Biodegradable multifunctional platform for potential treatment of vaginal candidiasis: In-vitro preparation, in-vivo assessment of antifungal efficacy in rats

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Abstract

The human vagina remains to be a relatively unexplored route of drug delivery despite its potential as a noninvasive route of drug administration [1]. This route has been traditionally used to provide women with therapy for local disorders. The administration of locally acting drugs comprises antifungal, antibacterial, antimicrobial, anti-protozoal, antichlamydial, antiviral agents, and labor inducing agents [2,3]. Recently, the vagina as a highly vascular tissue, has been explored as an alternative route for systemic administration, thus avoiding first-pass metabolism, gastrointestinal side effects and the inconvenience associated with the parenteral route [4,5].

For effective vaginal dosage form, the formulation should be comfortable with the ability to spread onto the vaginal mucosal surface and to achieve an intimate and prolonged contact at the site of application [6]. Various methods were investigated to increase the drug bioavailability by prolonging the contact time between drug and vaginal epithelium [7]. Among the adopted approaches was the use of highly viscous gels and ointments, which provided a sustained contact with the vaginal surface, but caused a sticky sensation and leakage which has been frequently considered as their major drawback [5,8]. Another approach to optimize bioavailability was the development of mucoadhesive delivery systems [9], using suitable polymers that can interact with the mucous layer coating the external surface of the vagina. The use of films or wafers was proposed to allow drug release over a long period [10]. Wafers are relatively novel formulations prepared by lyophilization/freeze drying of polymeric gels or solutions yielding solid porous cakes that are easily applied to mucosal membranes [11,12]. Freeze-dried wafers are lighter, easily handled, capable of avoiding the leakage of traditional compressed tablets, thus increasing the patients’ compliance [13,14]. Furthermore, the sponge-like nature ensures fast hydration and gelation of these wafers and thus alleviating the foreign body sensation [15]. Wafers can be easily applied being very effective compared to other dosage forms owing to low frequency of administration and lack of additives, that result in adverse effects on the vagina, leading to toxic keratitis manifested as punctuate lesions with a persistent epithelial defect [14,16]. Insoluble wafers suffers some disadvantages; they have to be removed manually, in addition to causing erosion of the vaginal wall and discomfort due to foreign body sensation, and other device related issues [17]. On the contrary, soluble wafers that dissolve or erode gradually after administration overcome the previously mentioned drawbacks, and employ ingredients that are well adapted for vaginal use [18,19].

Vaginal candidiasis is one of the most common gynecological condition affecting more than 130 million women worldwide [20]. About 85% of the reported cases are caused by Candida albicans, the most prevalent species of the Candida fungi [21]. Treatment of vaginal candidiasis involves the use of oral and topical medications, among which are the azole antifungal agents [22]. Clotrimazole [23] is a broad-spectrum antifungal azole acting by inhibiting ergosterol biosynthesis, the major constituent of the fungal membrane resulting in the damage of the cell structure and eventually fungal death [24]. Although have being widely investigated for treatment of various fungal infections, the poor aqueous solubility of CLT is still the main challenge in the formulation process [23].

Therefore, the main goal of this research was exploring an innovative approach for the preparation of mucoadhesive, biodegradable system that could be well tolerated by the patients for the vaginal delivery of CLT. The solubility of CLT was first improved using different solubilizers then formulated into vaginal wafers. The prepared wafers were then characterized regarding their weight variation, drug content, surface pH, swelling index, in-vitro drug release, solid sate characterization and porosity. Finally, the in-vivo performance and antifungal activity of the CLT vaginal wafer was evaluated against vaginal candidiasis.
2. Materials and methods

2.1. Materials

CLT was kindly supplied from Kahira Pharmaceuticals & Chemical Industries Company [25]. Low M. Wt chitosan (50,000–190,000 Da), medium M. Wt chitosan (190,000–310,000 Da), high M. Wt chitosan (310,000–375,000 Da), Solutol® HS15, Hydroxypropyl methyl cellulose (HPMC; M.wt 90,000) and Cremophore RH40 were purchased from Sigma Aldrich, USA. Glacial acetic acid was provided by United Company for Chemical and Medical Preparations, Cairo, Egypt. Inutec SP1 was generously provided by BENEOR, Oraflit, Tienen, Belgium. Gelucire® 13 and Gelucire® 14 were kindly donated by Gattefosse, France. All other reagents were of analytical grade.

2.2. Saturated solubility

To determine the solubility of CLT, excess amount of CLT was added to 5 mL of different solubilizers, namely; Cremophore® RH40, Inutec® SP1, HPMC, Solutol® HS15, Gelucire® 13, and Gelucire® 14. The vials were shaken at 37 ± 0.5 °C for 48 h in a thermostatically controlled water bath (Memmert Gmgh, Germany), then filtered through a 0.45 μm membrane filter. The concentration of CLT in the filtrate was determined spectrophotometrically at λmax 260 nm (Shimadzu UV spectrophotometer, 2401/PC, Japan).

2.3. Preparation of CLT vaginal wafers

Chitosan aqueous solutions (1 and 2% w/w) containing a 1:1 wt ratio mixture of CLT: Solutol® HS15 were prepared. Chitosan was first dissolved in 1% glacial acetic acid, then accurately weighed amounts of CLT and Solutol® HS15 were dispersed in the chitosan solution with a CLT concentration of 50 mg/mL. Following that, the prepared solution (1 mL) was poured into plastic PVC moulds (length:3 mm, diameter: 13.0 mm) and frozen at −22 °C for 24 h, followed by lyophilization (Novalyphile-NL 500; Savant Instruments Corp., Holbrook, NY, USA) for 24 h under a condenser temperature of −45 °C and a pressure of 7 × 10−2 mbar. The formulated sponge-like vaginal wafers containing the therapeutic dose (Martindale, 2007) of 50 mg CLT/tablet were stored in a desiccator until use.

2.4. In-vitro evaluation of the prepared CLT loaded vaginal wafers

2.4.1. Weight uniformity

Ten randomly selected wafers from each formulation were weighed individually (Precisa Balances, Dietikon, Switzerland) and average weight was determined.

2.4.2. Content uniformity

One wafer was dissolved in 10 mL 0.1 N acetic acid solution by stirring on a magnetic stirrer at 500 rpm. The absorbance of the solution was then measured spectrophotometrically (UV-1601, Shimadzu, Japan) at 260 nm to determine the content of CLT. The test was done on 10 individual wafers.

2.4.3. Surface pH

The prepared CLT loaded freeze-dried wafer was allowed it to swell in contact with 5 mL simulated vaginal fluid (pH 4.5) for 2 h at room temperature. The surface pH was determined by applying the electrode of the pH-meter (Jenway, Barloworld Scientific, UK) on the wafer surface and equilibrating for 1 min, and recording the pH (Thombre and Gaikwad, 2013). The surface pH was determined in triplicate.

2.4.4. Swelling index

The water uptake at room temperature was determined by gravimetric analysis [26] using electric balance (Precisa Balances). A small filter paper (d = 55 mm, Schleicher & Schuell GmbH, Dassel, Germany) was placed on a 1% w/v agar petri dish, and left to equilibrate for 60 min. An accurately weighed CLT loaded vaginal wafer was placed on the upper side of the filter paper in the covered petri dish and the weight of the swollen wafer was determined at different time intervals. The swelling index (%) was estimated using the following equation:

Swelling ratio (%) = \( \frac{W_t - W_0}{W_0} \times 100 \)

where \( W_0 \) is the initial weight of the sample, while \( W_t \) is the weight of the sample at different time intervals.

2.5. In-vitro drug release study

In-vitro release studies of CLT from the prepared CLT-loaded vaginal wafers were performed in bottles containing 100 mL acetate buffer (pH 4.5): ethanol in the ratio 6:4 as a release medium. The bottles were placed in oscillating water bath (Stuart SBS 40, Staffordshire, UK) operating at 100 rpm, with temperature adjusted at 35 °C. Aliquots of 1 mL were withdrawn at different time intervals and analyzed for CLT content by measuring the absorbance at 260 nm. The samples were replaced each time with fresh medium.

2.6. Characterization of selected vaginal wafer

2.6.1. Differential Scanning Calorimetry (DSC) studies

DSC measurements were performed for CLT plain powder, selected chitosan plain powder, and a selected CLT-loaded vaginal wafer, using a PerkinElmer DSC 6 (PerkinElmer, Beaconsfield, UK). The samples, weighting 8–12 mg, were placed into the DSC under a nitrogen flux (20 mL/min) and heated from 25 to 225 °C at a scanning rate of 10 °C/min.

2.6.2. Powder X-ray diffraction (XRD) studies

Diffract patterns of CLT plain powder, selected chitosan plain powder, and a selected CLT-loaded vaginal wafer were determined in a Scintag X-ray diffractometer [27] using Cu Kα radiation with a nickel filter, a voltage of 45 kV, and a current of 40 mA.

2.6.3. Scanning electron microscopy

The surface morphology and cross-sections of the selected optimum wafer were sputter coated with gold under argon atmosphere using Edwards Sputter coater. The samples were then examined using JEOL (JXA-840A, Japan) electron probe microanalyzer.

2.6.4. Porosity characterization and pore size distribution

The porosity of ten tablets was determined through the measurement of apparent and specific density. The specific density (\( \sigma_s \)) of each tablet was measured using a helium displacement density pycnometer (Quantachrome UltraPyc 1200e, Quantachrome Instruments, Florida, USA) with 5 purge/measurement cycles at 15.0 psig. The apparent density (\( \sigma_a \)) was then measured using the same instrument after closure of all the pore spaces. Porosity [28] was then calculated using the following equation [29].

\[
\phi = 100\% \frac{\sigma_a - \sigma_s}{\sigma_a - \sigma_f}
\]

where, \( \sigma_f \) is the density of the used fluid (helium), equals to 0.000164 g/cm³.

The pore size distribution of ten tablets was measured using mercury intrusion capillary pressure technique ‘MICP’ (Automatic Pore Size Analyzer, AutoPore IV, Quantachrome PoreMaster series). Samples were subjected to mercury intrusion pressures from 0.5 up to 60,000 psia. The pore size distribution and median pore size for each sample were analyzed.
2.7. Assessment of the antifungal activity of drug-loaded vaginal wafers

2.7.1. Animals

The study was performed on 20 female Wistar rats (80–100 g; National Research Centre, Cairo, Egypt). Before the experiment, the rats were accommodated for one week in metal cages with 12 h dark/light cycle, with free access to a standardized laboratory diet and water. The experimental procedure was approved by the Animals Ethics Committee of Cairo University, Egypt, and conformed to the EU Directive 2010/63/EU for use and treatment of animals in experiments.

2.7.2. Experimental rat vaginitis

Rat vaginitis was induced using C. albicans (NRRL Y-477) as previously described in the literature [30]. Sterile cotton ball was first soaked with 250 μL of Candida suspension (1 × 106 CFU/mL) then inserted into the rat vaginal tract for 5 min, then repeated at 24 and 48 h interval.

2.7.3. Experimental design

Rats infected with vaginitis were equally distributed into four groups and received different treatments; Group I: control untreated rats, Group II: the market product, Group III: the selected CLT-loaded vaginal wafer. The treatments were applied to the rat vaginal tract and a swap was taken using a sterilized cotton ball at 24, 48, 72, 96 and 168 h following the last day of Candida inoculation. The cotton ball was introduced into distilled water (5 mL) in sterile tubes and kept for 60 min at room temperature, then 1 mL of the infected solution was placed in a sterile Petri dish containing 20 mL potato dextrose agar medium. The Petri dishes were kept for 24 h at 37 °C in an incubator, then the formed colonies were counted and expressed as CFU/mL. The inhibition percent was calculated using the following equation:

\[
\text{Inhibition percent} (\%) = \frac{\text{CFU/mL of untreated group} - \text{CFU/mL of treated group}}{\text{CFU/mL of untreated group}} \times 100
\]

2.8. Statistical analysis

All measurements were expressed as mean ± SD and calculated data was statistically analyzed using SPSS®-17.0 (SPSS Inc., Chicago, IL). Statistical differences was evaluated by one way analysis of variance (ANOVA) followed by the least significant difference test. Values of \( P < 0.05 \) were considered statistically significant.

3. Results and discussion

3.1. Saturated solubility

Drug solubility is a crucial parameter affecting the bioavailability, a formulation problem that can be handled using different approaches among which is the choice of a proper solubilizer. The chosen one should enhance the solubilization of active agents in aqueous medium; promote the absorption and permeation at the biological membranes without affecting the release pattern or modifying the therapeutic activity [31]. With the goal of enhancing the solubility of CLT, a solubility study was conducted using various solubilizers aiming to select the optimum one for vaginal wafers preparation. Hence, the following solubilizers; Cremophore® RH40, Inutec® SP1, HPMC, Solutol® HS15, Gelucire® 13, and Gelucire® 14 were screened and the results are shown in Fig. 1. The aqueous solubility of CLT was reported to be 0.005 mg/mL [32]. The obtained data revealed that all the tested solubilizers succeeded to improve the solubility of CLT. The increase in solubility was in the following order: Solutol® HS15 > Gelucire® 13 > Gelucire® 14 > Inutec® SP1 > Cremophore® RH40 > HPMC. As observed, Solutol® HS15 was found to be most effective in enhancing the solubility of CLT, showing the highest solubilization capacity (1.6 ± 0.38 mg/mL) representing about 200-fold increase in solubility compared to CLT aqueous solubility. Solutol® HS15 is a nonionic surfactant, commonly used in many drug delivery systems due to its high capacity to solubilize poorly water soluble drugs in addition to the reported low toxicity [33,34]. Based on the above results, Solutol HS15 was selected as the optimum solubilizer for the preparation of CLT vaginal wafers.

3.2. Preparation and in vitro evaluation of the prepared CLT loaded vaginal wafers

During the past few years, chitosan, a natural biodegradable and biocompatible polymer derived from chitin, have been widely explored for mucosal and transmucosal delivery of drugs [25,35]. Owing to the mucoadhesive and penetration enhancement characteristics of chitosan, it is capable of enhancing drug absorption across both rich mucosa like intestinal and nasal as well as poor ones, such as vaginal and buccal mucosa [36]. Thus, in the present study, three molecular weights of chitosan (low, medium and high) at two different concentrations (1 and 2 %w/w) have been utilized for the preparation of six CLT-loaded vaginal wafers (Table 1). The wafers were fabricated using the lyophilization method. Generally, in the lyophilization method, during the freezing step, the produced water crystals are eliminated by sublimation resulting in a porous structure [37]. Accordingly, the prepared wafers were flexible and elastic, with highly porous spongy consistency and smooth surface. In addition, the highly porous structure facilitates the penetration of vaginal fluids upon application. The average weights, content and pH of the prepared CLT-loaded vaginal wafers were determined and presented in Table 1. As observed, the average weight of the prepared CLT-loaded vaginal wafers varied according to their actual content, ranging between 0.134 ± 0.008 and 0.191 ± 0.007 gm. The drug content of the

### Table 1

<table>
<thead>
<tr>
<th>Code</th>
<th>Chitosan Molecular weight</th>
<th>Average weight (gm)</th>
<th>pH</th>
</tr>
</thead>
<tbody>
<tr>
<td>CLT-GH-L1</td>
<td>Low 1</td>
<td>0.181 ± 0.007</td>
<td>4.4 ± 0.00</td>
</tr>
<tr>
<td>CLT-GH-L2</td>
<td>Low 2</td>
<td>0.192 ± 0.026</td>
<td>4.6 ± 0.14</td>
</tr>
<tr>
<td>CLT-GH-M1</td>
<td>Medium 1</td>
<td>0.180 ± 0.004</td>
<td>4.45 ± 0.07</td>
</tr>
<tr>
<td>CLT-GH-M2</td>
<td>Medium 2</td>
<td>0.182 ± 0.001</td>
<td>4.55 ± 0.07</td>
</tr>
<tr>
<td>CLT-GH-H1</td>
<td>High 1</td>
<td>0.135 ± 0.008</td>
<td>4.4 ± 0.00</td>
</tr>
<tr>
<td>CLT-GH-H2</td>
<td>High 2</td>
<td>0.185 ± 0.001</td>
<td>4.55 ± 0.07</td>
</tr>
</tbody>
</table>

Fig. 1. CLT saturated solubility in different solubilizers.
prepared vaginal wafers was higher than 85% in all the prepared wafers indicating homogenous distribution of CLT in the preparation (data not shown). The surface pH of the prepared wafers varied between 4.4 ± 0.00, and 4.6 ± 0.14, which is close to that of the vagina (pH 4.5). According to these results, all wafers provided an acceptable pH for the vagina and would not produce any local irritation to the mucosal surface upon application.

3.3. Swelling index

In order to have local vaginal effect, critical physiological parameters such as low volume of vaginal fluids have to be considered [38]. Therefore, the prepared vaginal wafers have to be rapidly hydrated in order to achieve rapid mucoadhesion to the vaginal mucosa, and avoid fast elimination from the waferion site. The swelling indices of the prepared vaginal wafers over a period of 8 h are presented in Fig. 2. The water uptake and subsequent wafer swelling was found to increase with time. The highest swelling index % after 8 h was achieved by the wafer containing low concentration of the low molecular weight chitosan (CLT–CH–L1), hence capable of absorbing and retaining high contents of water, thus providing favorable environment for vaginal application. As observed, the swelling characteristics of the wafers were affected by both the concentration and molecular weight of chitosan used. The results showed that increasing the concentration and molecular weight of chitosan resulted in slower swelling of the prepared vaginal wafers owing to the increase in binding capacity, and the formation of highly viscous gel layer surrounding the wafer, which retards the penetration of water inside the wafers.

3.4. In vitro drug release study

The release of CLT from the prepared CLT-loaded vaginal wafers was performed in acetate buffer (pH 4.5), in order to mimic the pH of human vagina (≈ 4.5). The release profiles of CLT from the prepared vaginal wafers are presented in Fig. 3. All prepared CLT-loaded vaginal wafers achieved a sustained drug release pattern for the duration of 8 h. Initially, all wafers showed burst drug release after 1 h, ranging from 13.52% (CLT–CH–H2) to 39.60% (CLT–CH–L1) followed by a much slower release rate. The results revealed that increasing the concentration and molecular weight of chitosan resulted in slower release of CLT from the prepared vaginal wafers which might be related to the increase in binding capacity, and the formation of a strong gel layer, hindering the matrix dissolution and drug release. As observed, among all the investigated wafers, CLT–CH–L1 showed the highest drug release profile owing to complete erosion or degradation of the chitosan matrix. The obtained data are in accordance with the results of swelling index, where wafers containing high concentration of the high molecular weight chitosan showed lower swelling, due to retardation of water penetration through the wafer, and hence lower percentage of drug released. Similar results were obtained by Kassem et al. [39], in their study on the preparation of chitosan buccal sponges for buspirone HCl delivery.

The drug release from chitosan matrices might occur by three different mechanisms; drug release from the sponge surface, or diffusion through the swollen matrix, or due to polymer erosion [40]. The observed burst drug release after 1 h could be attributed to the dissolution of the drug adhering to the surface and not entrapped within the inner matrix of the wafer. The drug release afterwards could be due to the diffusion process, which was much slower when compared to the initial release. In a previous study, Foda et al. [41] reported that a large percentage of tramadol HCl was released in the first hour for both uncross-linked and cross-linked matrices due to the presence of surface drug. Many authors stated that the release of active drugs from different chitosan dosage forms decreased with the increase in chitosan molecular weight. Polk et al. [42] showed that the molecular weight of chitosan was a key variable in the release of albumin from chitosan microspheres, where decreasing the molecular weight increased the release of albumin. Similarly, Ko et al. [43] observed that the release of felodipine from cross-linked chitosan microparticules decreased as the molecular weight of chitosan increased.

Accordingly, the kinetics analysis of the release data was performed using zero order, first order, Higuchi diffusion model and Korsmeyer-Peppas equation. All wafers depicted proper fitting to Higuchi diffusion kinetics showing good linearity (R²: 0.983–0.996). In order to provide better explanation of the mechanisms governing the release, Peppas equation was applied for the analysis of the release data and the release exponent “n” was determined. The “n” values varied between 0.43 and 0.85, pointing out to a non-Fickian release mechanism representing an anomalous diffusion where the drug release was governed by more than one process referring to a combination of diffusion and erosion mechanisms.

3.5. Characterization of selected vaginal wafer

3.5.1. Differential Scanning Calorimetry (DSC) studies

The DSC was applied to study the phase of transformation of CLT during the formation of the vaginal wafers. As illustrated in Fig. 4, the free drug was characterized by a single, sharp melting endothermic peak of 147.26 °C corresponding to its melting point, confirming its crystallinity [23]. The low molecular weight chitosan showed two characteristic degradation stages, a broad endothermic peak ranged from 57 to 155° C corresponding to a dehydration process while the other peak extended from 275 °C to 340 °C pointing to sample combustion [25]. On the contrary, the prepared lyophilized vaginal wafer;
CLT–CH–L1 did not show the characteristic peaks of CLT and chitosan indicating transformation of the crystalline drug into the amorphous form during the preparation of the wafers.

3.5.2. Powder X-ray diffraction

In order to further examine the physical form of the drug in prepared wafers, CLT, low molecular weight chitosan and the selected CLT-loaded vaginal wafer; CLT–CH–L1 were investigated using powder X-ray diffraction (Fig. 5). The diffractogram of CLT confirmed its crystalline nature as indicated by prominent intense diffraction peaks, while only a single characteristic peak at 2θ = 21.1° was observed for low molecular weight chitosan. On the other hand, the diffractogram of CLT–CH–L1 revealed a diffuse pattern with complete absence of the numerous distinctive peaks of CLT indicating the entirely amorphous nature of CLT in the prepared.

3.5.3. Scanning electron microscopy

In order to study the surface morphological characteristics of CLT–CH–L1 as well as CLT, SEM was performed and photomicrographs of are presented in Fig. 6 (A). SEM image of CLT–CH–L1 illustrated the complete disappearance of any drug crystals (Fig. 6 A). Meanwhile, the surface and cross-section view of CLT–CH–L1 demonstrated the spongy shaped nature of the wafer and the formation of a network of highly interconnected 3D porous structure. Such structure is a result of the lyophilization process during the preparation of the wafers, where chitosan is capable of absorbing high amounts of water forming ice crystals at the freezing step, producing pores after sublimation [44].

3.5.4. Porosity characterization and pore size distribution

The porosity of wafers plays an important role in controlling the diffusion of active ingredients and vaginal fluids upon application. Thus, the porosity and pore size distribution of the selected vaginal wafer; CLT–CH–L1 were determined. The obtained result showed that the average porosity of CLT–CH–L1 was 87.76 ± 0.618 with mean pore diameter of 100.7 ± 12.87 µm. A sample plot of incremental intrusion volume versus pore size (Fig. 6 Bi) illustrates a steep elevation initially followed by a plateau indicative of a narrow parameter range further confirmed by intrusion volume distribution versus pore diameter (Fig. 6 Bii). The recorded high porosity of CLT–CH–L1 can be attributed to the large sized pores of thin walls [44] as observed in SEM photomicrographs. Such feature provides enough space for the delivery of therapeutic agents and essential for the removal of unwanted vaginal secretions as well as increasing the adhesion.

3.6. Assessment of the antifungal activity of CLT-loaded vaginal wafers

Vaginal candidiasis is considered a common opportunistic microbial problem affecting approximately 75% of women at least one time during their life, about 50% of them will suffer a recurrence while a limited number will experience a chronic course [45,46]. Generally, vaginal candidiasis infection reaches deeper epithelial layers [47], thus efficient treatment is not only dependant on the selection of appropriate antifungal compound but also on the development of an appropriate delivery system. Such system should be able to penetrate deep into the epithelium, localize at the infection site as long as possible capable of releasing the active agent according to the treatment protocol [21,48]. Accordingly, the in-vivo efficacy of CLT-loaded vaginal wafer; CLT–CH–L1 was assessed compared to the available market product using Wistar rats infected by C. albicans to mimic vaginal candidiasis pathological condition. After infection, colonies of C. albicans were counted along the treatment days and the percentage of fungal infection inhibition was calculated and presented in Fig. 7. As observed, along the treatment days, both treated groups showed significant inhibition of the fungal growth compared to the control untreated rats. However, CLT–CH–L1 was noticed to be therapeutically more active showing greater reduction of fungal count, represented in superior inhibition of C. albicans propagation since the first day of treatment reaching 89.12% ± 2.01 at the end of the study compared to 76.30 ± 3.14 in case of market product group. The obtained results reflect the ability of CLT-loaded vaginal wafer in the efficient clearance of CFU confirming the enhanced antifungal inhibitory effect. This promoted efficacy could by particularly related to the composition of the produced wafer based mainly on chitosan. Being a natural polymer, chitosan is well known for its biocompatibility and safety in addition to the reported bioadhesive capacity and antimicrobial activity, such collective properties favors vaginal delivery [49]. Upon application, wetting and swelling of chitosan took place allowing an intimate contact with the mucosal layer. Then, the positively charged polymer form electrostatic interactions with negatively charged sialic groups in mucin of the mucosal tissue allowing the interpenetration of the polymer chains and entanglement with mucin chains resulting in enhanced mucoadhesiveness [50]. Moreover, the cationic character of chitosan allow the effective interaction with the negatively charged fungal cell membranes leading to the disruption of their normal function causing the leakage of intracellular constituents and finally to cell death [51]. Thus, the present study and the obtained microbiological data revealed that CLT formulated as chitosan based vaginal wafer can inhibit C. albicans cell
populations, suggesting that the proposed formulation could potentiate CLT therapeutic activity and pointing out to the future applicability of such formulation against vaginal candidiasis.

4. Conclusion

In the present study, CLT-loaded vaginal wafers based on chitosan of different molecular weights and concentrations were successfully developed using freeze-drying technique. The prepared wafers were fully characterized and optimized. The vaginal wafer, CLT–CH–L1, composed of 1% low molecular weight chitosan showed the highest swelling index and suitable release profile was selected for further studies. The wafer revealed spongy shaped highly porous structure of large size pores with narrow parameter range. The DSC and XRD studies confirmed the amorphous nature of CLT in the prepared lyophilized vaginal wafer. The in-vivo antifungal activity of the vaginal wafer was compared to the market product showing superior antifungal effect in eradicating Candida infection in rats. The present study revealed the feasibility to produce an easily handled effective vaginal dosage form providing greater convenience and better patient compliance serving as a potential delivery system for management of vaginal candidiasis.

CRediT authorship contribution statement

Rehab Shamma: Conceptualization, Methodology, Formal analysis, Investigation, Writing - original draft, Writing - review & editing. Mona Basha: Conceptualization, Methodology, Investigation, Resources, Data curation, Writing - original draft, Writing - review & editing. Ghada Awad: Conceptualization, Methodology, Formal analysis, Investigation.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.
Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jddst.2020.101561.

References


