	0%	0%
Musk, 25%	3%	15%	0%	0%	0%	0%	0%	0%
Musk,30%	0%	0%	0%	0%	0%	0%	0%	0%

Light microscopy observations revealed that both metronidazole and musk promoted changes in the shape of great part of trophozoites of Trichomonas vaginalis in the form of transformation to round-shaped cells with disappearance of the flagellae. Electron microscopy study showed swelling and distortion of trophozoite morphology (Fig.1) rounded cells with loss of flagellae (Fig.2) wrinkling and membrane blebbing (Fig.3) with formation of large empty areas with altered vacuoles distributions with damaged plasma membrane and extensive cytoplasmic destruction (Fig.4-6).Results were compared to normal scanning microscopy results (Cheon et al., 2013).

By spectrophotometric study; the linearity of the response of the musk was verified at 10, 15, 20, 25 and 30% (vol/vol). The calibration curve was obtained by plotting the OD versus the concentration data and was treated by linear regression analysis. The calibration curve was found to be linear in the aforementioned concentrations. The equation of the calibration curve for musk obtained was $y = -1.72x + 2.498$ and the correlation coefficient (R^2) of determination was 0.942.

Fig. 1: SEM of the effect of 20% musk on *Trichomonas vaginalis* after 24 hours incubation showing swelling and distortion of trophozoite

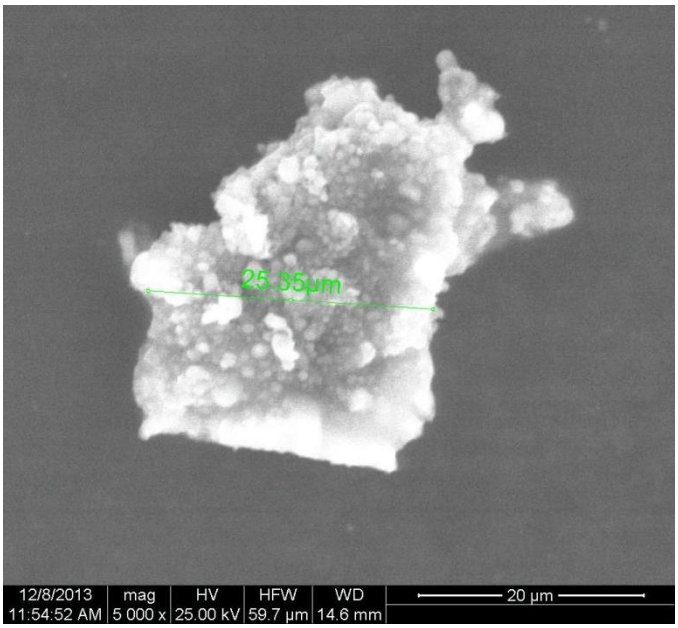


Fig. 2: SEM of the effect of 20% musk on *Trichomonas vaginalis* after 24 hours incubation showing rounded cells with loss of flagellae

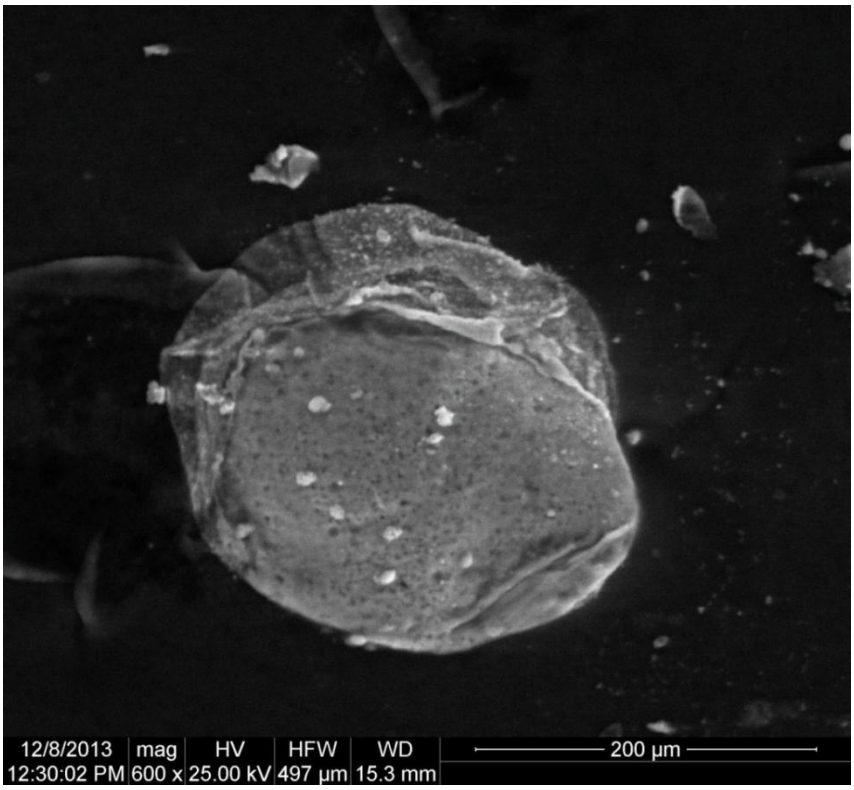


Fig. 3: SEM of the effect of 20% musk on *Trichomonas vaginalis* after 24 hours incubation showing wrinkling and membrane blebbing

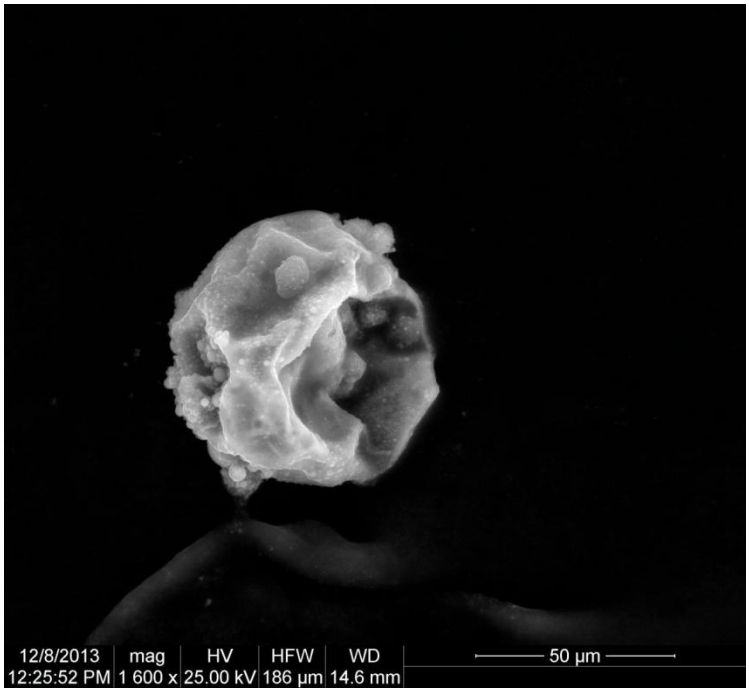


Fig.4: TEM of the effect of 20% musk on the ultra- structure of the *Trichomonas vaginalis* after 24 hours incubation showing swelling and distortion of trophozoite morphology (rounding of cells).

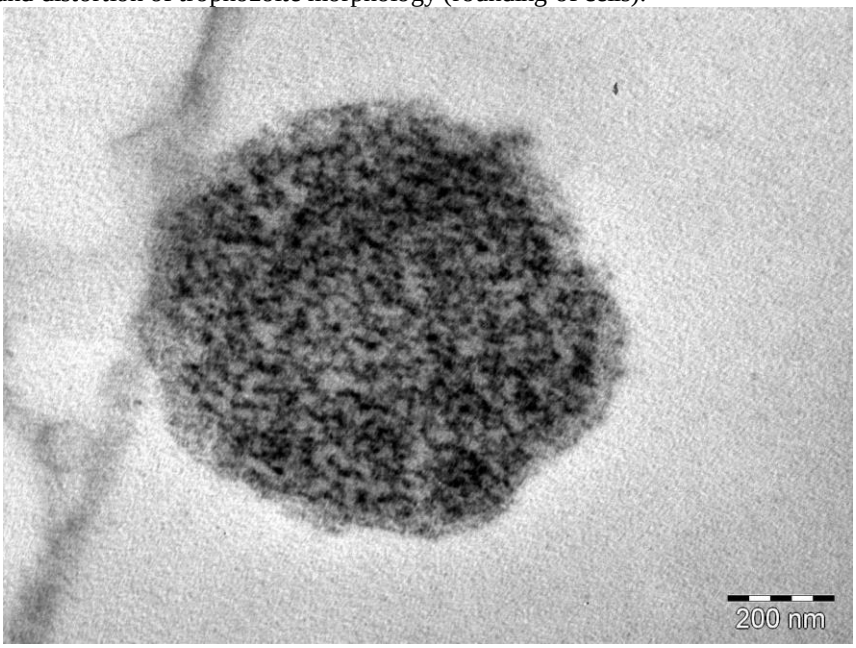


Fig.5: TEM of the effect of 20% musk on the ultra- structure of the *Trichomonas vaginalis* after 24 hours incubation showing formation of large empty areas with altered vacuoles distributions.

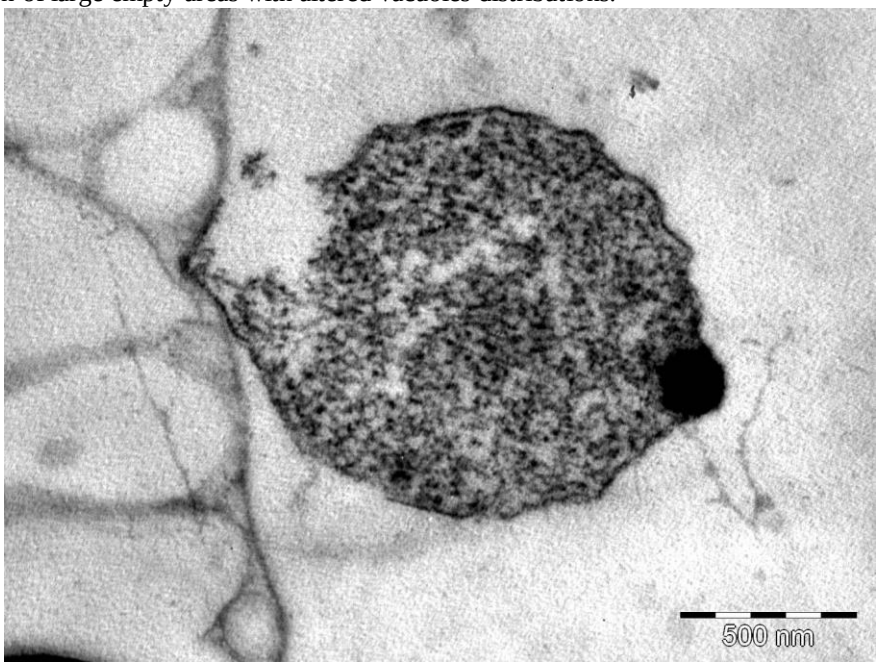


Fig.6: TEM of the effect of 20% musk on the ultra- structure of the *Trichomonas vaginalis* after 24 hours incubation showing damaged plasma membrane and extensive cytoplasmic destruction

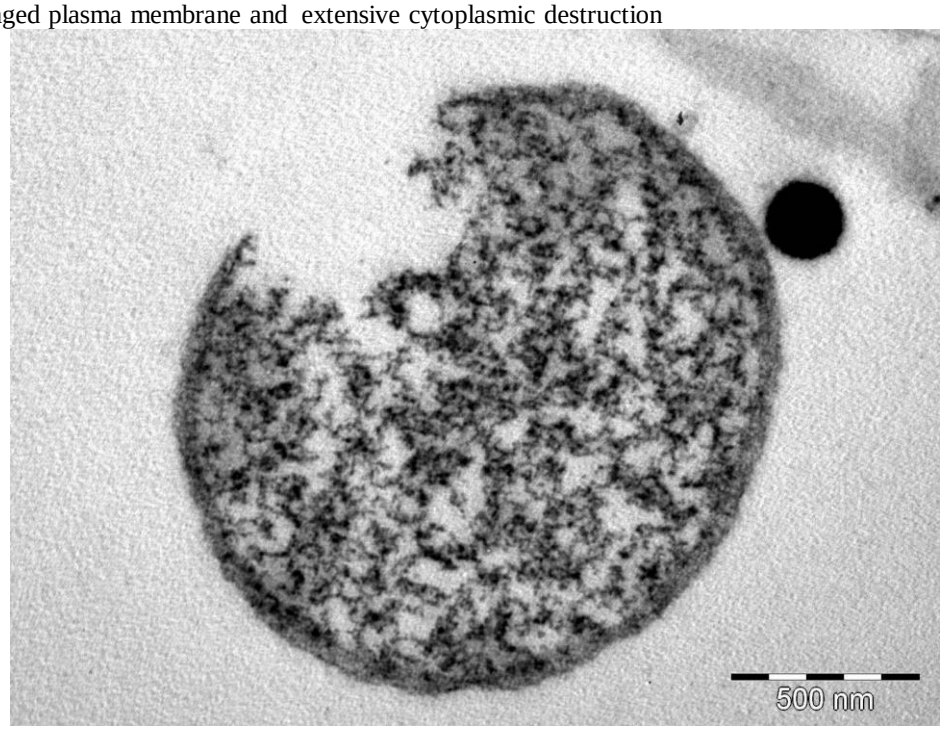
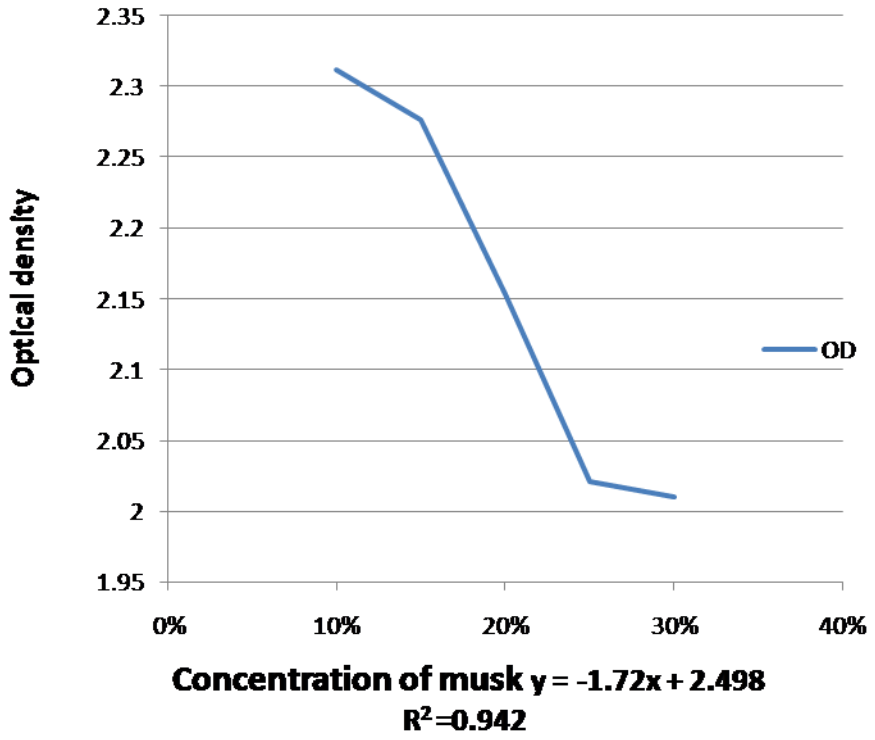


Fig.7: Standard curve graph plotted between studied concentrations of musk (vol/vol) in and optical density at a wavelength of 492nm



DISCUSSION:

Trichomoniasis is a STD afflicting many women worldwide with annual incidence of trichomoniasis 57.0 /1000 according to Kenyon et al. (2014) highest in Sub-Saharan Africa and lowest in East Asia and Pacific regions. The disease is usually not life-threatening, but has been associated with the development many serious health problems. The development of resistant *Trichomonas vaginalis* strains necessitates the development of other nontraditional medications (Gehrig and Efferth, 2009). Considerable attention to alternative therapies, particularly using natural sources derived compounds for the treatment of diseases has been paid as many studies reviewed the effect of different medical plants on *Trichomonas vaginalis* in culture medium (Sharafi et al., 2013).

Studies on deer musk showed its anti-bacterial and anti-fungal affects (Saddiq, 2004; 2008; 2011 ; 2014; and Saddiq El-Eliany, 2009 and Cheng et al., 2011) . In this study the deer musk showed the potential anti *Trichomonas vaginalis* effect as it showed growth inhibition of cultures at a musk concentration of 10% and 15% gradual decrease in growth of the trophozoites occurred till complete inhibition of the parasitic growth was observed after 96h of incubation. At a concentration of 20% a decrease in growth of the trophozoites was observed after 24h and complete inhibition of the parasitic growth was observed after 48h of incubation and a complete inhibition of the parasitic growth was observed with concentration of 25% and 30% after 48 h and 24 h incubation respectively. The effect of all concentrations showed a statistical significant difference compared to control (Table 1)

As for metronidazole-treated culture showed gradual inhibition of the parasitic growth with concentrations of 20 and 30 µg/ml and complete inhibition of the parasitic growth was observed after 96h and 72 h of incubation respectively, at a concentration of 40 µg/ml after 24 h. The effects of all concentrations showed a statistical significant difference compared to the control (Table 1).

Musk also revealed motility inhibition of *Trichomonas vaginalis* trophozoite as musk-treated cultures showed gradual inhibitory effect on the motility of the trophozoites was noted till complete inhibition of the parasitic motility was observed with concentrations of 30% after 24 h, 25% - 20% after 48 h, 15% after 72 h and 10% after 96 h of incubation. Metronidazole-treated culture had an inhibitory effect on the motility of the trophozoites with complete inhibition of the motility with concentration of 40µg/ml after 24 h, 30µg/ml after 72 h and 20µg/ml after 96 h. (Table 2)

The MIC of musk was 10% at 96h, 15% at 72h, 20% and 25% at 48h and 30% at 24h and MLC was 20% in re-growth assessment (Table 2).

Both TEM and SEM results showed considerable damage of the membrane system of the trophozoites, abnormal vacuolization and extensive destruction of the cytoplasm other degenerative changes associated with cell death and decline growth are also likely to occur ending up by complete loss and disintegration of trophozoites (Fig. 1-6).

OD measurements have the enormous advantages of being rapid, low cost, and nondestructive and it can be used both qualitatively as the turbidity of a culture and quantitatively as a measure of the intensity of light transmitted through the culture. OD correlates directly with biomass so that cell concentration can be monitored without having to conduct tedious procedures for measuring concentration of cells by hemocytometry (Myers et al., 2013). The calibration curve of musk studied concentrations was found to be linear with a R^2 indicating large positive association between the concentration of musk and the OD of the culture (Fig.7) as OD decreases with the increase in musk concsntration.

The common methods used to assess post drug effect on *Trichomonas vaginalis* mainly focuses on motility and viability of the trophozoite. While the traditional use of a cell counting chamber hemocytometer is time-consuming and leads to considerable error as it depends on the number of cells which are counted in each area of the reticule therefore a standard spectrophotometer can also measure the concentration of cells and determining the inhibitory action of compounds on microorganisms. It has much quicker and easier to use and to replicate with several studies showing no statistically significant differences between the results obtained with the spectrophotometeric method and viable count of bacteria in agar (Domvnguez et al., 2001 and AL-Janabi, 2010). This method has been performed to monitor the growth of bacteria or fungi (Valdez and Piccolo, 2006) but not yet on *Trichomonas vaginalis* culture.

Musk is a safe natural product used in medicine and as a fragrance for over 5000 years (Homes, 1999) that provides a promising source for new drugs development for *Trichomonas vaginalis* as well as its antifungal and antibacterial properties that requires further investigations on identification of its mode of action as well as determination of resistance formation after extensive application and of possible cytotoxicity of the compounds to mammalian cell lines is required despite its animal origin. Further research is needed to develop a convenient formulation for musk to be used in a clinical trial to evaluate its Trichomonocidal activity in vivo.

Ethical consideration;

An informed consent was taken from all patients before taking vaginal samples. The study was approved by the Research Ethics Committee, Faculty of Medicine, Ain Shams University in compliance with the relevant laws and guidelines in accordance with the ethical standards of the Declaration of Helsinki.

Conflict of interest:

Authors declare no Conflict of interest and no funding was received.

REFERENCES:

Ali, N.M. (2007): In vitro activity of commercially available Egyptian propolis on *Trichomonas vaginalis*. The New Egyp. J. Med., 36:7– 15.

AL-Janabi, A.A. (2010): In Vitro antibacterial activity of ibuprofen and acetaminophen. J. global. Inf. dis., 2(2): 105-8.

Bhunu, C.P. and Mushayabasa, S. (2011): Transmission dynamics of *Trichomonas vaginalis*: A mathematical approach. J. Math. Anal. Appl., 379:852–60.

Calzada, F., Y´epez-Mulia, L., Tapia-Contreras, A. (2007): Effect of Mexican medicinal plant used to treat trichomoniasis on *Trichomonas vaginalis* trophozoites. J. Ethnopharmacol. , 113: 248–51.

Cedillo-Rivera, R., Chavez, B., Gonzalez-Robles, A., et al. (2002): In vitro effect of nitazoxanide against Entamoeba histolytica, Giardia intestinalis and *Trichomonas vaginalis* trophozoites. J. Eukaryot. Microbiol., 49: 201–8.

Cheng, D.H., Wang, J., Zeng, N., et al. (2011) : Study on drug property differences of shexiang (moschus) and bingpian (borneolum synthcticum) based on analysis of biothermodynamics. J. Tradit. Chin. Med. ,31(1):21-6.

Cheon, S.H., Kim, S.R., Song, H.O., et al. (2013):The Dimension of *Trichomonas vaginalis* as Measured by Scanning Electron Microscopy. Korean. J. Parasitol ., 51(2): 243-6.

Cudmore, S.L., Garber, G.E. (2010): Prevention or treatment: the benefits of *Trichomonas vaginalis* vaccine. J. Infect. Public Health.,3: 47–53.

Diamond, L.S . (1957): The establishment of various trichomonds of animals and man in axenic cultures. J. Parasitol.,43:488–90.
Domvnguez, M.C., de la Rosa, M., Borobio, M.V. (2001): Application of a spectrophotometric method for the determination of post-antibiotic effect and comparison with viable counts in agar. J. Antimicrob. Chemoth.,47:391-8.
Gehrig, S. and Efferth, T. (2009): Development of Drug Resistance in *Trichomonas vaginalis* and its Overcoming with Natural Products. Open Bio. Com. J., 2: 21-8.
Homes, V. (1999): On the Scent: Conserving Musk Deer – the Uses of Musk and Europe's Role in its Trade. TRAFFIC Europe, Brussels, Belgium.
Kenyon, C., Buyzea, J., Colebunders, R. (2014): Classification of incidence and prevalence of certain sexually transmitted infections by world regions. Int. J. Inf. Dis., 18 : 73–80.
McClelland, R.S., Sangare, L., Hassan, W.M. (2007): Infection with *Trichomonas vaginalis* increases the risk of HIV-1 acquisition. J .Infect. Dis.,195 (5): 698–702.
Meingassner, J.G. and Thurner, J. (1979): Strain of *Trichomonas vaginalis* resistant to metronidazole and other 5-nitroimidazoles. Antimicrob. Agents Chemother.,15(2):254–7.
Meri, T., Jokiranta, S.T., Suhonen, L. and Meri, S. (2000): Resistance of *Trichomonas vaginalis* to metronidazole: Report of the first three cases from Finland and optimization of the In vitro susceptibility testing under various oxygen concentrations. J. Clin. Microbiol., 38: 763-7.
Myers, J., Curtis, B.S., Curtis, W.R. (2013): Improving accuracy of cell and chromophore concentration measurements using optical density. BMC Biophysics., 6(1): 4 doi:10.1186/2046-1682-6-4.
Palmas, C., Wakelin, D., Gabriele, F. (1984): Transfer of immunity against Hymenolepis nana in mice with lymphoid cells or serum from infected donors. Parasitol.,89(2):287–93.
Rabiee, S., Bazmani, A., Matini, M., Fallah, M . (2012): Comparison of resistant and susceptible strains of *Trichomonas vaginalis* to metronidazole using PCR method. Iran. J .Parasitol.,7(3):24–30.
Rosa,A., Rocha,D.A., deSouza,W., et al., (2011): Ultrastructural alterations induced byΔ(24(25))- sterol methyltransferase inhibitors on *Trichomonas vaginalis*. FEMS Microbiol. Lett. ,315(1):72-8.
Saddiq, AA. (2004): The use of musk as an antibiotic against fungi and yeasts. Patented. King Abdul Aziz City for Science and Technology.Riyadh,SA..
Saddiq, A.A. (2008): Use of natural AMSC Lung product for the treatment of lung cancer Patented (No. 0829539).King Abdul Aziz City for Science and Technology.
Saddiq, A.A. (2011): Potential Effect of Natural Musk and Probiotic on Some Pathogens Strain. Int Res J Microbiol.,2(5) : 146-52.
Saddiq, A.A (2014); Antiagnostic Effect of Musk and Sidr Leaves on Some of the Opportunistic Fungi that Cause Lung Toxicity. Life Sci J.,11(2s) :99-108.
Saddiq, A.A. and El-Eliany, R.A . (2009): Fungi hepatotoxicity and treatment with musk extract and lotus. Egy J biol exp.,2:21-7.
Scopel, M., dos Santos, O., Frasson, A. P., et al. (2013): Anti-*Trichomonas vaginalis* activity of marine-associated fungi from the South Brazilian Coast. Exp. Parasitol., 133: 211–6.
Sebitloane, H.M., Moodley, J., Esterhuizen, T.M. (2011): Pathogenic lower genital tract organisms in HIV-infected and uninfected women and their association with postpartum infectious morbidity. S. Afr Med J.,101:466-9.
Sharafi, S.M., Yousefi, M., Yousefi, H.A., et al. (2013): In vitro effects of various plants extracts on the growth of *Trichomonas vaginalis*. Infect Disord Drug Targets. ,13(5):322-7.
Sherrard, J., Ison, C., Moody, J., Wainwright, E., Wilson, J., Sullivan, A. (2014): United Kingdom National Guideline on the Management of *Trichomonas vaginalis*. Int. J. STD. AIDS. , 25(8):541-9.
Soper, D. (2004):Trichomoniasis: Under control or undercontrolled?. Amer.J. Obstet Gynecol.; 190 : 281–90.
Sutton, S. (2011): Measurement of Microbial Cells by Optical Density Microbiology Topics. J Val Tech.,17 (1) 46-9.
Takaoka, H . (2007): Structure-odour relationships of macrocyclic musks and harmony with the environment. IFEAT International Conference in Budapest, Hungary. , 36-43 in the printed Conference Proceedings.
Thevis, M., Schänzer, W., Geyer, H., et al. (2013): Traditional Chinese medicine and sports drug testing: identification of natural steroid administration in doping control urine samples resulting from musk (pod) extracts. Br. J .Sports Med. , 47(2):109-14.
Valdez, J.G. and Piccolo, R.J. (2006): Use of Spectrophotometry as a Tool to Quantify the Sporulation of Penicillium allii in Garlic Lesions. Fitopatol. Bras.; 31(6):595-7.
Viikki, M., Pukkala, E., Nieminen, P., Hakama, M. (2000): Gynaecological infections as risk determinants of subsequent cervical neoplasia. Acta. Oncol., 39: 71–5.

World Health Organization (2001): Global prevalence and incidence of selected curable sexually transmitted infections. overview and estimates.WHO, Geneva Switzerland.
Ye, T., Jin, C., Zhou, J., et al. (2011): Can odors of TCM be captured by electronic nose? The novel quality control method for musk by electronic nose coupled with chemometrics. J Pharm Biomed Anal. 55(5):1239-44.