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Original paper

Promising potent *in vitro* activity of curcumin and quercetin nano-niosomes against *Trichomonas vaginalis*

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ABSTRACT. Trichomonosis, caused by infection with a motile protozoan parasite called *Trichomonas vaginalis*, is the most common non-viral sexually transmitted disease worldwide. Since the 1960s, metronidazole has been used as a drug of choice. Considering increased resistance to anti-trichomonial drugs, alternative treatments are urgently needed. In this study, the standard strain of *T. vaginalis* was cultured in TYM medium. Curcumin and quercetin loaded with hyaluronic acid niosomes were prepared by the thin film hydration method. The mean vesicle size, polydispersity index, and zeta potential of each prepared formulation were characterized, and its anti-*Trichomonas* activity was assessed by concentrations of 0.01, 0.1, 1, 10 and 100 mg/ml. The cytotoxicity effects of the mentioned drugs were determined using a MTT assay on L929 fibroblast cell viability. The particle sizes of curcumin, quercetin, and curcumin-quercetin entrapped modified nano-niosomes were characterised as 243 ± 5.28, 223 ± 7.21 and 266 ± 4.81 nm. The results showed that quercetin and curcumin at a concentration of 100 mg/ml after 24 h had *anti-T. vaginalis* activity. However, curcumin at a concentration of 100 at time 3h with 97% growth inhibition had better performance than positive control (metronidazole). According to the results of the MTT assay, all drugs, even at the highest concentration (400 mg/ml), had no toxic effect on the fibroblast cell line. According to potent *in vitro* activity of curcumin and quercetin nano-niosomes *against T. vaginalis* in comparison with metronidazole, it can be concluded these compounds could be promising therapeutic candidates for trichomonosis in future.

Keywords *Trichomonas vaginalis*, curcumin, quercetin, nano-niosomes, treatment, *in vitro*

Introduction

Trichomonas vaginalis (*T. vaginalis*) is the causative agent of trichomonosis and the most common sexually transmitted infection (STI) worldwide [1]. This parasite lives in the urinary and genital tracts, and humans are the only natural hosts of this protozoan. Although the disease has been reported in both sexes [2,3]. Clinical symptoms are more common in women, and the infection is asymptomatic in 75% of men [4]. According to World Health Organization (WHO) reports, the incidence of new cases of *trichomonosis* per year is 276 million and its prevalence is 187 million per year. In North America, more than 8 million new cases are reported each year, which is estimated to be up to 50% asymptomatic [5–7].

Clinical manifestations include vaginal discharge, which is the most common clinical symptom of the disease and can be accompanied by profuse discharge, burning, itching, frequent urination, and pain from urination or sexual

intercourse in women. The infection is often asymptomatic in men, but in acute cases, it can affect the seminal vesicles and the upper urogenital tract [7] and can cause urethritis, prostatitis, epididymitis, cervical cancer, infertility and pelvic inflammatory disease [8–11]. The gold standard and drug of choice for the treatment of this disease is metronidazole [12]. The results of various studies have shown that metronidazole has carcinogenic effects on human cells, and drug resistance has been developed against this drug. Other side effects of metronidazole include nausea, vomiting, abdominal pain, metallic taste in the mouth, skin rashes, constipation, swollen tongue, dizziness, fatigue, and turbid urine [1,13]. It is generally recommended that treatment with metronidazole should be avoided during the first trimester of pregnancy. These reasons indicate the need for an effective and affordable drug. Various drugs, including chemicals and natural products, have been used to treat trichomonosis *in vivo* and *in vitro* [14–17]. Natural products usually have many medicinal applications due to their low cytotoxicity. This has prompted researchers to do more research to replace these compounds with chemical drugs [18,19].

Curcumin is a natural compound with a hydrophobic polyphenolic structure that is extracted from the rhizomes of *Curcuma longa* belonging to the Zingiberaceae family [20] and has also been approved as a food additive by the WHO [21]. In recent years, it has attracted the attention of researchers in various fields of medical sciences, and to date, antibacterial, anti-tumor, anti-inflammatory, and antioxidant effects of this compound have been reported [21,22]. Quercetin is currently one of the most popular compounds among researchers due to its very low toxicity and its wide range of therapeutic applications [23]. Quercetin is one of the main flavonoids present in many fruits and vegetables, such as apples, grapes, tea, and tomatoes [24]. This compound is also found in medicinal plants such as *Sambucus canadensis*, *Ginko biloba*, and *Hypericum perforatum* [24]. Due to its various therapeutic properties, it has received a lot of attention today as a new medicinal biomolecule. Therapeutic applications of this compound include anti-cancer [24], anti-inflammatory [23], antioxidant [23], antiviral [25], antibacterial and antifungal properties [26]. Novel delivery systems like niosomes can enhance drug efficiency and decrease side effects and toxicity [27]. Recently, niosomes have been used as a vesicular drug delivery system to increase the rate of drug delivery, particularly in skin drug delivery, sustained drug delivery, or controlled drug release, with greater stability and lower cost than liposomes. Drug encapsulation in niosomes is another strategy to overcome drug resistance in different resistant pathogens. Studies have also shown that curcumin nanoparticles also inhibit the growth of *Staphylococcus epidermidis* [28]. Also, these nanoparticles have antibacterial activity against *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli*, *Pseudomonas aeruginosa* and antifungal activity against *Penicillium notatum* and *Aspergillus niger* [29,30].

Modified nano-niosomes are also more stable than simple niosomes and more efficient. Furthermore, creating these novel drug delivery systems on a nano scale improves drug bioavailability and therapeutic effectiveness even further. Due to the wide and considerable therapeutic applications of curcumin and quercetin compounds and the similar therapeutic properties in the treatment of fungal and bacterial infections and other infectious diseases [25], and given that no alternative drug to metronidazole has been found in the treatment of trichomonosis, this study was conducted to evaluate the effect of curcumin and quercetin on the trophozoite stage of *T. vaginalis*. So, to enhance curcumin and quercetin's therapeutic effectiveness, nano-modified nano-niosomes were prepared by incorporating hyaluronan as an alternative therapeutic strategy.

Materials and Methods

Parasite

In this study, the standard strain of *T. vaginalis* (Type: I and actin gene with No. KP400513) was used. The standard *T. vaginalis* strain was cultured in TYM media containing fetal calf serum (FBS) and antibiotics including ceftriaxone, liquid ciprofloxacin, and amphotericin B at 37°C. Each day, the samples were passaged with fresh culture medium until the parasite count reached 500,000 per milliliter, and viability and motility were assessed using Neobar slide and trypan blue staining [17].

Preparation of modified nano-niosome containing curcumin and quercetin

Curcumin, quercetin, and curcumin-quercetin co-entrapped modified nano-niosomes were prepared as described in our previous study

[31,32,34]. Modified nano-niosomes were prepared by the thin film hydration method. First, a mixture of ethanol and chloroform was used to dissolve the hydrophobic phase; then, a uniform layer was prepared by drying in a rotary evaporator. Finally, the thin film layer was hydrated by a hydration medium using a rotary evaporator (60°C at 270 rpm). The hydration medium contained hyaluronan, Tween 80, and curcumin or quercetin, or both of them, according to each formulation. To uniform the dispersion probe, a sonicator was used for 8 minutes (60 s on, 60 s off). Purification was performed by centrifugation, and purified modified nano-niosomes were used for further analysis [32].

Primary characterization (*particle size, polydispersity index, zeta potential and entrapment*)

Mean vesicle size, polydispersity index and zeta potential of each prepared formulation were measured by the Dynamic Light Scattering method (DLS Zetasizer Nano ZPS Instrument, Malvern, Worcestershire, UK). This process was repeated three times and average values were reported [32].

Modified nano-niosomes morphology characterization

To analyze the lamellarity and shape of modified nano-niosomes TEM analysis (ZEISS, Germany) was employed. Samples, after drying on the copper–gold carbon grid, were mounted on the device for taking different photographs [32]. The analysis of surface morphology and shape of the modified nano-niosomes was carried out using JPK-AFM (JPK Instruments AG, Berlin, Germany) [33].

Drug entrapment and drug loading

To measure the amount of entrapped drugs, first the un-entrapped values of the drug were determined. Then, by subtracting the measured un-entrapped values from the total amount of the drug, the entrapped values of the drug were calculated. A UV spectrophotometry method (Jasco, v630, spectrophotometer, Japan) was used to measure the amount of un-entrapped drugs. The curcumin and quercetin were determined based on absorbance at 428 and 256 nm, respectively.

$$\text{EE (\%)} = \frac{\text{Amount of drug entrapped in the vesicles}}{\text{total amounts of drug}} \times 100$$

Calculation of drug loading for modified nano-niosomes was done after drying specific amounts of each formulation using a freeze dryer (alpha 1-2 ld plus, Martin Christ GmbH, Germany). The amounts of the drugs in the vesicles were determined by UV-Vis spectrophotometer; after lysing modified nano-niosomes in excess amounts of ethanol.

$$\text{Drug loading \%} = \frac{\text{weight of drug in the vesicles}}{\text{weight of the vesicles}} \times 100$$

Differential scanning calorimetry analysis (*DSC*)

Perkin Elmer, PYRIS 6 DSC INTRACOOLER, Netherland was used for DSC analysis of pure curcumin, pure quercetin, cholesterol, span 60, hyaluronan, curcumin, quercetin and curcumin-quercetin co-entrapped modified nano-niosomes. All samples were analyzed in sealed aluminum pans, under a nitrogen atmosphere with a heating range from 30°C to 300°C and a scanning rate of 10°C/min. A blank aluminum pan was used as a reference.

Exposure of the parasite to curcumin, quercetin and curcumin-quercetin co-entrapped modified nano-niosomes

Experiments were performed on 24-well plates with metronidazole (50 µg/ml) as a positive control group and culture media containing *Trichomonas* as a negative control group, under sterile conditions in a standard biologic hood. First, 200 µl of TYM culture medium was added to each well, and then 100 µl of *Trichomonas* te with a concentration of 5×10^5 per ml was added. After that, concentrations of 0.01, 0.1, 1, 10 and 100 mg/ml were added to the wells. The plates were then placed in an incubator with 5% CO_2 and incubated at time intervals of 1, 3, 6, 24, and 48 h at 37°C. The parasite was tested for motility and viability using trypan blue dye, and parasites were counted using a Neobar slide and were microscopically examined using a 40× magnification. The experiments were performed in double-blind and triplicate and all steps were performed under completely sterile conditions under a laminar hood [35].

Cell culture

L929 as a normal mouse fibroblast cell line is purchased from Pasteur Institute of Iran. This cell line is cultured in RPMI-1640 medium with 10% bovine fetal serum (FBS) and L-glutamine at the rate of 100 units per milliliter at 37°C, the pressure of 5% CO_2, and humidity of 95%.

Table 1. Physicochemical properties of different modified nano-niosomes

Formulation name	Size (nm)	PDI (value)	Zeta potential (mv)	EE (% w/w)	DL (% w/w)
Curcumin entrapped modified nano-niosomes	243 ± 5.28	0.3 ± 0.09	-31. 3 ± 0.65	97.32 ± 0.47	1.88 ± 0.32
Quercetin entrapped modified nano-niosomes	223 ± 7.21	0.48 ± 0.03	-36. 4 ± 0.31	91.16 ± 0.95	1.45 ± 0.41
Curcumin-quercetin co-entrapped modified nano-niosomes	266 ± 4.81	0.32 ± 0.05	-35. 1 ± 0.93	Curcumin 96.41 ± 0.67 Quercetin 90.89 ± 2.32	Curcumin 2.13 ± 0.18 Quercetin 2.48 ± 0.15

Determining the effect of drugs on cell viability by MTT assay

The effects of curcumin and quercetin entrapped modified nano-niosomes on L929 fibroblast cell viability are determined by MTT staining. To perform this test, cells were first cultured in RPMI 1640 medium. Then, 200 µl of culture medium containing 10,000 cells is added to each well of the 96-cell culture plate. After 24 h of incubation, the cells are placed in the different groups of treatment with curcumin, quercetin, and curcumin-quercetin co-entrapped modified nano-niosomes at concentrations of 100, 200, and 400 mg/ml. a group without treatment and a DMSO group as control are considered. Groups are incubated triplicate at 24 and 48 h. After the desired times, 20 µl of ready MTT solution (5 mg MTT in 1 ml PBS) is added to the wells and the plates are incubated for 4 h at 37°C

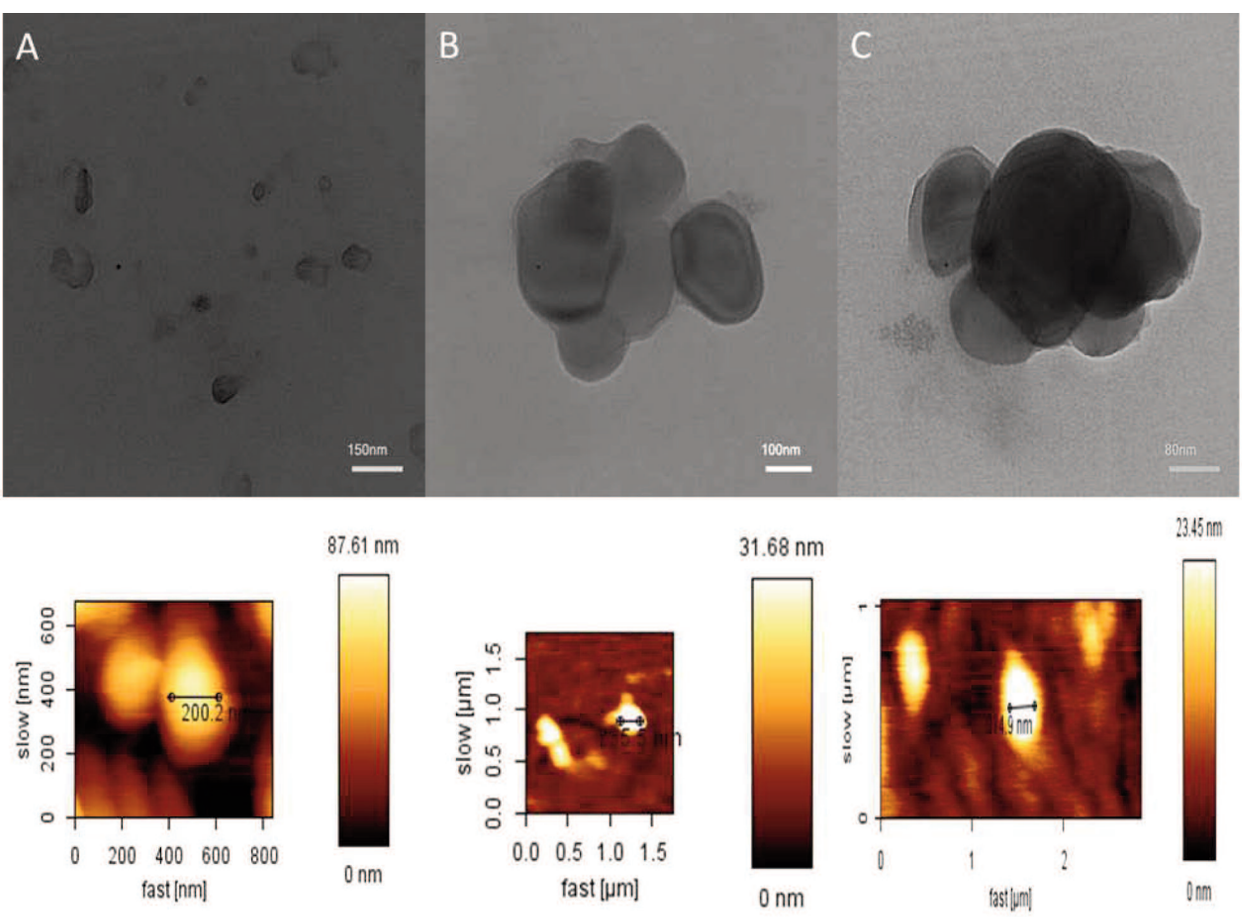

Figure 1. Transmission electron micrographs and Atomic Force microscopy images of quercetin entrapped modified nano-niosomes (A), curcumin entrapped modified nano-niosomes (B), and curcumin-quercetin co-entrapped modified nano-niosomes (C)

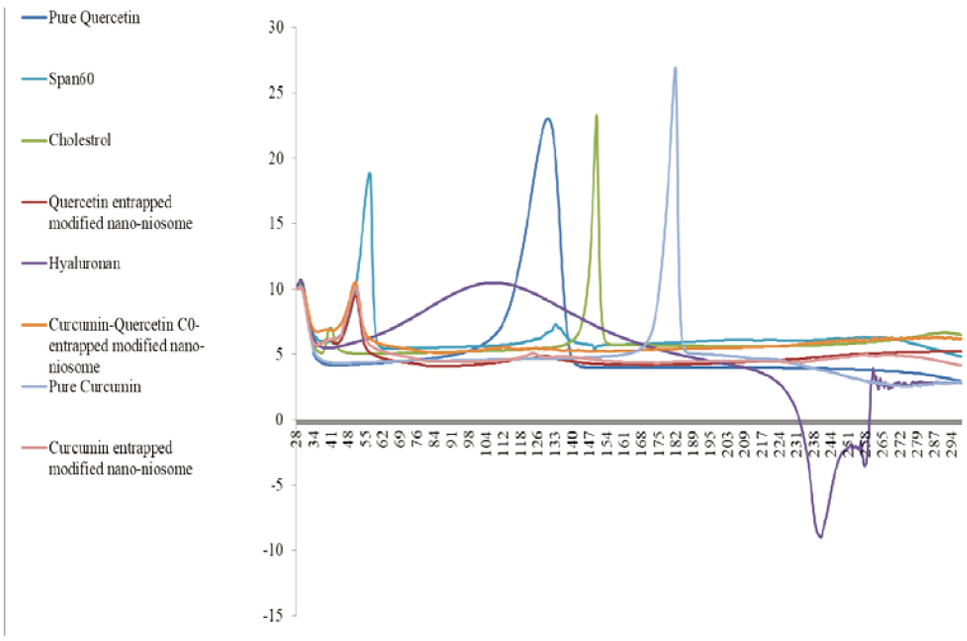

Figure 2. DSC thermograms of pure curcumin, pure quercetin, cholesterol, span60, hyaluronan, curcumin entrapped modified nano-niosomes, quercetin entrapped modified nano-niosomes, and curcumin-quercetin co-entrapped modified nano-niosomes with heat flow endothermic up

and humidity of 95%, and at the end of the incubation time, the supernatant of the wells is drained. Then, 200 µl of DMSO is added to each well to dissolve the formazan deposits and the plates are incubated away from light for 10 min. Finally, light absorption will be read at 570 nm using the ELISA Reader [36].

Statistical analysis

Data obtained from this study was analyzed using SPSS software version 22. To evaluate the effect of time in each treatment environment, repeated measures analysis of variance (between groups and intragroup) was used in general. A one-way analysis of variance was used in order to study the groups separately in each time period according to the drug and concentration. For comparison between treatments, the Bonferroni post hoc test was used.

Ethical approval

This study was approved by Mazandaran University 198 of Medical Sciences, Sari, Iran (IR.MAZUMS.REC.1399.7970).

Results

Modified nano-niosomes characterization
Primary characterization

Physicochemical characteristics (mean vesicle size, polydispersity index, and zeta potential), drug entrapment and loading percent of different modified nano-niosomes (mean ± SD, n=3) were reported in table 1.

Modified nano-niosomes morphology characterization

According to figure 1, based on TEM images, modified nano-niosomes were spherical and their sizes were well compatible with the values measured by DLS method (Tab. 1). The spherical shapes of modified nano-niosomes were also confirmed by AFM images

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Differential Scanning Calorimetry Analysis (*DSC*)

According to figure 2, melting point of pure curcumin, pure quercetin, cholesterol and span 60 can be seen as an endothermic sharp peak in DSC thermogram of each component. DSC thermogram of pure hyaluronan had two different peaks, a broad endothermic peak (showing melting point of hyaluronan) and a narrow, sharp exothermic peak due to the destruction of the polymer that is in agreed with previous study.

Thermograms of curcumin, quercetin, and curcumin-quercetin co-entrapped modified nano-niosomes showed similar peaks. In brief, the characteristic peak of pure curcumin, pure quercetin, span 60, cholesterol, and hyaluronan disappeared, which showed the interaction between

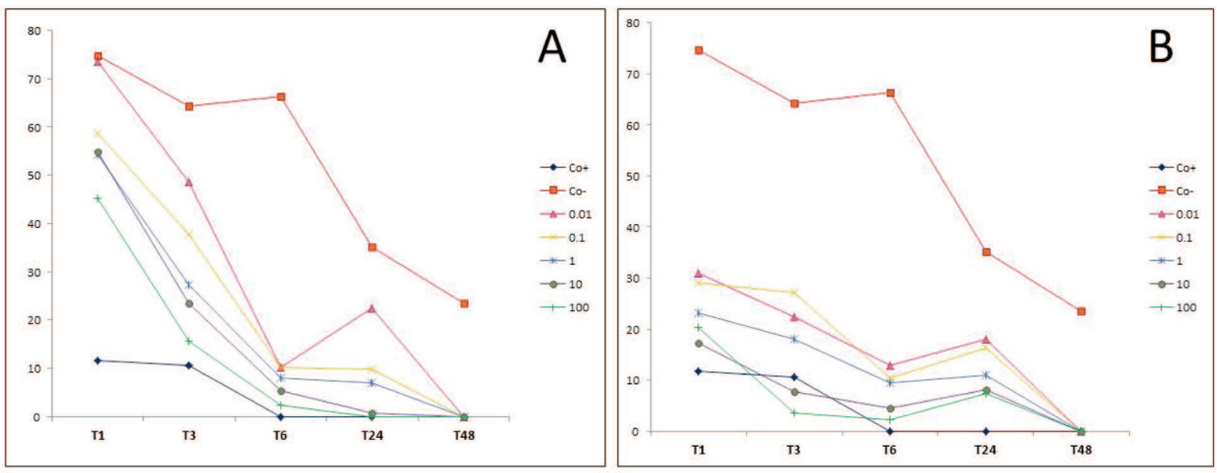

Figure 3. Viability rate of *T. vaginalis* parasite in the presence of quercetin (A) and curcumin (B) at concentrations of 0.01, 0.1, 1, 10, and 100 mg/ml after 3, 6 , 24 and 48 h *in vitro*

hyaluronan, cholesterol, and span 60 that led to niosome preparation and drug entrapment.

Determination of the effect of curcumin, quercetin and curcumin-quercetin co-entrapped modified nano-niosomes on T. vaginalis

In this study, the cytotoxic effect of curcumin and quercetin and curcumin, quercetin and curcumin-quercetin co-entrapped modified nano-niosomes at concentrations of 0.01, 0.1, 1, 10, and 100 mg/ml was examined on *T. vaginalis* trophozoite in TYM culture medium after 1, 3, 24 and 48 h. Metronidazole (50 µg/ml) was considered as positive control and the non-treated group as negative control and the efficacy of the treatments were assessed using trypan blue stain as vital staining by Neobar slide. The parasite growth inhibition was observed in all concentrations, but the greatest effect for all the above compounds was observed in the concentration of 100 mg/ml after 24 h. The results of the study showed that with increasing concentration and time, the trichomonacide activity was increasing (Figs 3–6).

The results of this study showed that quercetin at a concentration of 100 mg/ml caused 84.38%, 97.56%, and 100% effectiveness at times of 3, 6, and 24 h, respectively. Moreover, at a concentration of 10 mg/ml and after 24 h, it has 99% cytotoxicity and was statistically similar to metronidazole as a positive control (P>0.05) (Fig. 3A). Curcumin at a concentration of 100 mg/ml caused the *T. vaginalis* cytotoxicity of 96.42% after 3 h, which had significantly better efficacy than the positive control (P<0.05). Also, at a concentration of 10 mg/ml after 3 h, it caused 92.35% of the parasite cytotoxicity and showed a similar performance to metronidazole (P>0.05) (Fig. 3B).

Quercetin entrapped modified nano-niosomes at

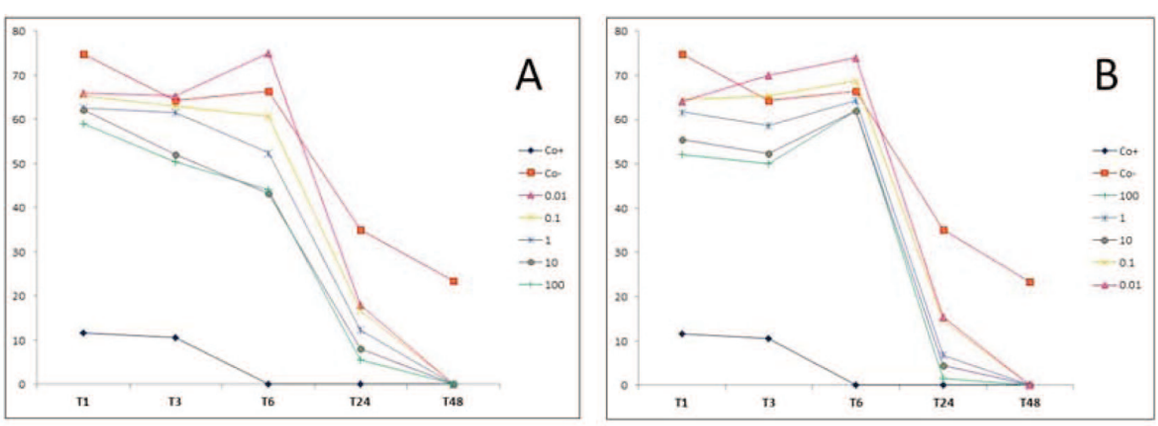

Figure 4. Evaluation of viability rate of *T. vaginalis* parasite in the presence of curcumin, quercetin and curcumin-quercetin co-entrapped modified nano-niosomes (A) and nano-curcumin (B) at concentrations of 0.01, 0.1, 1, 10, and 100 mg/ml after 3, 6, 24, and 48 h *in vitro*

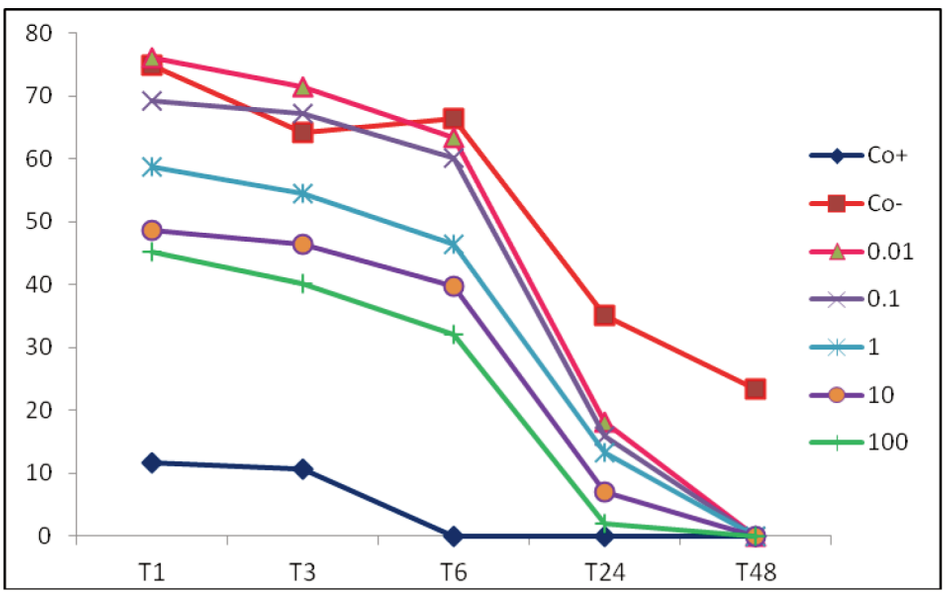

Figure 5. Evaluation of viability rate of *T. vaginalis* in the presence of curcumin-quercetin co-entrapped modified nano-niosomes at concentrations of 0.01, 0.1, 1, 10, and 100 mg/ml after 3, 6, 24, and 48 h *in vitro*

concentrations of 0.1, 1, 10, and 100 mg/ml caused 100% cytotoxicity on *T. vaginalis* after 48 h and had a positive efficacy similar to the control group, but within 24 h caused 87–95% cytotoxicity on *T. vaginalis*, which showed a lower efficacy than metronidazole; however, this difference was not statistically significant ($P>0.05$) (Fig. 4A). Curcumin entrapped modified nano-niosomes at concentrations of 1, 10, and 100 after 24 h had the same ability as metronidazole (the gold standard drug) to treat trichomonosis with 95 to 100% of its effectiveness (Fig. 4B).

Curcumin and curcumin-quercetin co-entrapped modified nano-niosomes at concentrations of 100 mg/ml could provide the cytotoxicity of 100% of the parasites at 24 and 48 h, which had the same ability as metronidazole against *T. vaginalis* (Fig. 5).

In the study of the effect of curcumin and quercetin and curcumin, quercetin and curcumin-quercetin co-entrapped modified nano-niosomes based on time, it was found that at 24 h, curcumin-quercetin co-entrapped modified nano-niosomes had the best efficacy compared to other drugs. At 6 h, with a significant difference, the best efficacy was related to quercetin and curcumin ($P<0.05$), and at 3 h, curcumin was significantly better than other drugs ($P<0.05$), which indicates that the hybrid drug requires more time to be effective than the normal form of the drug (Fig. 6).

IC50 values were calculated for each of the times and compounds and are given in table 1. The results of curcumin showed a minimum concentration of 50% cytotoxicity after 1 and 3 h. At 6 h, quercetin and curcumin had the lowest concentrations of compounds to provide an inhibition rate of 50%, respectively. At the time of 24 h, the lowest concentration occurred in the curcumin compound to achieve a 50% inhibition. To evaluate the toxicity of the studied drugs, the drugs at concentrations of 100, 200, and 400 mg/ml were tested on the L929 cell line, and the results showed that all drugs, even at the highest concentration (400 mg/ml), had no toxic effect on the fibroblast cell line (Tab. 1).

Discussion

In the current study, the cytotoxicity of curcumin, quercetin, and curcumin-quercetin co-entrapped modified nano-niosomes was evaluated against *T. vaginalis* protozoa for 3, 6, 24, and 48 h. The results showed that curcumin and quercetin at a concentration of 100 mg/ml cause 96.42% and 84.38% cytotoxicity after 3 h and have higher effectiveness than the positive control. The comparison of the efficacy of curcumin with quercetin in inhibiting the growth of *T. vaginalis* at concentrations of 10 and 100 after 3, 6, and 24 h revealed results similar to those of metronidazole, but curcumin at a concentration of 100 mg/ml after 3 h had a better performance compared to quercetin, hybrid, and positive control.

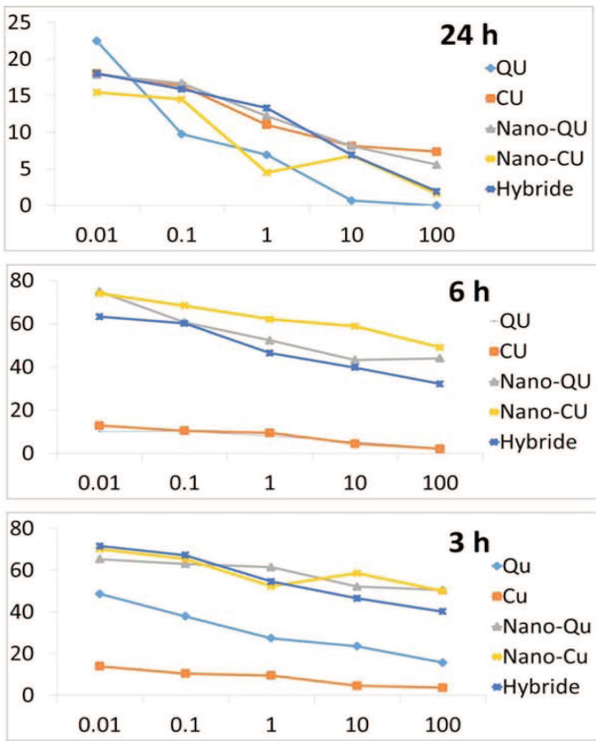

Figure 6. Evaluation of viability rate of *T. vaginalis* parasite in the presence of curcumin and quercetin and curcumin, quercetin and curcumin-quercetin co-entrapped modified nano-niosomes at concentrations of 0.01, 0.1, 1, 10, and 100 mg/ml after 3, 6, and 24 h

Researchers have reported different doses of curcumin for its anti-parasitic effects. A study by Hussein et al. [38] was conducted to evaluate the schistosomacidal effects of *Curcuma longa* (*C. longa*) in comparison to praziquantel in mice infected with *Schistosoma mansoni* (*S. mansoni*). The results showed that *C. longa* methanolic extract at a concentration of 400 mg/kg body weight (twice a week for 8 consecutive weeks) significantly reduced the burden of *S. mansoni*. In another study, Elamin et al. [39] anti-proliferative and apoptotic induction activities of curcumin at optimal concentrations of 20, 40, 60, and 80 µM on *Leishmania major* promastigotes were examined in a 96-well plate medium. The viability of promastigotes in curcumin-treated groups was statistically compared with the untreated control group, and the results showed that promastigotes were very sensitive to curcumin, especially in high doses of treatment.

After 1 and 3 hours of incubation, curcumin and quercetin showed better effectiveness than nano and hybrid forms of the drugs and were similar to the positive controls. But, after 6 and 24 hours, the hybrid form revealed better cytotoxicity effects. These results are due to the controlled and suspended release of the drugs in hybrid form. In a study conducted by Sadeghi Ghadi et al. [31] with the aim of describing and evaluating *in vivo* niosome nanoparticles for the encapsulation of curcumin and quercetin, they found a higher anti-inflammatory effect compared to the simple suspension of curcumin and quercetin. However, since the drugs become encapsulated, the drugs are released slowly, which could be due to vesicle preparation methods, so that the complete release of quercetin from the niosome takes 41 hours. Due to the low solubility and stability of curcumin and quercetin, which reduces the therapeutic effect of these compounds, and considering the wide and considerable therapeutic applications of these compounds and the similar therapeutic properties of these two compounds, a special system such as nanoparticles has been used to improve pharmacokinetics and pharmacodynamic properties. Significant research has been done in the field of drug release using special release systems as carriers for large and small molecules [37], and various studies have compared the anti-inflammatory, antibacterial, antifungal, and anti-parasitic potential of natural compounds with their nanoparticles, and the results show that the nanoparticles of these compounds provide better elucidation. For instance, in a study conducted by Dende et al. [40], the effect of curcumin on entrapped modified nano-niosomes prevented degenerative changes in rat cerebral malaria was evaluated. It was shown that neurological symptoms and cytotoxicity caused by cerebral malaria were better treated with nano-formulated curcumin (PLGA curcumin) than native curcumin.

The results of the study showed that curcumin entrapped modified nano-niosomes at concentrations of 1, 10, and 100 mg/ml could provide growth inhibition rates of 41.45, 47.73, and 50.01% after 3 h. For quercetin entrapped in modified nano-niosomes, growth inhibition rates were 38.54, 47.86, and 49.55% at the same concentration and time. Moreover, for hybrid forms at the same concentration and time, the *Trichomonas* growth inhibition rates of 45.35, 53.52, and 59.83% were obtained. However, over time, the *Trichomonas* growth inhibition has increased, so that for curcumin entrapped modified nano-niosomes at concentrations of 1, 10, and 100 mg/ml, growth inhibition was 93.16, 95.50, and 98.4% after 24 h and for quercetin entrapped

modified nano-niosomes, at the same concentration and time, it was 87.74, 91.87, and 94.41%, and in hybrid forms at the same concentration and time, it was 86.68, 93.1, and 98.08%, respectively.

Curcumin, in spite of being a natural polyphenolic compound with numerous drug activities, is associated with several problems with bioavailability due to poor solubility and stability, and many nanostructures are designed to overcome these disadvantages. In another study conducted by Sadeghi Ghadi et al. [37], curcumin with a neosomal structure containing hyaluronan was designed to improve the performance of curcumin, and the results showed that 500 µl of the formulation prepared with 4.00 00×10^{-7} mol of curcumin had an antioxidant effect of 100%. Also, the anti-inflammatory effect of niosomes was greater than the anti-inflammatory effect of simple curcumin suspension.

According to the results of this study, it has been proven that curcumin, quercetin and curcumin-quercetin co-entrapped modified nano-niosomes not only have a positive effect after 24 h, but also have a very beneficial and effective effect on anti-inflammation and antioxidants, according to current and many published studies. Therefore, these compounds can be used intravaginally as an anti-inflammatory with the same anti-*Trichomonas* activity. Although curcumin inhibits 96.42% of parasites at a concentration of 100 at a time of 3 hours, due to its low solubility and stability, its effectiveness in treating diseases is limited. As a result, it is better to be in the form of a niosome where the drug is released slowly [31,32,37].

In conclusion, considering the potent *in vitro* activity of curcumin and quercetin nano-niosomes against *T. vaginalis* in comparison with metronidazole, as well as lack of toxic effects on the fibroblast cell, it can be concluded these compounds could be favorable therapeutic candidates for trichomonosis in future. As a whole, further investigations regarding anti-trichomonal effects of the curcumine and its derivative niosomes on animal models is highly suggested.

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