Original Article
Metronidazole-loaded nanoparticulate thermoreversible gel for gynecologic infection of *Trichomonas vaginalis*

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Abstract: Objective: Trichomoni asis is a common sexually-transmitted disease that is associated with increased perinatal morbidity and human immunodeficiency virus (HIV) transmission. This study aimed to develop a Metronidazole-loaded nanoparticulate thermoreversible gel for gynecological infection of *Trichomonas vaginalis* (*T. vaginalis*). Methods: The optimized nanoparticulate formulation was used in thermoreversible gel and characterized for physico-chemical properties, antiparasitic activity, and *in vivo* efficacy in the BALB/c mouse model. Result: A nearly threefold rise in antiparasitic activity of the optimized formulation was observed as compared to that of regular gel. Formulation F5 successfully cured the trichomoni asis within 3 days, while regular gel and pure Metronidazole (MTDZ) failed to cure this infection (*P*<0.05). Conclusion: The present investigation confirms the ability of thermoreversible gel containing nanoparticulate metronidazole against the infection by *T. vaginalis*. The developed gel could be an alternative to the existing drug delivery system for the treatment of trichomoni asis.

Keywords: *Trichomonas vaginalis*, poloxamer, chitosan, thermoreversible, trichomoni asis, vaginal, Metronidazole, nanoparticles, drug delivery

Introduction

Trichomoni asis, caused by infection with a flagellated protozoan called *Trichomonas vaginalis* (*T. vaginalis*), is a parasitic disease spread by sexual contact. Females with trichomoni asis present with foul-smelling vaginal discharge, genital itching, and painful urination. Males, on the whole, show no signs or symptoms. The risk factors include multiple sexual partners and not using condoms during intercourse [1].

Trichomoni asis is a non-viral sexually transmitted disease (STD) that is easily curable and treatable.

There are more than 143 million cases of trichomoni asis each year around the world, according to a data sheet published by the World Health Organization [2]. Global statistics and estimates indicate that, in developed countries, a substantial number (more than 50%) of patients admitted to STD clinics suffer from trichomoni asis, and the rate of infection is rising [3]. *T. vaginalis* infection is related to a two to three-fold increased risk of human immunodeficiency virus (HIV) infection, as well as premature birth and other adverse pregnancy outcomes for pregnant women. Although trichomoni asis is a STD that is simple to diagnose and treat, the current public health STD control programs pay too little attention to infection prevention [4].

Metronidazole (MTDZ), a 5-nitroimidazole used for the treatment of infections caused by parasitic protozoa and anaerobic gram-negative bacilli, is widely used for trichomoni asis. MTDZ (Flagyl) has been shown to be highly effective in the treatment of many anaerobic bacteria and protozoal infections. It is administered orally for the treatment of trichomoni asis using a single dose of 2 gm or 500 mg twice a day for 7 days [5, 6]. However, it also leads to some side effects such as nausea, vomiting, bad after-
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Table 1. CS-MTDZ formulation compositions

<table>
<thead>
<tr>
<th>Formulation</th>
<th>NP1</th>
<th>NP2</th>
<th>NP3</th>
<th>NP4</th>
</tr>
</thead>
<tbody>
<tr>
<td>MTDZ (mg)</td>
<td>3.5</td>
<td>3.5</td>
<td>3.5</td>
<td>3.5</td>
</tr>
<tr>
<td>Ethanol (ml)</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Chitosan (mg)</td>
<td>25</td>
<td>50</td>
<td>75</td>
<td>100</td>
</tr>
<tr>
<td>3% Acetic acid (ml)</td>
<td>15</td>
<td>15</td>
<td>15</td>
<td>15</td>
</tr>
<tr>
<td>Tween 80 (ml)</td>
<td>0.2</td>
<td>0.4</td>
<td>0.6</td>
<td>0.8</td>
</tr>
<tr>
<td>TPP (mg)</td>
<td>0.5</td>
<td>1</td>
<td>1.5</td>
<td>2</td>
</tr>
<tr>
<td>Purified water</td>
<td>q.s.</td>
<td>q.s.</td>
<td>q.s.</td>
<td>q.s.</td>
</tr>
</tbody>
</table>

q.s., Quality Standard; TPP, Tripolyphosphate; MTDZ, Metronidazole.

Taste, gastrointestinal disturbances, exanthema, hives, angioedema, vertigo, peripheral neuropathy and intermittent neutropenia, which lower patients' treatment compliance. MTDZ resistance to T. vaginalis infection has been shown to be associated with a rising number of refractory cases. Vaginalis infection is a problem since MTDZ is still the only drug approved by the United States for the treatment of trichomoniasis. Therefore, refractory patients are typically treated with higher doses of the drug [7, 8]. Considering all these drawbacks of the current MTDZ therapy, an efficient alternate drug delivery technique is urgently needed.

To improve the aqueous solubility of poorly soluble drugs, innovations in nanoparticulate drug delivery have already been developed and recorded for pharmaceutical applications, which in turn contributes to bioavailability. Nanoparticle innovation makes it possible to formulate poorly soluble drugs alone or with a mixture of pharmaceutical excipients as particles. There is a substantial increase in the surface area by growing the particle size from a micron to a nanometer-scale [9, 10].

In the ophthalmic, vaginal, transdermal and other drug delivery methods, the use of a nanocarrier has been extensive, and it can significantly enhance pharmacokinetic and pharmacodynamic properties, and improve corneal, mucosal, dermal permeability along with solubility and bioavailability [11]. By increasing the residence time of medicine in the vagina by using a thermoreversible gel system, the successful dosage administered can be altered. Such gels increase the duration of residency and also preserve the drug's release process. Vaginal in-situ gelling requires biocompatible polymers that are structurally altered under conditions such as pH, temperature, and ionic strength with minor adjustments. During installation into the vaginal cavity, in-situ formation gels are liquids and then undergo rapid gelation in response to body temperature [12, 13]. The present invention aims to develop a thermoreversible gel containing nanoparticulate MTDZ and to test its efficiency against the infection of T. vaginalis.

Materials and methods

Materials

MTDZ was obtained as a gift sample from Baoji Guokang Bio-Technology Co., Ltd. (Baoji, Shaanxi, China). Tripolyphosphate (TPP), Chitosan (CHS), and Poloxamer (PLR) were obtained as gift samples from Sigma Aldrich, USA. Acetic acid was donated by Shanghai Chemical Co. (Shanghai, China). T. vaginalis samples were obtained from the Institutional Cell Culture Lab. Trichomonas dehydrated culture media Trichomonas Medium Base CM0161 was purchased from Thermofisher Scientific, USA.

Development of CHS-MTDZ nanoparticles (NPs)

CHS-MTDZ NPs were formulated using the ionotropic gelation method with TPP as the crosslinking agent. CS (25-100 mg) was dissolved in 3% v/v acetic acid solution (15 ml) under continuous magnetic stirring for nearly 45 minutes. After complete solubilization of CHS, Tween 80 was added at a concentration of 0.2 to 0.8 ml under continuous magnetic stirring for 45 minutes. MTDZ (3.5 mg/ml) was dissolved separately in ethanol and added dropwise to the polymeric solution of CHS using a syringe fitted with a needle. Triphenyl phosphate (TPP) (0.5 to 2 mg) previously dissolved in 5 ml of purified water was added dropwise to result in dispersion. This dispersion was kept overnight for crosslinking of CHS and TPP. The resulting nanoparticulate suspension was centrifuged (at 50-700 C; 30,000 rpm) to separate the NPs from the solution. The separated NPs were freeze-dried using trehalose as a cryoprotectant. The resulted NPs were used for further characterization [14]. The formula composition of the NPs batches is presented in Table 1.

Development of thermoreversible gel containing CHS-MTDZ-NP

The thermoreversible gel was prepared by using the cold hydration method. The poloxamer 407 was added to cold purified water
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Table 2. Formulation compositions of thermoreversible gel

<table>
<thead>
<tr>
<th>Excipient</th>
<th>Batch Code</th>
</tr>
</thead>
<tbody>
<tr>
<td>CHS-MTDZ-NP eq. to 50 mg</td>
<td>F1 F2 F3 F4 F5</td>
</tr>
<tr>
<td>Poloxamer 407 (mg)</td>
<td>80 80 80 80 80</td>
</tr>
<tr>
<td>Polyethylene glycol 400 (mg)</td>
<td>200 250 300 350 400</td>
</tr>
<tr>
<td>Hydroxypropyl methyl cellulose K15 (mg)</td>
<td>5 10 15 20 25</td>
</tr>
<tr>
<td>Triethanolamine (ml)</td>
<td>q.s. q.s. q.s. q.s. q.s.</td>
</tr>
<tr>
<td>Distilled water (mg)</td>
<td>q.s. to 2 gm</td>
</tr>
</tbody>
</table>

q.s., Quality Standard; MTDZ, Metronidazole.

(5-70°C) slowly under continuous stirring and kept overnight in the refrigerator for proper and complete hydration of the polymer. The optimized formulation of NPs equivalent to 50 mg of MTDZ was dispersed in 10 ml purified water, followed by the addition of PEG 400 and HPMC and stirred continuously for 30 minutes to mix it properly. This dispersion was slowly added to poloxamer 407 solutions under overhead stirring for 1 hour. The final pH of the gel was adjusted to between 3.8 to 4.5 by dropwise addition of triethanolamine. The formulated gels were used for further characterization [15]. The formula composition of the thermoreversible gel is presented in Table 2.

Characterisation of CHS-MTDZ-NP

Particle size, Zeta potential and polydispersity index (PDI): The developed NPs were characterized for particle size and zeta potential using a Malvern particle size analyzer (Zetasizer, Worcestershire, UK). The nanoparticulate suspension was diluted 10 times using purified water and sonicated for 20 minutes to get a proper dispersed nanoparticulate suspension. This suspension was loaded in particle-size analyzer at room temperature and particle size, zeta potential, and PDI were determined [16, 17].

MTDZ loading efficiency

The MTDZ concentration in supernatant solution was determined after centrifugation, and samples were analyzed by the high performance liquid chromatography (HPLC) method. The loading efficiency was determined by using the following formula [18]. %DLE = Target Loading - Unloaded NLX/Target Loading ×100.

Surface characteristics using FESEM

The surface morphology of the developed NPs was determined using field emission scanning electron microscopy (FESEM SU9000, Hitachi, Japan). The dry NPs powder was kept on an aluminium stub covered with a double-sided adhesive disc and sputter-coated with gold at 20 kV for 3 min. The images were observed and captured at various locations.

Characterisation of thermoreversible gel containing CS-MTDZ-NP: gel clarity and appearance

The clarity and appearance of the formulated gels were estimated by visual observations against dark black and white backgrounds [19].

pH

The pH of all formulations was determined using a calibrated pH meter (Shinwa 72788 Digital PH Meter, Japan). The pH was determined at three different locations, and the average was considered the final pH of the formulation.

Gelation temperature

Gelation temperature of the formulation was determined by placing 3 ml refrigerated sample in test tubes properly sealed with parafilm. These test tubes were placed in a hot water bath and the temperature was increased at the rate of 50°C/minute. The gelation temperature was noted at the point when gel did not come down at the inverted position of the test tube. The experiment was performed in triplicate for each formulation and the average was determined as final gelation temperature.
MTDZ content

MTDZ content from the gel was determined in triplicate by dissolving it in pH 6.8 phosphate buffer followed by extraction in 0.3% acetic acid solution with continuous stirring. The solution was filtered through 0.45-micron disc filters to get a clear solution without any undissolved particles. The samples were analyzed by HPLC at 280 nm, and drug content was estimated.

Viscosity measurement

The viscosity of the formulation was determined by a Brookfield viscometer (CAP 1000+ Viscometer, USA) using spindle No. 62 at 100 rpm. Initially, the viscosity of the solution was measured followed by measurement of the gel viscosity after conversion into gel form. The measurements were carried out in triplicate, and the average viscosity was calculated.

Mucoadhesive strength

The mucoadhesive strength of the gel formulation was determined by calculating the minimum force of detachment required from the biological mucosal membrane. This membrane was fixed to a small glass slide using adhesive tape, attached to the bottom of the beaker (100 ml) from outside and then kept in a 500 ml glass beaker. Phosphate buffer pH 6.8 was poured into the beaker, and the level was adjusted just above the mucosa. Accurately weighed 1 gm of gel was applied on the lower side of the biological membrane followed by placing a preload of 100 gm on a glass slide for 10 min to ensure proper adhesion between gel and biological membrane. The weight required to detach the test sample from the biological mucosal membrane was determined, and an average value was calculated.

Ex-vivo permeation of MTDZ

The MTDZ permeation was studied using a Franz diffusion cell fitted with a mucosal membrane obtained from the local slaughterhouse. The membrane was properly maintained in normal saline solution at a controlled temperature of 6-80°C. This well-maintained membrane was placed between the donor and receptor compartment of the diffusion cell. Formulated gels equivalent to 50 mg of MTDZ was applied uniformly on the membrane. The receptor compartment was filled with pH 6.8 phosphate buffer, and the temperature was maintained at 37±0.5°C with continuous stirring at 30 rpm. The samples were withdrawn (0.5 ml) at a specific time interval up to 1 hour and replenished with an equal volume of fresh buffer to maintain the sink condition. The samples were analyzed using HPLC at 280 nm wavelength, and the amount of drug diffused through the membrane was calculated.

Antiparasitic activity assay

Antiparasitic activity of the optimized formulation was determined using the Petri plate method by measuring the zone of inhibition (ZOI). Nearly 30 ml Trichomonas Medium Base CM0161 suspension was poured in Petri plates. After complete solidification of the media, pure MTDZ (Std, 50 mg), regular gel formulation without NPs (eq. to 50 mg) and optimised gel formulation with NPs (test, eq. to 50 mg) were poured into the bores in Petri plates already seeded with T. vaginalis. The plates were kept for incubation at 37°C for 24 hours, and ZOI was measured using a standard. All aseptic conditions were maintained during experimental procedures [21]. In vivo efficacy testing was done in female BALB/c mice (20-25 g) to test in vivo efficacy of the optimised formulation.

All animal handling procedures and experiments were performed following the Institutional Animal Ethical Committee guidelines with prior approval (Ethical approval number: LLS2020ky036). The animals were divided into four groups (n=40), with 10 mice in each group. All the animals were infected with the injection of 0.5 ml T. vaginalis suspension intravaginally and kept at controlled room temperature for 7 days to induce the vaginal infection. The vaginal fluid from all animals was collected and cultured in Trichomonas Medium Base CM0161 as described above, and the growth of the T. vaginalis was observed. The first animal group (Group I) was treated with 5 mg equivalent optimised gel formulation; Group II was treated with 5 mg pure MTDZ, and Group III was treated with regular gel without NPs, while Group IV was used as the control without treatment. Mice were administered vaginally by syringe. The vaginal fluid from all animals was collected up to five days at the interval of one day and cul-
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Table 3. Characterisation of MTDZ-CHS-NP

<table>
<thead>
<tr>
<th>Code</th>
<th>Drug loading (%)</th>
<th>Particle size (nm)</th>
<th>Zeta potential (mV)</th>
<th>PDI</th>
</tr>
</thead>
<tbody>
<tr>
<td>NP1</td>
<td>11.38</td>
<td>140</td>
<td>19.12</td>
<td>0.21</td>
</tr>
<tr>
<td>NP2</td>
<td>13.24</td>
<td>160</td>
<td>19.89</td>
<td>0.27</td>
</tr>
<tr>
<td>NP3</td>
<td>15.26</td>
<td>195</td>
<td>20.27</td>
<td>0.19</td>
</tr>
<tr>
<td>NP4</td>
<td>17.45</td>
<td>220</td>
<td>23.14</td>
<td>0.20</td>
</tr>
</tbody>
</table>

PDI, polydispersity index.

The NPs were formed due to the interaction between CHS (Positive charge) and TPP (Negative charge) [22]. The ionotropic gelation method was found to be a suitable method for the preparation of MTDZ-CHS-NP. The drug loading efficiency was found to be between 11.38 to 17.45% with particle size ranging from 140-220 nm. The loading efficiency was directly associated with the concentration of CHS used in the formulation. Increasing the CHS concentration was beneficial to the maximum loading of the MTDZ. The PDI values showed that the formulated NPs had uniform size distribution within nanoparticulate suspension. Also, the zeta potential values (19.12 to 23.14 mV) of the suspension confirmed the physical stability of the NPs (Table 3).

Morphologic observation of MTDZ-CHS-NPs

The surface characteristics of the NPs were studied using FESEM, and it was found that the NPs (NP4) were spherical and smooth without a crack in shape. From all these observations, NP4 formulation was considered as the optimized nanoparticulate formulation, so it was further used in the development of thermoreversible gel (Figure 1).

Physico-chemical evaluation of thermoreversible gel

The thermoreversible gel was developed by using Poloxamer 407 polymer due to its unique property of gelation with temperature rising. The various physico-chemical properties of the gels are presented in Table 4.

Permeability of drugs through mucosal membranes

The formulation F5 showed an excellent permeation profile over 12 hours as compared to other formulations. This permeation profile was also found to be dependent on the polymeric concentration. A higher polymeric concentration played a better role in the retardation of drug permeation through the mucosal membrane (Figure 2).

Activity of the formulations

The antiparasitic activity against the T. vaginalis of the developed gel formulations was tested by measuring the ZOI in Trichomonas Medium Base CM0161. The results were compared to the ZOI of pure MTDZ observed in experiments (Table 5).
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In vivo efficacy testing in female BALB/c mice

The in vitro antiparasitic activity of the formulated gel was confirmed in female BALB/c mice with gynecological infection of *T. vaginalis*. Initially, all 40 animals were injected with a suspension of *T. vaginalis*, and the vaginal fluid from all animals were collected and cultured in Trichomonas Medium Base CM0161 after 7 days. All the culture media showed the growth of *T. vaginalis* (with grade 3 as very high growth) indicating the infection by trichomoniasis. These observations confirmed the induction of trichomoniasis in all animals. The animal vaginal fluid in Group I showed reduced *T. vaginalis* infection from day 1 and complete eradication by day 3, showing excellent potential in the treatment of trichomoniasis. It can be observed from Figure 3 that initially vaginal fluids obtained from all four groups of animals showed Grade 3 infection, indicating very high growth. Since day 1, vaginal fluid obtained from group I animals showed a decline in the growth of *T. vaginalis*, and since day 3 there was no growth of microorganisms in Trichomonas Medium Base CM0161. This observation indicated that the F5 formulation successfully cured the trichomoniasis within 3 days, while regular gel and pure MTDZ failed. At the end of the 5th day, the pure gel still showed grade 1 growth, and pure MTDZ showed grade 2 growth (Figure 3).

Table 4. Physico-chemical properties of MTDZ-CHS-NP loaded thermoreversible gel

<table>
<thead>
<tr>
<th>Formulation</th>
<th>Visual Appearance</th>
<th>Clarity</th>
<th>pH</th>
<th>MTDZ Content (%)</th>
<th>Gelation temperature (°C)</th>
<th>Viscosity (cPoise)</th>
<th>Mucoadhesive strength (dyne/cm²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>F1</td>
<td>Transparent</td>
<td>Clear</td>
<td>3.9</td>
<td>90.95</td>
<td>47</td>
<td>750</td>
<td>1645.90</td>
</tr>
<tr>
<td>F2</td>
<td></td>
<td></td>
<td>4.1</td>
<td>93.94</td>
<td>45</td>
<td>820</td>
<td>1917.17</td>
</tr>
<tr>
<td>F3</td>
<td></td>
<td></td>
<td>4.5</td>
<td>95.47</td>
<td>40</td>
<td>890</td>
<td>2015.24</td>
</tr>
<tr>
<td>F4</td>
<td></td>
<td></td>
<td>4.4</td>
<td>97.40</td>
<td>38</td>
<td>950</td>
<td>2240.41</td>
</tr>
<tr>
<td>F5</td>
<td></td>
<td></td>
<td>4.4</td>
<td>99.49</td>
<td>37</td>
<td>1059</td>
<td>2411.50</td>
</tr>
</tbody>
</table>

MTDZ, Metronidazole.

Table 5. Comparison of the ZOI of pure drug and optimised formulation

<table>
<thead>
<tr>
<th>Formulation</th>
<th>ZOI (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Standard drug Solution (Metronidazole)</td>
<td>8.54</td>
</tr>
<tr>
<td>Regular Gel without NPs</td>
<td>15.4</td>
</tr>
<tr>
<td>Optimized formulation (F5) with NPs</td>
<td>44.25</td>
</tr>
</tbody>
</table>

Note: ZOI, zone of inhibitions.

Discussion

Metronidazole (MTDZ) is known as a classic treatment for *T. vaginalis*-induced vaginitis. However, in recent years, the resistance of MTDZ has become the focus of current vaginitis research, with more studies reporting weakened treatment effects of MTDZ due to vaginal cells’ resistance to MTDZ [23, 24]. As a high end new technology in modern medical research, nano-chitosan has been used as a carrier for multiple drugs and achieved remarkable results in improving the utilization rate of drugs and reducing the drug resistance of the human body [25]. The study of Thurner et al. indicates that drug delivery by NPs will be a new direction for the future treatment of osteoporosis in the elderly [26]. For MTDZ, a previous study confirmed the feasibility of nano-drug delivery [27]. Therefore, this may also be a breakthrough for the treatment of *T. vaginalis* infection in the future.

This study identified another option, a thermoreversible gel containing nanoparticulate MTDZ, for the effective treatment of vaginal infections.
caused by *T. vaginalis* to provide new ideas in the field of vaginal infection treatment. Also, this study intended to help researchers uncover key areas of developing thermoreversible gels with NPs that many researchers have failed to explore. Therefore, a new theory on the treatment of vaginal infection with a novel thermoreversible gel containing drug-loaded NPs may be applied to drug delivery.

The present work deals with the development of thermoreversible gel containing nanoparticulate MTDZ for the cure and management of *T. vaginalis*-induced gynaecological infections. The thermoreversible gel was developed by using Poloxamer 407 polymer due to its unique temperature-rising gel property. This polymer forms a gel when it comes in contact with any body cavity including the nose, eye and vagina. Due to its unique property, this polymer is widely used in ophthalmic, nasal, and vaginal drug delivery systems [28, 29]. The NPs were prepared by using chitosan polymers as they have excellent biocompatibility in drug delivery systems. The ZOI of the optimized F5 was found to be 44.25 mm and that of regular gel with NPs was 15.4 mm. A nearly threefold rise in antiparasitic activity was observed in an optimized formulation as compared to regular gel. The enhancement in this activity was attributed to the presence of MTDZ NPs in the gel formulation. In virtue of their very small size, NPs helped to increase the diffusion of the drug through the medium and resulted in maximum inhibition of the growth of the parasites. On the other hand, regular gel failed to diffuse through the nutrient medium. These observations indicated the potential of thermoreversible gel with NPs against *T. vaginalis* infection. In previous studies, we also found that nano-coated MTDZ can effectively reduce the activity of Trichomonas [30, 31], which can also corroborate the results of this experiment. Moreover, Vazini et al. found that MTDZ had an antibacterial capacity of 85% in an *in vitro* experiment on the resistance of MTDZ-loaded nano-Micana cordifolia to *T. vaginalis* infection [30], which again fully demonstrated that the application effectiveness of MTDZ can be improved through nanotechnology.

In animal experiments, we found that the formulation F5 successfully cured trichomoniasis within 3 days, while regular gel and pure MTDZ failed. The complete recovery from this infection was achieved by the formulation F5 due to the nanoparticulate MTDZ in the formulation, which helped to enhance the solubility and permeability of the released drug and rapidly controlled the growth of *T. vaginalis*. This observation indicated the potential of novel thermoreversible gel loaded with nanoparticulate MTDZ for the prevention and control of *T. vaginalis*-induced gynaecological infections.

However, this study is still based on animal experiments, and the effect of the new thermoreversible gel loaded with nanoparticulate MTDZ on *T. vaginalis* infection is not clear in clinical application, so this needs to be confirmed by *in vivo* human experiments as soon as possible. In addition, due to the structural differences between animals and humans, the specific dosage of the drug also needs further study. Furthermore, the mechanism of the effect of the novel thermoreversible gel loaded with nanoparticulate MTDZ on *T. vaginalis* infection is still unclear, so more *in vitro* experiments are needed for confirmation and analysis. In a follow-up study, we will continue to improve the application analysis of the new thermoreversible gel loaded with nanoparticu-
late MTDZ in view of the above limitations, so as to provide a reliable clinical reference.

Conclusion

The present investigation confirms the potential of thermoreversible gel containing nanoparticulate MTDZ against infection by *T. vaginalis*. The developed gel could be an alternative to the existing drug delivery system for the treatment of trichomoniais.

Acknowledgements

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Disclosure of conflict of interest

None.

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