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Original Article

Green synthesis of nano-liposomes containing *Bunium persicum* and *Trachyspermum ammi* essential oils against *Trichomonas vaginalis*

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KEYWORDS
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Abstract *Background: Trichomonas vaginalis*, a parasitic flagellated protozoan, is one of the main non-viral sexually transmitted diseases worldwide. Treatment options for trichomoniasis are limited to nitroimidazole compounds. However, resistance to these drugs has been reported, which requires the development of new anti-*Trichomonas* agents that confer suitable efficacy and less toxicity.
Methods: In the present work, we assessed the effectiveness of the liposomal system containing essential oils of *Bunium persicum* and *Trachyspermum ammi* against *T. vaginalis in vitro*. The chemical composition of *B. persicum* and *T. ammi* were analyzed using gas chromatography-mass spectrometry (GC—MS). Liposomal vesicles were prepared with phosphatidylcholine) 70%) and cholesterol)30%) using the thin-film method. The essential oils of *B. persicum* and *T. ammi* were loaded into the liposomes using the inactive loading method. Liposomal vesicles were made for two plants separately. Their physicochemical features were tested using Zeta-Sizer, AFM and SEM. The anti-*Trichomonas* activity was determined after 12 and 24 h of parasite cultures in TYI-S-33 medium.
Results: After 12 and 24 h of administration, the IC_{50} of the *B. persicum* essential oil nano-liposomes induced 14.41 μg/mL and 45.19 μg/mL, respectively. The IC_{50} of *T. ammi* essential oil nano-liposomes induced 8.08 μg/mL and 25.81 μg/mL, respectively.
Conclusions: These data suggested that nano-liposomes of the essential oils of *B. persicum* and *T. ammi* may be a promising alternative to current treatments for *Trichomonas* infection.

Introduction

Trichomonas vaginalis (*T. vaginalis*), a flagellated parasitic protozoan, is one of the leading non-viral sexually transmitted infections worldwide, with approximately 248 million new cases detected annually causing trichomonosis.[1] However, most infected women and men are asymptomatic, but *T. vaginalis* is an important cofactor in promoting the spread of the human immunodeficiency virus (HIV) and may have a major impact on the dynamics of the HIV epidemic in some countries.[2–4] Furthermore, *T. vaginalis* has been associated with severe consequences, such as adverse pregnancy outcomes and preterm birth, infertility, predisposition to cervical cancer, premature rupture of membranes and pelvic inflammatory disease.[5] Moreover, this parasite can cause intellectual disability in children and brain abscess in neonates.[6–9] The diagnosis of trichomoniasis is made by different approaches, including clinical diagnosis, wet mount examination, culture in medium, cell culture, nucleic acid detection and antibody-based technique. Non-culture-based tests often provide an increase in the ability to detect infections, because viable organisms are no longer required, but the culture system is potentially useful in resource-constrained settings where clinic-based microscopy may not be feasible, but a central laboratory may be able to provide low-tech needs, such as incubation and microscopic evaluation. Furthermore, it is inexpensive, so we used the Diamond's TYI medium in this study.[1]

Treatment options for trichomoniasis are limited to Nitroimidazole compounds. However, treatment failure occurs, mainly because of significant gastrointestinal adverse effects, which disappear after treatment. Furthermore, resistance to Metronidazole has been reported in at least 5% of clinical cases of trichomoniasis, which could lead to higher and sometimes toxic doses in these patients. Consequently, potent new alternatives with low toxicity against *T. vaginalis* are urgently needed.[10,11]

In recent years, there have been growing studies of the therapeutic potential of natural or herbal products. Medicinal plants are generally considered to be safe and have low toxicity compared with synthetic drugs.[12] *Bunium persicum* and its derivatives have reported multi-pharmacological effects, including antimicrobial, antioxidant, anti-inflammatory, Antinociceptive, antidiabetic, antifungal and

antibacterial.[13–18] *Trachyspermum ammi* has impressive biological and pharmacological properties, including antimicrobial, antihyperlipidemic, anthelmintic, antibacterial and insecticidal activities.[19–23]

Liposomes, small vesicles produced by the dispersion of phospholipids in the aqueous medium, trap the aqueous medium between their closed concentric spheres of the phospholipid membranes.[24,25] Liposomes can contain hydrophilic and lipophilic drugs.[26] Furthermore, they are endurable and safe in clinical trials, and some formulations have been approved by the FDA.[27,28] Some methods are generally used to prepare liposomes for diagnosis and drug delivery purposes.[29] Phospholipid vesicles enhance the penetration of compounds incorporated and/or encapsulated in them.

These liposomes containing essential oils with greater permeability and concentrations, and then address concerns such as side effects, low drug solubility in water, and lack of proper drug delivery to protozoa. In the present study, the nano-liposomal lipid carriers of essential oils of *B. persicum* and *T. ammi* were used against *T. vaginalis in vitro*.

Materials and methods

Plant collection

B. persicum and *T. ammi* were collected from the mountain Bahabad, located in the Province of Yazd, in Iran, and their species were identified and authenticated in the botanical section of the Yazd Agricultural Research Center, with the voucher numbers 1141 and 24,985 respectively. A total of 100 g of seeds of *B. persicum* and *T. ammi* were harvested, dried, and mechanically powdered using an electrical blender. Clevenger device was used to extract essential oils of two plants. The chemical composition of *B. persicum* and *T. ammi* was analyzed using gas chromatography-mass spectrometry (GC—MS) (Agilent19091S-433, USA).

Preparation of liposomal systems containing essential oils of *B. persicum* and *T. ammi*

The liposomal system containing essential oils was prepared by thin-layer coating with a combination of soybean phosphatidylcholine) 70% (and cholesterol)30%). Soybean phosphatidylcholine choline, cholesterol and essential oil were dissolved in a chloroform solvent at 45 °C on a rotary (Heidolph Instruments GmbH & Co. KG, Schwabach, Germany) and dried under vacuum. Hydration was then performed by adding sterile distilled water for 1 h at 55 °C. The prepared nano-liposomes were then reduced in size using a bath sonicator for 40 min. At the end of the mixture, the size reduction was filtered using 0.45 and 0.20 μm filters to homogenize the constituent particles.

Determination of essential oil loading percentage in liposomal systems

Liposomes containing essential oils were inserted into the dialysis bag after size reduction and filtration and incubated with distilled water for 4 h at 4 °C to remove the free and uninspired essential oil. Liposomes were mixed with 1:20 isopropyl to break the lipid wall around the essential oil and release the essential oil. The amount of essential oil absorbed in the liposomal system was determined by a wavelength spectrophotometer. Finally, using the after relationship, the percentage of *B. persicum* and *T. ammi* load of essential oils in liposomal systems was determined separately.

$$\text{Encapsulation efficiency}\ (\%) = \frac{\text{Essential oil in liposome}\ (\text{mg})}{\text{Total of essential oil}\ (\text{mg})} \times 100$$

Essential oils release from liposomal systems

The release of essential oils from liposome system was performed by the dialysis bag method. In this method, liposomes containing the essential oils were poured into the dialysis bag and placed in the vicinity of the PBS buffer at 35 °C and pH = 6 for 24 h. The absorbance was read by spectrophotometer)570 nm (and based on this, the release chart of liposome essential oils was drawn. All steps were performed separately to release from the liposome system containing *B. persicum* and *T. ammi*. The release results were analysed according to the standard deviations.

Size and zeta potential characterization of essential oils in liposomal systems

The particle size distribution range, and particle size peak, was determined using Dynamic Light Scattering (DLS) by nanosizer (Brookhaven Instruments Corp, NY, USA). Nano-liposomes were measured at an angle of 90° and laser light irradiation at 657 nm at 25 °C was used. The sample was prepared diluted to 0.1 mg/mL. Measurements were performed immediately after preparation. Samples were measured 3 times and each time for 30 s. Furthermore, surface charges zeta potential of nano-liposomes containing essential oils were measured by Zeta Sizer (Brookhaven Instruments Corp, NY, USA).

Morphology of liposomal systems containing essential oils

The liposomal system was imaged using an Atomic Force Microscope (AFM) (Nano wizard II, JPK instruments, Berlin) Germany (to confirm the formation of essential oil-bearing nano-liposomes containing essential oil. Furthermore, 25 μL of liposome sample was poured onto a slide and air dried. Samples were then coated with gold for a few seconds to be conductive. At the end, the surface morphology of the nano-carriers (roughness, shape, smoothing and mass) was investigated using a Scanning Electron Microscope) SEM) (EM3200, KYKY Technology Development Ltd, Beijing, China).

Cytotoxicity studies by MTT assay

The cytotoxicity of the empty liposomal system was determined by the MTT assay. To measure toxicity, cells were cultured separately at 1 × 104 cells in a 96-well plate for 24 h. Healthy human foreskin fibroblast) HFF (cells were then

treated with the same volume of fresh culture medium. Then, HFF fibroblasts were cultured in four replicates at 100—1000 μg/mL, respectively, and incubated again for 48 h. Then, 20 μL of MTT solution at a concentration of 5 mg/mL were added to each well and incubated for 3 h. The supernatant was then removed and 150 μL of DMSO was added to dissolve the Formosan crystals. At each step, centrifugation was performed to remove the liquid. The absorbance was recorded at 570 nm using a spectrophotometer. Finally, cell viability was calculated according to the relationship below:

Mean optical absorption in the test group - Average light absorption in culture medium.

Mean optical absorption in the control group - Average light absorption in culture medium.

Determination of IC_{50} nano-liposomes containing essential oil on *T. vaginalis*

The strain of *T. vaginalis* was isolated from the vaginal discharge of a 34-year-old woman. Inclusion criteria of the subjects recruited in this study were: 1) The patient who presented with the classic symptoms of trichomoniasis, such as a purulent, foul-smelling vaginal discharge, dysuria and dyspareunia, 2) The motile protozoa were observed in the wet mount prepared from her vaginal discharge, and 3) To confirm the *T. vaginalis* infection, we cultured the swab in TYI-S-33 medium) Merck, Darmstadt, Germany) and incubated at 37 °C for 48 h. Smears were prepared to observe parasites. Patients were at the laboratory of Isfahan University of Medical Sciences and serial sterile swabs were taken from vaginal fornix, and those samples were immediately cultured in TYI-S-33 medium.

This study was approved by the ethical committee of the University of Medical Sciences of Isfahan, Isfahan Province, Iran (Ref. No. 1396.851) to obtain human biological samples. The parasite isolate was cultured in TYI-S-33 medium and incubated at 37 °C until reaching the number of parasites in the logarithmic phase. Smears were preparedto determine the number of parasites. To count *T. vaginalis* using a hemocytometer, 15—20 μL of the parasite suspension was added between the hemocytometer and covered glass using a P-20 Pipetman. We counted the number of parasites in all four outer squares divided by four the mean number of parasites/square. The parasites of the logarithmic phase (10^5 parasites) were then exposed to different concentrations of *B. persicum* and *T. ammi* nano-liposomes at a concentration of 100,000 (per mL) grouped according to Table 1.

All groups were kept at 37 °C and after 12 and 24 h the number of live parasites was counted by Trypan blue using a Neubauer smear and light microscope Haemocytometer method. Growth inhibition was calculated for each of the different concentrations (1.95—1000 μg/mL) of liposome-containing essential oils in the parasite strain by the formula GI = (a-b/a (Where **a:** the number of live parasites in the negative control sample, **b:** the number of live parasites in the sample containing *B. persicum* and *T. ammi* nanoliposomes. Then, SigmaPlot™ 13 software was used to determine IC_{50} (50% inhibitory concentration) of the above nano-liposomes. It should be noted that all experiments were performed in three replicates separately for liposomal nano-carriers containing essential oils of *B. persicum* and *T. ammi* and the results were shown in an average level.

Selectivity Index SI is obtained from the IC_{50} value of liposomal nano-carriers containing essential oils of *B. persicum* and *T. ammi* against HFF cells divided by the IC_{50} value of *T. Vaginalis*. Selective activities of the compounds were calculated as follows:

Selectivity Index (SI) = IC_{50} Cells HFF/IC_{50} Cells *T. vaginalis*

Cellular uptake study

A total of 10^5 HFF cells in 6-well plates were grown in a monolayer for 24 h. Then, the cells with the optimal nano-liposomes formula contained DIL color without essential oil. For the fluorescence of the Newsome system and, to a large extent, 0.1% M in the organic phase to dry the structure, the additives were followed by the steps of the thin film method, as described by Alemi A et al., 2018.[30] Subsequently, fluorescent colored nano-liposomes attached to the DIL. These were incubated with essential oils for 3 h and washed with PBS. Subsequently, the 95% alcoholic solution was stabilized and the nuclei were stained with DAPI (1 mg/mL) for 15 min. The cells were harvested and then examined under a fluorescent microscopy (Olympus, Okayama-shi Okayama, Japan).

Statistical analysis

In this study, all data were recorded, edited, and entered in the Excel software to plot the results which were reported as mean and standard deviation. Meanwhile, the SigmaPlot™ 13 software (Systat Software Inc., San Jose, CA, USA) was also used to calculate IC_{50}.

Results

Release patterns of essential oils from the nano-liposomal systems

The caryophyllene (25.51%), γ-terpinene (13.81%), cuminyl acetate (11.29%), *p*-cymene (7.09%), β-pinene (6.23%) and α-pinene (4.56%) were identified the major chemical constituent of the essential oil of *B. persicum* seeds by GC/MS technique. The thymol (55.87%), γ-terpinene (20.52%), *p*-cymene (19.09%), α- and β-pinene (9.45%) were identified the major chemical constituent of the essential oil of *T. ammi* seeds.

Fig. 1 shows the release results of the essential oils containing *B. persicum* and *T. ammi* according to the standard deviations. The release charts for essential oils containing *B. persicum* and *T. ammi* from the liposomal system were plotted during 24 h at 35 °C and pH = 6 based on the calibration chart. Examination of the essential oil release pattern of the liposomal system (Table 2) shows that the explosive release of essential oil began in the early hours. Furthermore, the release charts for the two plants of essential oils were removed from both nano-liposomal systems in 24 h (100% release in 24 h).

Table 1 Effects of *B. persicum* and *T. ammi* nano-liposomes against *T. vaginalis*.

Number of groups	TYS-I-33 medium culture group	Final concentration of *B. persicum* and *T. ammi* nano-liposomes (μg/mL)	*T. ammi* number of parasites after 12 h	*T. ammi* number of parasites after 24 h	*B. persicum number of parasites after 12 h*	*B. persicum number of parasites after 24 h*
Negative control	Containing unexposed parasites	–	107,000	132,705	102,894	127,252
Positive control	Containing the 10^5 parasites (*T. vaginalis*) and metronidazole (64 μg/mL)	–	–	–	–	–
1	Contains the 10^5 parasites	1.95	103,792	120,761	94,662	106,892
2	Contains the 10^5 parasites	3.9	93,091	100,857	84,373	80,169
3	Contains the 10^5 parasites	7.81	81,320	84,935	68,939	55,991
4	Contains the 10^5 parasites	15.62	69,553	61,044	57,621	39,448
5	Contains the 10^5 parasites	31.25	60,992	35,832	47,331	20,361
6	Contains the 10^5 parasites	62.5	52,436	19,906	39,099	10,184
7	Contains the 10^5 parasites	125	40,667	1327	29,839	1273
8	Contains the 10^5 parasites	250	28,896	0	17,492	0
9	Contains the 10^5 parasites	500	17,125	0	5145	0
10	Contains the 10^5 parasites	1000	3211	0	0	0

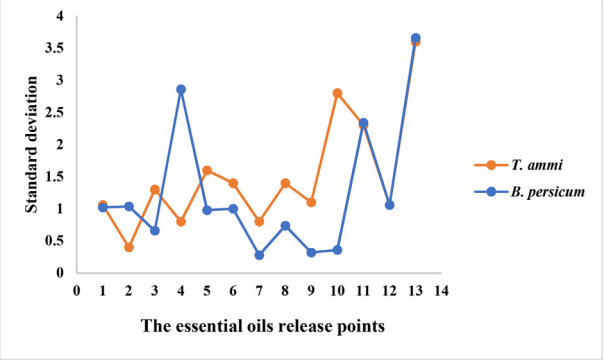

Figure 1. The release results according to the standard deviations. The release of essential oils from liposome system was investigated by measuring the absorbance at 570 nm.

Characterization of the size and zeta potential of essential oils in the nano-liposomal systems

Figs. 2 and 3 show the DLS results of essential oils containing *B. persicum* and *T. ammi*, 121 nm and 111 nm, respectively. Furthermore, the surface charge (zeta potential) of the nano-liposomal systems of two plants of essential oils was calculated to be −16.7 mV and −9 mV, respectively (Figs. 4 and 5). The DLS results and zeta potential were also calculated for the nano-liposomal systems containing *T. ammi* were 111 nm and −9 mV, whereas for *B. persicum* they were 121 nm and −16.7 mV, respectively. The release of two plants essential oils from the liposomal system was slow in 24 h and after 24 h were completely released.

The present study led to the production in the nano-liposomal systems of essential oils containing *B. persicum* and *T. ammi*. The results showed that the load of essential oils containing *B. persicum* and *T. ammi* in the nano-liposomal systems was 51.64 ± 1.24 and 40.12 ± 2.71%, respectively.

Morphology of nano-liposomal systems containing essential oils

The morphology on the nano-liposomal systems of both essential oils was investigated by the Atomic Force Microscope (AFM), Scanning Electron Microscope (SEM), and the formation of liposome was confirmed (Figs. 6 and 7). The SEM image shows that the morphology of the constituent particles are liposome systems containing essential oil, are spherical with smooth surface and average particle size from 25 to 40 nm.

Table 2 The release of the essential oil containing *B. persicum* and *T. ammi* in PBS.

Point number	1	2	3	4	5	6	7	8	9	10	11	12	13
Time (hour)	0.5	1	1.5	2	2.5	3	3.5	4	4.5	5	6	12	24
B. persicum release (%)	39.17	44.42	48.47	55.16	62.39	66.99	68.42	69.09	69.32	69.56	77.79	83.61	95.25
Std	1.02	1.04	0.66	2.86	0.98	1	0.28	0.74	0.32	0.36	2.34	1.06	3.06
T. ammi release (%)	16.55	19.62	21.41	29.11	37.12	40.07	45.84	53.03	57.84	65.74	76.42	95.82	100
Std	1.06	0.4	1.3	0.8	1.6	1.4	0.8	1.4	1.1	2.8	2.3	1.06	3.6

*Std: Standard deviation.

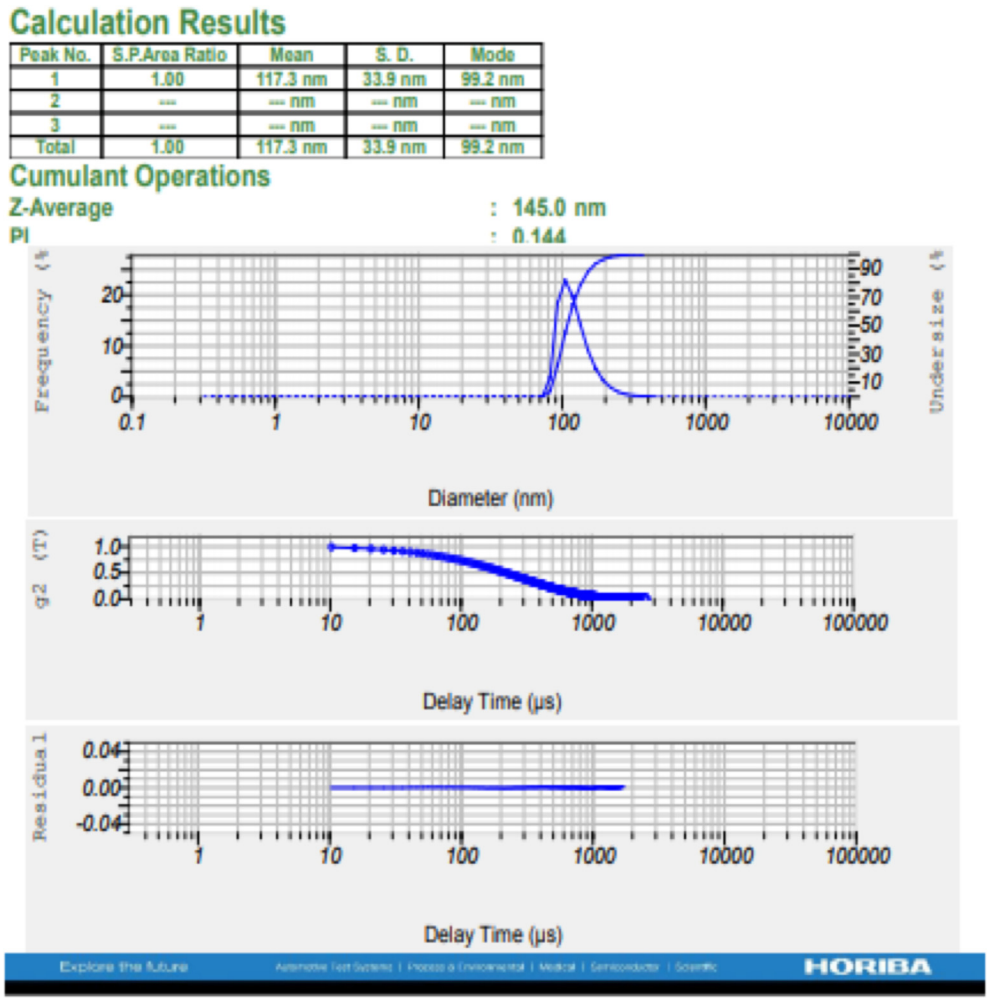

Figure 2. Size of the liposomal system containing essential oil of *B. persicum*. The particle size distribution range, and particle size peak, was determined using Dynamic Light Scattering (DLS) by nanosizer. Nano-liposomes were measured at an angle of 90° and laser light irradiation at 657 nm at 25 °C was used. The data are presented as mean ± SD.

Furthermore to electron microscopy images, it also confirms the formation of liposomal systems, spherical morphology and the appropriate constituent particles of liposomal systems. Cellular studies also showed that the nano-liposomal systems had low toxicity for HFF cells, and the nano-liposomal systems of essential oils containing *T. ammi* and *B. persicum* for 24 and 48 h showed toxicity for *T. vaginalis* and were furthermore prevented its growth.

Cytotoxicity studies by MTT assay on human foreskin fibroblasts (HFF) cell line

Human Foreskin Fibroblast (HFF) cells were examined by MTT-assay to determine the of the essential oil-free liposomal system cytotoxicity. The cells showed that the viability of these cells in the presence of 10 and 100 µg/mL of an empty liposomal system is 96.7% and 97.2%, respectively. Thus, the empty liposomal system has little toxicity to HFF cell lines.

IC_{50} of nano-liposomes containing the essential oil against T. vaginalis

The IC_{50} of *B. persicum*-containing liposome essential oil was calculated to be 45.19 µg/mL and 14.41 µg/mL in *T. vaginalis* after 12 and 24 h, respectively. The IC_{50} value of the nano-carrier containing *T. ammi* essential oil was determined after 12 and 24 h against the parasite, *T. vaginalis*, at 25.81 µg/mL and 8.08 µg/mL, respectively (Figs. 8 and 9). The value of SI (Selectivity Index) were 3.1 for *T. ammi* and 2.49 *B. persicum*.

The lethal effect of *B. persicum* on *T. vaginalis* at 12 and 24 h has a significant difference (P-value = 0.046), but the lethal effect of *T. ammi* on *T. vaginalis* was not significantly different (P-value = 0.052). The statistical analysis of the results obtained from the effect of *B. persicum* on *T. vaginalis* showed that all concentrations, except 0.0019 and 0.0039, were significantly different from the negative control (P-value <0.05) and concentration of 0.12 µg/mL and above there was no significant difference with positive

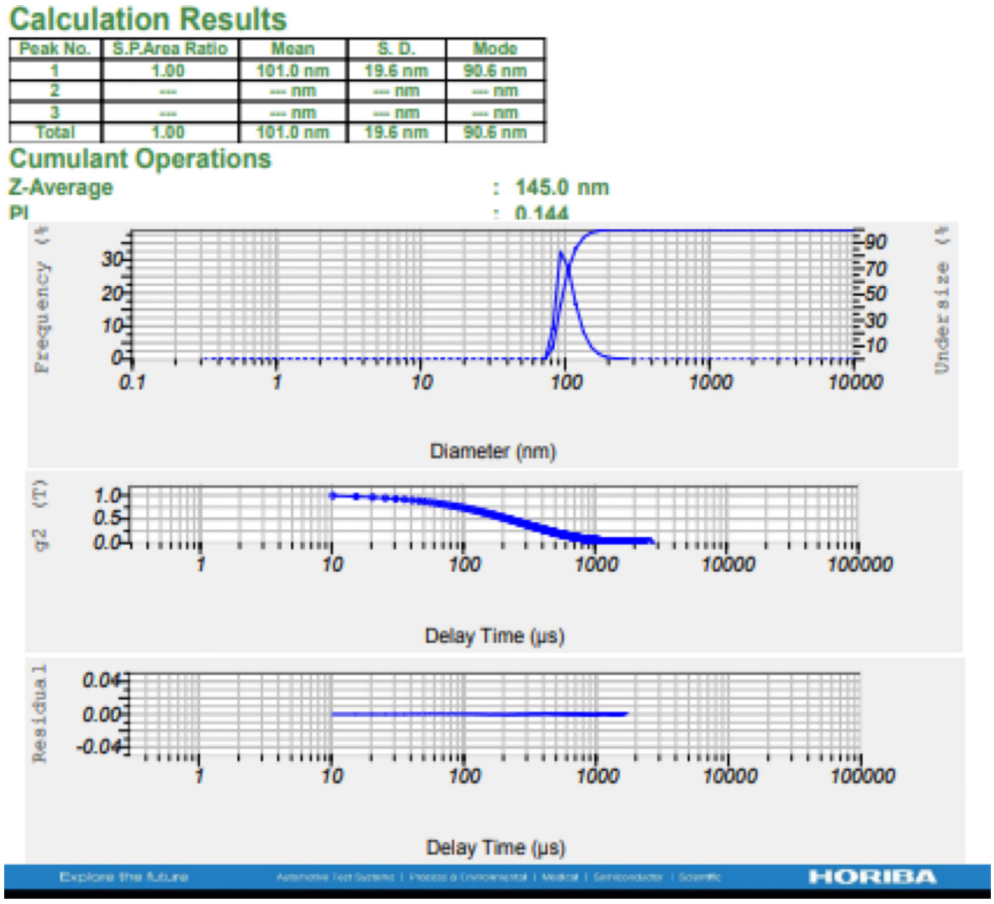

Figure 3. Size of the liposomal system containing essential oil of *T. ammi*. The particle size distribution range, and particle size peak, was determined using Dynamic Light Scattering (DLS) by nanosizer. Nano-liposomes were measured at an angle of 90° and laser light irradiation at 657 nm at 25 °C was used. The data are presented as mean ± SD.

control. The statistical analysis of the results obtained from the effect of *T. ammi* on *T. vaginalis* showed that all concentrations, except 0.0019, were significantly different from the negative control (P-value <0.05) and concentration of 0.12 µg/mL and above there was no significant difference with the positive control.

Cellular uptake study

Highly successful nano-liposomes cell extract containing essential oils without fluorescent dyes DIL was synthesized by fluorescent microscopy. According to Fig. 10, the intensity of green fluorescence in healthy HFF skin cells indicates the cellular uptake of nano-liposomes essential oils, nano-liposomes are well absorbed by the cell. Core-shaped with DAPI color (blue) and colored DIL nanoliposomes (green) were stained.

Discussion

Trichomoniasis is one of the most common sexually transmitted diseases in humans. The issue of drug resistance among microbial pathogens is an important and serious problem. Metronidazole has been the only approved drug combination for the treatment of trichomoniasis, and tinidazole in recent years in some countries, but failure to treat trichomoniasis with metronidazole has been reported because the early years. On the other hand, this drug also has side effects. Different reports of treatment of trichomoniasis failure have been published in different communities.[10] In recent years, the use of plants and natural compounds has been considered by researchers. The aim of this study was to investigate the anti-Trichomonas activity of the nano-liposomal lipid carriers of essential oils of *B. persicum* and *T. ammi* against *T. vaginalis in vitro*. The results of this study showed that after 12 and 24 h of administration, the IC_{50} of the *B. persicum* essential oil nano-liposomes induced 14.41 µg/mL and 45.19 µg/mL, respectively. The IC_{50} of *T. ammi* essential oil nano-liposomes induced 8.08 µg/mL and 25.81 µg/mL, respectively.

So far, previous studies have been performed on the production of lipid systems containing drugs and essential oils, each of which investigates different physicochemical factors of lipid systems, including loading efficiency, drug release pattern, particle size and surface charge zeta potential.[31,32] This physicochemical index depends on several factors, including the size of the constituent system, the type and molar percentage of phospholipids used in the structure of the liposomal system, the molar percentage of cholesterol used in the structure of the liposomal system and the nature of the loaded material.

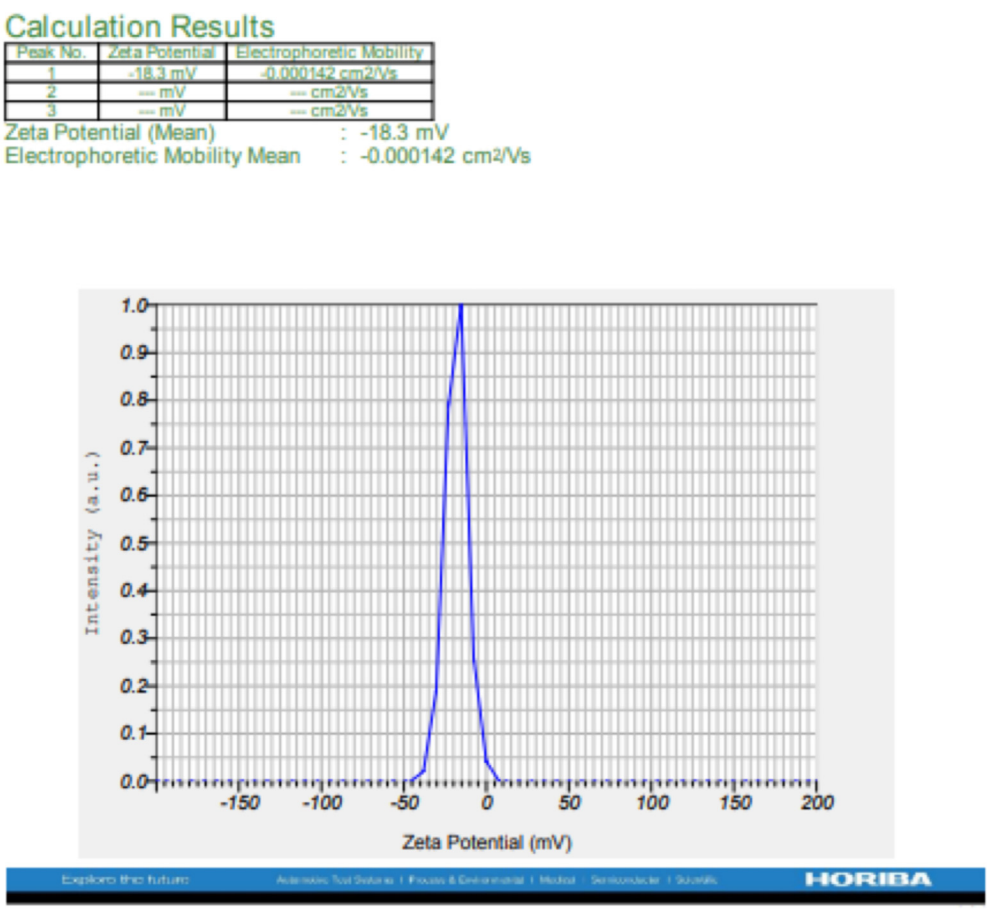

Fig. 4. Zeta potential of the liposomal system containing essential oil of *B. persicum*. Zeta potential of nano-liposomes containing essential oils were measured by Zeta Sizer. Zeta potential of the nano-liposomal systems of essential oils containing *B. persicum* was −16.7 mV.

In the present study, the amount of essential oils containing *B. persicum* and *T. ammi* in the liposomal system was measured as 40.12 ± 2.71 and 51.64 ± 1.24%, respectively. Other studies have yielded similar results, for example, Majdizadeh et al., 2018, reported loading of essential oil of *Mentha piperita* by 61.38%.[26] Furthermore, Haghiralsadat et al., 2016 reported the loading rate of essential oil of *T. ammi* in the liposomal system as 35.6± 7.4.[33] Other features studied in liposomal systems are the patterns of drug release. The present study showed that the release of essential oils of *B. persicum* and *T. ammi* from liposomal systems was slow in 24 h.

The pattern of release of these essential oils from the liposomal systems shows that, because of the high concentration of essential oils between the liposomal systems and the buffer that surrounds them, the release of essential oils from the system was explosive in the first hours and decreased more than with time. This difference in concentration decreased the release of the essential oil and caused the slope of its release chart to tilt to zero.[34]

Another important feature evaluated in this study was the level of superficial charge of the liposomal systems, which is one of the factors that influence the stability of the liposome. There is a direct relationship between the surface charge and the chance/stability of the liposomal systems. Therefore, the greater the surface load of the liposomal system, the greater the chance that these liposomal systems will form and aggregate, increasing their stability.[35–37]

Wachter et al.(2014) (investigated the effects of different concentrations of curcumin on *T. vaginalis* and reported that curcumin had an anti-*Trichomonas* effect at all concentrations.[38] Jabari et al.)2015) investigated the effects of *Chaerophyllum macropodum* extract on *T. vaginalis* and reported an inhibitory effect at all concentrations 2–150 mg/mL. Because the concentration of the extract increased, the inhibitory effect also increased.[39] Vazini et al.) 0.2017 (investigated the effect of the nano-emulsion of the extract of *Micana cordifolia* on the growth of *T. vaginalis* and results of their study showed that the concentration of 100 ppm of nano-emulsion of *M. cordifolia* at the times of 12, 24 and 72 h has the anti-Trichomonas activity of 44 ± 1.66, 37 ± 1 and 25 ± 2, respectively.[40] Fakhrieh-Kashan et al.(2017) evaluated the combined anti-*Trichomonas* effects of *Ginger officinale* and *Verbascum thapsus* extracts and reported that these extracts could increase apoptosis in *T. Vaginalis*.[41]

Majdizadeh et al.) 2018(developed a liposomal system containing peppermint essential oil in a thin layer with a particle size of 247 nm, a zeta potential of −34.54, a loading efficiency of 61.38% and a slow release at 62 hours[28]. It seems

Calculation Results

Peak No.	Zeta Potential	Electrophoretic Mobility
1	-17.8 mV	-0.000138 cm2/Vs
2	--- mV	--- cm2/Vs
3	--- mV	--- cm2/Vs

Zeta Potential (Mean) : -17.8 mV
Electrophoretic Mobility Mean : -0.000138 cm²/Vs

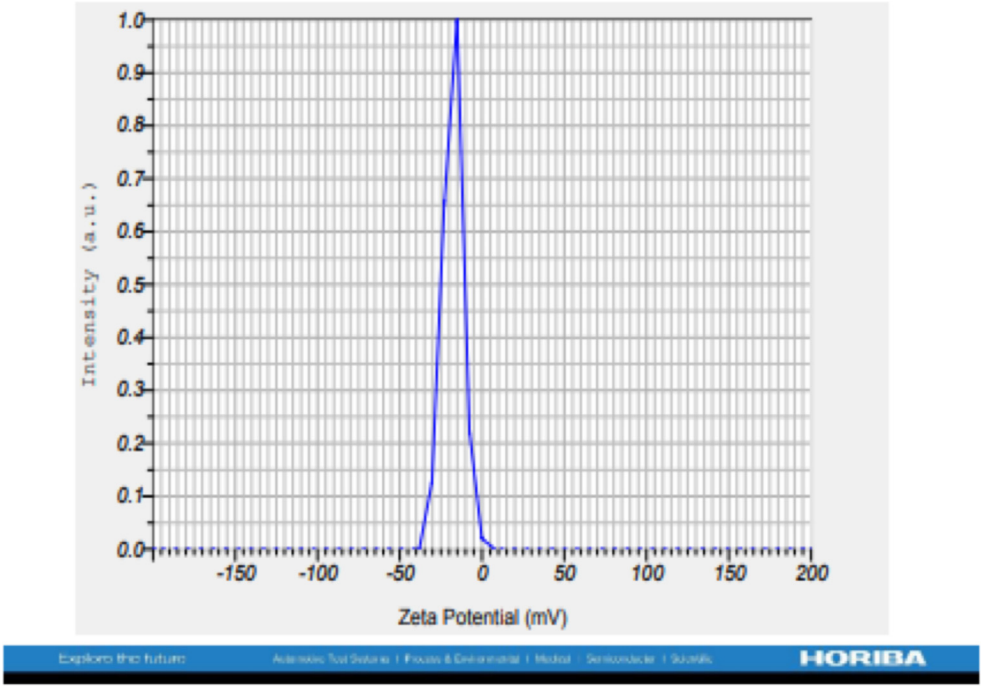

Figure 5. Zeta potential of the liposomal system containing essential oil of *T. ammi*. Zeta potential of nano-liposomes containing essential oils was measured by Zeta Sizer. Zeta potential of the nano-liposomal systems of essential oils containing *T. ammi* was −9 mV.

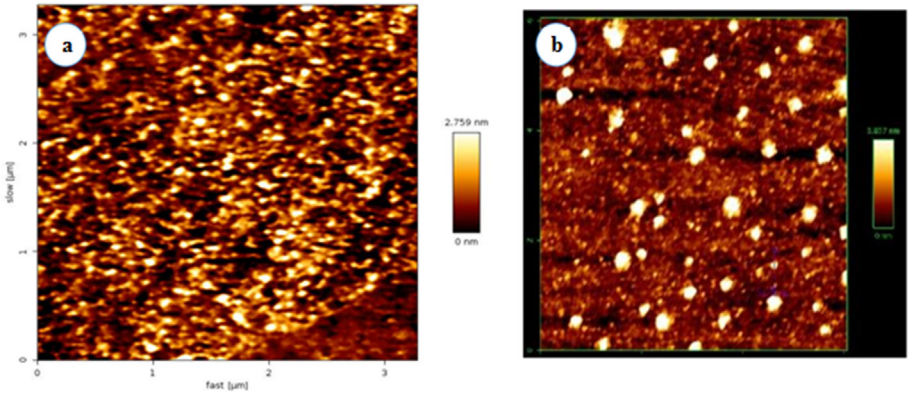

Figure 6. Atomic Force Microscope (AFM) photographs of liposomal system containing essential oil of *B. persicum* (**a**) and *T. ammi* (**b**).

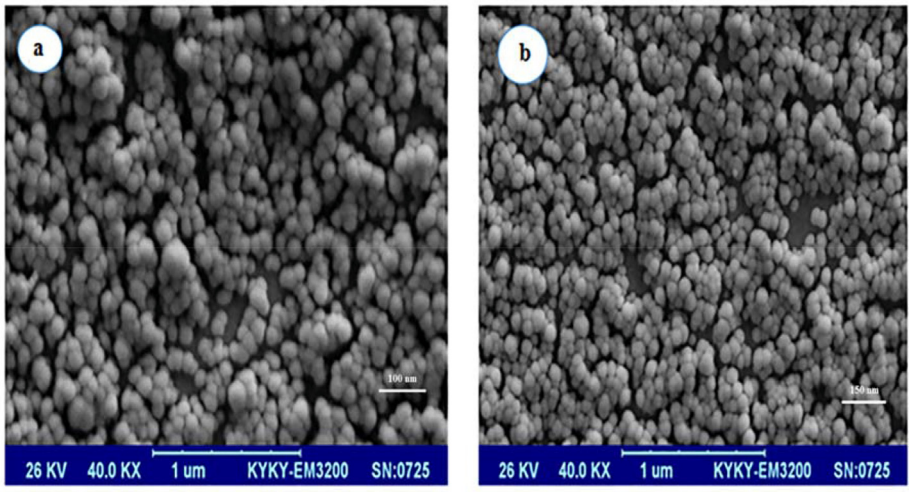

Figure 7. Scanning Electron Microscope (SEM) photographs of liposomal system containing essential oil of *B. persicum* (**a**); SEM photograph of liposomal system containing essential oil of *T. ammi* (**b**). The SEM image shows that the morphology of the constituent particles are liposome systems containing essential oil, are spherical with smooth surface and average particle size from 25 to 40 nm.

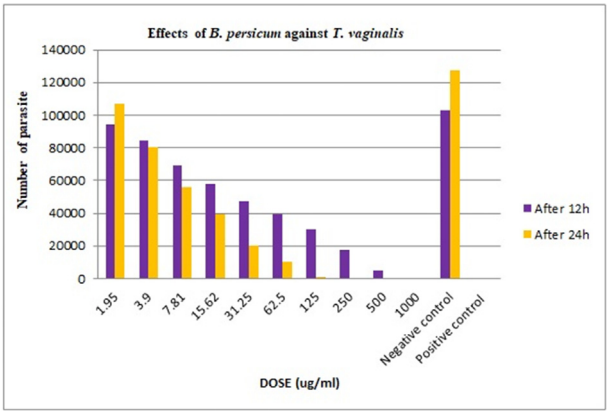

Figure 8. IC_{50} content of nano-liposomes containing essential oil of *B. persicum* against *T. vaginalis* after 12 and 24 h incubation. The parasitic strain of the logarithmic phase (10^5 parasites) was exposed to different concentrations of *B. persicum* nano-liposomes. Growth inhibition was performed by trypan blue assay, and calculated for each of the different concentrations (1.95—1000 μg/mL) of liposome-containing essential oils. SigmaPlot™ 13 software was used to determine IC_{50} of the above nano-liposomes. The data are presented as mean ± SD.

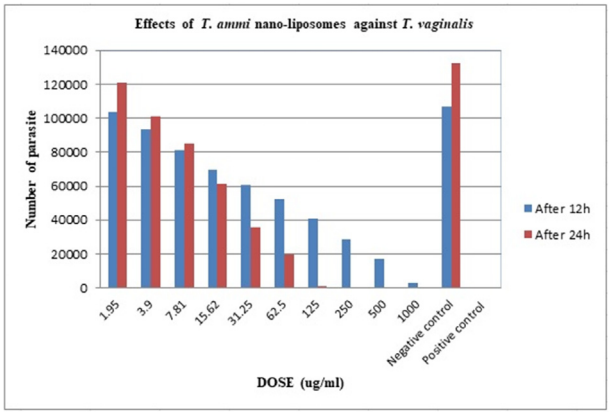

Figure 9. IC_{50} content of nano-liposomes containing essential oil of *T. ammi* against *T. vaginalis*after 12 and 24 h incubation. The parasitic strain of the logarithmic phase (10^5 parasites) was exposed to different concentrations of *T. ammi* nano-liposomes. Growth inhibition was performed by trypan blue assay, and calculated for each of the different concentrations (1.95—1000 μg/mL) of liposome-containing essential oils. SigmaPlot™ 13 software was used to determine IC_{50} of the above nano-liposomes. The data are presented as mean ± SD.

that similarities in the construction of the lipid system, the type of materials used in the lipid system and the nature of the loaded material are some of the factors that supports the results of the present study. In a study of the effect of rosemary on *T. vaginalis*, Saeidi et al.) 2012 (reported inhibitory effects on the growth of this parasite at concentrations of 0.0001 and 0.0002 mg/mL.[42] Recently, Mohammadi et al. (2019(developed a liposomal system that uses ginger essential oil to investigate the antifungal effects of this system and is related to the essential oil containing 95 nm liposomal system, having significant antifungal effects on *Aspergillus parasiticus*.[43] This similar finding with our study confirms the antimicrobial activity of lipid systems containing essential oils.

In this study, DIL-liposome solution treatment group, a very weak green signal was detected in the cell cytoplasm, whereas a relatively stronger signal was observed in the *T. ammi* essence of DIL-liposome and in the DIL-liposome treatment group. A similar uptake pattern has been reported for calcein-loaded liposomes,[44] and for hyaluronic acid-magnetic-loaded liposomes.[45] It has been reported that

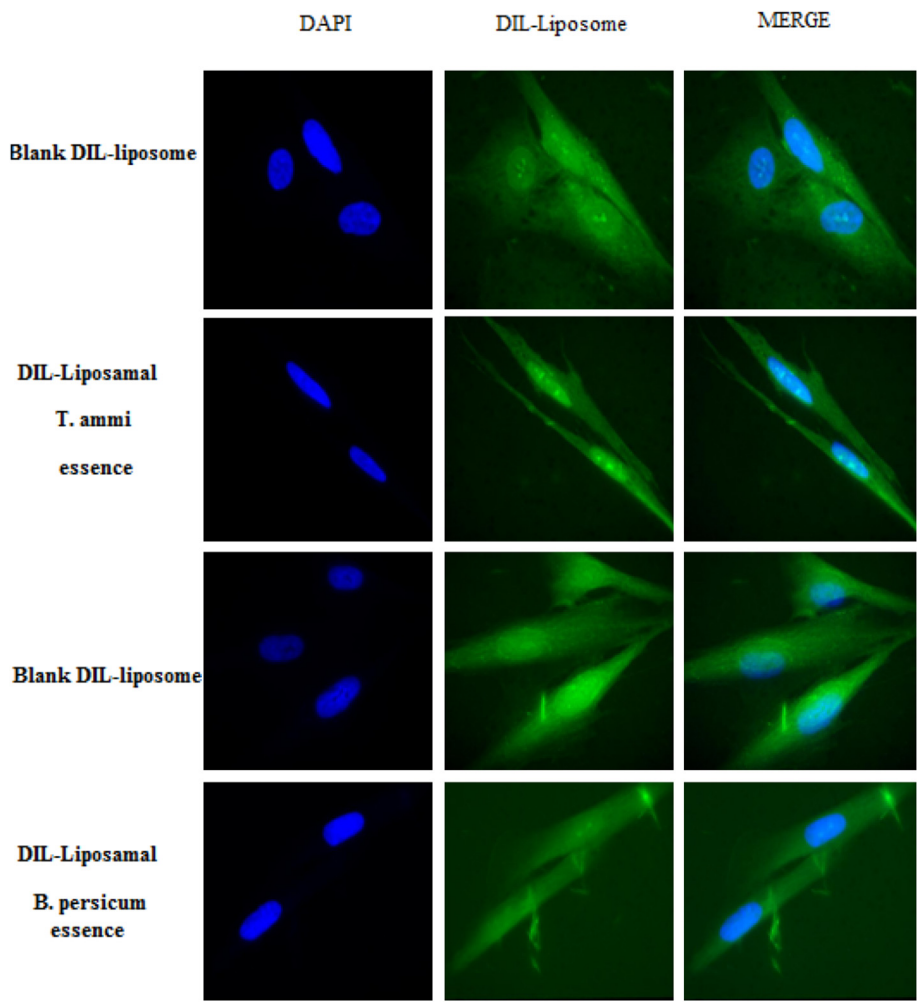

Figure 10. Fluorescent microscopic images of cellular uptake of nano-liposomes containing essential oils of *T. ammi* and *B. persicum* by human foreskin fibroblast under magnification of 400X. Cellular uptake was examined under a fluorescent microscopy, Core-shaped with DAPI color (blue) and stained DIL nanoliposomes (green) were seen. Abbreviations: DAPI, 6,4 diamidino-2-phenylindole; DIL, tetramethylindocarbocyanine perchlorate.

liposome can interact with cells by four mechanisms, including endocytosis, adsorption to the cell surface, transfer of liposomal lipids to the cell membrane and lipid-mediated fusion with the intracellular membranes, the main pathways for liposomal internalization.[46–48] Moreover, factors such as size because of aggregation, surface charge and surface affinity seem to have a major impact on nanoparticle binding to the cell membrane and subsequent cell uptake.[49] The results indicated that the cellular uptake of the nano-liposome by the skin cells was enhanced by the presence of the intensity of fluorescence signal in the skin cells.

Conclusions

The results significantly showed improvement in the co-entrapment of *B. persicum* and *T. ammi* for the liposomal systems, which had suitable stability, controlled release rate of essential oils, good surface zeta potential and suitable mean diameter (<50 nm). The results of this study confirmed the appropriate physical and chemical properties of the liposomal of *B. persicum* and *T. ammi*, essential oils, and demonstrate for the first time of their high potential in the treatment of Trichomonas infection. Furthermore studies are recommended to gain a better understanding of the mechanisms involved.

Author Contributions

FM, BFH and FR conceived, designed the study and conducted the laboratory work. RN and AS analyzed the data and interpreted the results. RN, VN, WM, MN, MLP, SAH, MM and MM wrote the manuscript. All authors read and approved the final manuscript.

Declaration of competing interest

The authors declare that they have no competing interests.

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