

Anti-*Trichomonas vaginalis* activity of nano *Micana cordifolia* and Metronidazole: an in vitro study

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Received: 31 December 2016 / Accepted: 23 May 2017 / Published online: 30 May 2017
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Abstract *Trichomonas vaginalis* is a flagellate parasite living in the genital tract and it is accounted as a sexually transmitted disease. The clinical symptoms vary from the asymptomatic to the severe form which is usually associated with the irritation, itching and infertility in some severe cases. Many drugs have been applied to treat this disease and Metronidazole is the gold standard for treatment; however, it has also detected that this medicine has many side-effects which it has been motivated the researchers to find an appropriate alternative for this medicine. One of the treatment options is the use of the herbal medicines and natural compounds. Thus, the aim of this study was to compare the in vitro anti-*T. vaginalis* activity of nano-emulsion of *Micana cordifolia* and Metronidazole. In this study, *T. vaginalis* was isolated from the clinical samples and were cultured on a modified Dorsate medium. The nano-emulsion of *M. cordifolia* was prepared by heating technique. The effect of nano-emulsion of *M. cordifolia* was separately investigated on the *T. vaginalis* at the times of 12, 24 and 72 h and the obtained data were analyzed by the Spss 20 using the ANOVA test. The results indicated that the concentration of 100 ppm of nano-emulsion of *M. cordifolia* at the times of 12, 24 and 72 h has the anti-*T. vaginalis* activity of 44 ± 1.66 , 37 ± 1 and 25 ± 2 , respectively. It is also observed that the concentration of 500 ppm of the extract has the best effect and was able to eliminate the 85% of *T. vaginalis*. Furthermore, the anti-*T. vaginalis* activity of nano *M. cordifolia* was observed to be 100% in the

concentrations of 1000 ppm. It can be concluded, based on the results, that the nano *M. cordifolia* has acceptable efficacy on the elimination of *T. vaginalis* and it can be a suitable alternative for Metronidazole after implementation of complementary tests on laboratory animals and human cells.

Keywords *Micana cordifolia* · *Trichomonas vaginalis* · Extract · Metronidazole

Introduction

Trichomonas vaginalis is a flagellate parasite which is living in the genital tract. Several studies have shown that about a third of the world population is annually affected with a sexually transmitted disease (STD) (Edwards et al. 2016). *T. vaginalis* has known as one of important causative agents of these diseases so that approximately 180 million women are annually infected by this parasite in the entire of the world (Lin et al. 2015; Workowski and Bolan 2015). In developing countries, more than 50% of the patients who is admitted to the STD clinics are suffering from the *trichomoniasis* (Workowski and Bolan 2015). This parasite can cause the vaginal and cervical infections in the women and also can cause the urinary tract infection in both men and women (Doxtader and Elsheikh 2016). Vaginal discharge is of most common clinical symptom of this disease which it can be associated with the abundant secretion, irritation, itching, frequent urination and lower abdominal pain (Kissinger 2015). This infection is often clinically asymptomatic in the men, but it can affect the prostate, seminal vesicle and the upper parts of the urogenital system in severe cases (Doxtader and Elsheikh 2016).

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The disease is often transmitted through sexual intercourse and it can easily transmit between sexual partners; it has a significant prevalence between the female prisoners and prostitutes and is considered as a major health problem among these populations. This disease has been reported from all regions of the country and its prevalence varies from 3 to 70%. The people without clinical symptoms has key role in the transmission of this disease and can be extremely important as a carrier from an epidemiological point of view (Poole and McClelland 2013). The presence of clinical symptoms is the diagnosis basis for the physicians to determine the infection; but, in some cases, the *trichomoniasis* cannot be certainly diagnosed by only its clinical symptoms such as irritation, itching and smelly discharge and the definite diagnosis of this disease should be performed by vitro methods (Mehlhorn 2009).

The selective drug and gold standard used to treat the *trichomoniasis* is the Metronidazole. The results of the previous studies have indicated that Metronidazole has carcinogenic effects on the human cells and the drug resistance has established against to this drug which can be considered as the reasons to conduct the studies for new cost-effective and available drug (Workowski and Bolan 2015). So far, various drugs have examined for the in vivo and in vitro treatment of *trichomoniasis* infection. Of the drugs used, the herbal medicines, as natural products, have found more popularity than other drugs for researchers (Abdali et al. 2015; Ziaei Hezarjaribi et al. 2016).

Micana cordifolia belongs to the order of Asterales. The antimicrobial and antifungal effects of this plant have been proven in various studies and an acceptable effect of this plant has been reported (Muelas-Serrano et al. 2000; Laurella et al. 2012; Kar et al. 2013). Given the importance of *trichomoniasis* in our country and around the world, this study was conducted to detect the efficacy of the nano *M. cordifolia* in elimination of *T. vaginalis*. On the other hand, this study was aimed to investigate the in vitro anti-*T. vaginalis* activity of Nano *M. cordifolia*.

Materials and methods

Preparation of *Micana cordifolia* extracts

In this study, *M. cordifolia* was provided from Mountainous area of Mazandaran province, Iran. The leaves of *M. cordifolia*, after plant herbarium confirmation, were separated and were dried in the shade and crushed into powder. This powder was maintained in the dark containers and the extracts of the plant was prepared by distillation method. The extracts of *M. cordifolia* (1% w/w) in span (0.5% w/w) as oil phase were combined with the Tween 80 (1% w/w) in deionized water as the aqueous phase. The nano-emulsion was prepared by

following method: Tween 80 was dispersed in deionized water and the Span 60 as mineral oil was added. The water and oil phases were heated at 40 °C and Oil phase was added to the water phase by homogenization and were mixed for 5 min at 22,000 rpm (D-91126 Schwabach, Heidolph, Germany). The mixture was sonicated for 40 min using an Ultrasonicator (Bandelinsonopuls Berlin, Germany) and then, the obtained nano-emulsion of oil in water was cooled in the ice bath. The concentrations of 100, 500 and 1000 ppm of the extracts were applied in this study (Rahimi et al. 2015).

Gas chromatography

Gas chromatography–mass spectrometry (GC–MS) was carried out by Hewlett Packard 6890 series and the DB-5 capillary column (30 m × 0.25 mm, film thickness 0.25 μm) which it was initially performed for 5 min at 60 °C and then, it was continued with increasing the temperature of the device up to 220 °C (4 °C per each min). The Helium was used as the carrier with the speed of 2 ml/min.

Cultivation of *T. vaginalis*

Parasites isolated from patients, after confirmation of species, were cultured in the Dorsate medium and subsequently, the parasite was counted by Neubauer slide. The number of 500,000 parasites per ml was used for this study.

The effect of nano *M. cordifolia* on *T. vaginalis*

In the present study, the different parasite concentrations with Metronidazole were used as a positive control and also the medium with DMSO and Tween 80 were used as the negative control. The tubes containing 500,000 parasites were incubated at 37 °C and the herbal nano-emulsion were consequently added. Finally, the number of the living *Trophozoite* in positive and negative controls and in different concentration was counted by the microscopy technique and methylene blue 1%. The percentage growth inhibition was calculated by the following Eq. 1:

$$\%GI = \left(1 - \frac{GR_{\text{extract}}}{GR_{\text{control}}} \right) 100 \quad (1)$$

Statistical analysis

The data obtained from this study were analyzed by Spss 20 software using the ANOVA test.

Cytotoxicity assay

The 2,5-diphenyl tetrazolium bromide (MTT) colorimetric technique established by Rahimi (Rahimi et al. 2015) with

revision was utilized to display the cytotoxic activity of nano *M. cordifolia*. Summery, the macrophage cells (1×10^4 cells/ml) were cultured for 24 h on cell culture plates, and were then exposed to 3 different concentrations (100, 500 and 1000 ppm) of nano *M. cordifolia*. Additionally; metronidazole and DMSO were used as positive and negative controls, respectively. For analysis of the viability, 10 μ l MTT reagents in 5.0 mg/ml PBS was added to each well and then kept at 37 °C for 3 h after incubation time, centrifuged at 1300 rpm for 5 min.(Sariego et al.

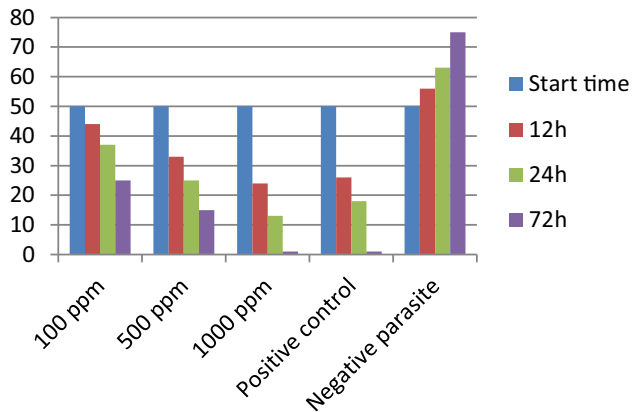


Fig. 1 Effect of the concentrations of nano-emulsion of *M. cordifolia* (100, 500 and 1000 ppm) on the *T. vaginalis* after 12, 24 and 72 h compared with the positive controls (Metronidazole) and negative control (culture medium with DMSO and Tween 80)

(2014) DMSO was added to the Sediment and after 20 min. The optical density (OD) of each well was measured at 550 nm. All experiences were done in triplicate.

Results

The nano *M. cordifolia* extracts were used in this study. The nano-emulsion of the *M. cordifolia* in the concentrations of 100, 500 and 1000 ppm has an acceptable effect against *T. vaginalis*, which it has detailed in the Table 1 and Fig. 1. The best results are observed in the concentration of 1000 ppm.

According to Table 1, there is a significance difference between the effects of different concentrations of nano-emulsion at different times ($P < 0.05$). The results of this study have shown that the concentration of 1000 ppm of nano-emulsion has an appropriate ability equal with the Metronidazole, the gold standard drug for the treatment of trichomoniasis, against the *T. vaginalis*.

Discussion

In present study, the anti-*T. vaginalis* activity of nano-emulsion of *M. cordifolia* were compared to Metronidazole ability in the elimination of this parasite which it indicates

Table 1 Effect of the concentrations of nano-emulsion of *M. cordifolia* (100, 500 and 1000 ppm) on the *T. vaginalis* after 12, 24 and 72 h compared with the positive controls (Metronidazole) and negative control (culture medium with DMSO and Tween 80)

	Parasite count	First time 10,000 parasite/ml	12 h 10,000 parasite/ml	24 h 10,000 parasite/ml	72 h 10,000 parasite/ml	P value
Nano plant	100 ppm	50	42	37	23	<0.05
	100 ppm	50	46	39	28	<0.05
	100 ppm	50	45	36	24	<0.05
	Mean (\pm)		44 \pm 1.66	37 \pm 1	25 \pm 2	
	500 ppm	50	36	24	18	<0.05
	500 ppm	50	31	29	14	<0.05
	500 ppm	50	33	23	14	<0.05
	Mean (\pm)		33 \pm 1.66	25 \pm 2.33	15 \pm 1.66	
	1000 ppm	50	26	17	3	<0.05
	1000 ppm	50	19	11	0	<0.05
	1000 ppm	50	28	13	1	<0.05
	Mean (\pm)		24 \pm 3.6	13 \pm 2	1 \pm 0.3	
Negative control	50	56	63	75	<0.05	
Positive control Metronidazole	50	31	19	0	<0.05	
	50	26	21	1	<0.05	
	50	21	16	2	<0.05	
	Mean (\pm)		26 \pm 3.33	18 \pm 2	1 \pm 0.33	

the a considerable efficacy of the nano-emulsion in this case. The use of herbal medicines to treat a variety of diseases has achieved a large attention recently; so that approximately 20,000 plants are used for this purpose in the world. Many well-known and effective drugs have the plant origin. For example, the quinine, an anti-malarial drug, is provided from cinchona bark which it also has the synthetic derivatives such as chloroquine, amodiaquine and (Swartzwelder et al. 1955; Sapp 1964; Genc 1976).

There are various studies towards the use of the herbal medicines for the elimination of *T. vaginalis* parasite. *Artemisia*, *Zataria multiflora*, *Myrtus* (Simbar et al. 2008), *Lavandula* (Moon et al. 2006), *Allium sativum*, *Asafoetida* (Ahmed 2010), *Artemisia absinthium*, *Achillea* (Rafeian et al. 2011), *Eucalyptus* (Hassani et al. 2013) and *Lavandulifolia Stachys* (Youse et al. 2012) are of the plants which has been used to destroy the *T. vaginalis* in Iran. Furthermore, other plants such as *Polygala decumbens*, *Maytenus imbricate*, *Harungana madagascariensis*, *Hypericum polyanthemum* and etc. have been used in the various researches towards the elimination of the *T. vaginalis* in other parts of the world (Iwalewa et al. 2008; Cornejo and Janovec 2010; Frasson et al. 2012; Cargnin et al. 2013). Azadbakht and Ziaei has conducted two separate studies to investigate the effects of the *Artemisia*, *Z. multiflora* and *Myrtus* in elimination of the *T. vaginalis* (Azadbakht et al. 2003). In these studies, the concentration of 0.001 mg/ml of *Artemisia* was able to destroy the all of parasites at the beginning of the cultivation; however, the *Z. multiflora* and *Myrtus* has completely destroyed the parasites in the concentration of 0.004 ppm. In addition, the most efficiency of the methanolic extracts of plants tested was observed in concentrations of 0.01 (Azadbakht et al. 2003). Kazemian et al. has examined the inhibition activity of *Eucalyptus* on the *T. vaginalis* and has shown that the hydroalcoholic extracts of this plant in concentrations of 60 and 90 mg/l can reach to 100% of efficacy against this parasite (Kazemian et al. 2012). Mehdi et al. in the In vitro studies has demonstrated that the *Eucalyptus* in pH = 4.65 and *Myrtus* in pH = 5.35 after 24 h are able to eliminate all parasites in an environment (Mahdi et al. 2006).

M. cordifolia is orally used as a diuretic and carminative medicine and is topically used as an anti-inflammatory and analgesic medicine. Recent studies have suggested that the *M. cordifolia* due to its effective components can successfully eliminate the infectious agents such as bacteria, fungi and parasites (Sen and Batra 2012).

In addition, the anti-parasitic effects of *M. cordifolia* have been surveyed in various studies and it has detected that this plant has also an anti-parasitic potential (Muschiatti et al. 2013).

In this study, the nano-emulsion of *M. cordifolia* was used in concentrations of 100, 500 and 1000 ppm and it was observed that the concentration of 1000 ppm of this nano-emulsion has outstanding effect against the *T. vaginalis* so that its effect is equal to the ability of Metronidazole as a Gold standard drug in elimination of these parasites. Recently, the nano-science has gained a variety of applications in various fields which application of this science in medicine and pharmacy industries is considered as a most important application of the nano-science (Koushik et al. 2016). For example, nano-silver compounds have effectively utilized in the treatment of Giardiasis and Leishmaniasis and the results of these studies has been totally satisfactory (Shahcheraghi et al. 2016). Medical Nano-science has provided a new path for treatment, diagnosis, monitoring and biological control of the diseases. This material can be involved alone or have been involved in drug delivery into cells. This material can target the specific cell or intracellular organs and these capabilities are extensively used in medical and pharmaceutical sciences. Given the role of nanoparticles, the drug can bind to their target within the cell or on its surface and are able to have good efficacy. In addition, it is led to avoid the non-specific binding of the drug and to reduce the side-effects of the drug (Want et al. 2016).

Typically, the target of the pharmaceutical compounds is the *monocytes*, *macrophages*, *dendritic cells*, *endothelial cells* and *cancer cells*. Parasitic infections such as *malaria*, *Leishmaniasis*, *Toxoplasmosis* and *trypanosomiasis* can be suitable targets for this family of drugs. However, the application of nano-science has not largely applied in medical Parasitology which it is due to lack of a comprehensive study in this area. Leishmania is an obligate intracellular parasite that it causes the *cutaneous leishmaniasis*, *mucocutaneous leishmaniasis* and visceral infection in the human and the Pentavalent compounds are the selective drug for the treatment Leishmania but it has enormous side effects due to its high toxicity. Several studies have revealed that the liposomes compound along with nanoparticles, due to its small size and specific shape, can easily enter into the cells and can play an acceptable anti-Leishmania activity (Want et al. 2016).

Furthermore, the nano-materials have been applied to eliminate intracellular tachyzoites of *Toxoplasma* and have demonstrated superior effects. In different studies, the researchers has been injected the nano-gold with specific antibody of the cell surface antigens into the body; these compounds were bonded to the cell surface and then were entered into the cell and were led to destroy the intracellular tachyzoite. This feature can be employed to eliminate other intracellular parasites which there are in the blood for long time such as malaria (Fernandes-Cunha et al. 2016).

Bavand et al. (2014) has studied the effect of gold nanoparticles in the three concentrations of 0.05, 0.1 and 1.3 ppm on the human isolates of *Giardia lamblia* cyst. In that study, the researchers have compared the effects of gold nanoparticles with Metronidazole (positive control group) (Golami et al. 2016). The results of this study have shown that gold nanoparticles in concentration of 0.3 were able to destroy 96% of this *G. lamblia* within 180 min while the Metronidazole destroyed 99% of this parasite which there is no statistically significant difference. In another study, Jaffari et al. has used the nano silver for the treatment of *cutaneous leishmaniasis* caused by *L. major* and has observed the same effect with the positive control group (Jaffary et al. 2016).

In conclusion, given the importance of the *trichomoniasis* and its considerable prevalence in the world, the finding of the effective treatment with minimal side effects is accounted as important health care goals. The results of this study have shown that *M. cordifolia* as a natural compound has a suitable potential for elimination of the *T. vaginalis* and it can be an appropriate alternative to Metronidazole and other synthetic drugs. It is also proposed that the side-effects of *M. cordifolia* on the human cells and also it be used in vivo.

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