ABSTRACT

Objective: The principal objective of this research work was to formulate tioconazole into mucoadhesive microspheres which were inserted into the vagina for sustaining the anti-fungal activity and protecting the liver and the kidney from the harmful drug side effects.

Methods: Microspheres were prepared by the emulsion solvent evaporation method, using different ratios of the drug with either ethyl cellulose N14 or hydroxypropylmethylcellulose K100M (HPMC K100M) as mucoadhesive polymers. The formulated microspheres were evaluated for the particle size, entrapment efficiency, yield percentage, mucoadhesion strength, swelling percentage, pH, in vitro release of the drug and finally in vitro antifungal activity.

Results: The optimized formulae were F3 and F6, which showed drug entrapment of 88.4±2.5 % and 97.8±1.6 %, yield percentage of 85.0±2.3% and 95.2±1.2%, mucoadhesion strength of 31.5±1.6 and 38.1±1.6 *10^3 dyne/cm², swelling percentages of 143.1±1.3 and 154.2±0.8, pH of 4.7±0.1 and 4.6±0.5, and particle size of 400±3.5 µm and 435±7.8 µm respectively. F3 and F6 released about 72.8%±3.2 and 58.4%±2.7 of the drug after 8 h respectively. F3 and F6 showed a significant anti-fungal activity.

Conclusion: Ethyl cellulose and hydroxyl propyl methylcellulose mucoadhesive microspheres are considered a good way to increase the duration of the anti-fungal activity of tioconazole with minimum side effects.

Keywords: Microspheres, Tioconazole, Mucoadhesion, Vagina

INTRODUCTION

Vaginal cavity is an important and alternative area for the drugs that suffer from either first pass metabolism or extensive destruction in the stomach. Increased drug administration area, rich blood supply and poor enzyme activity, encourage the researchers to choose the vagina as a route for application of several drugs, especially those have harmful impacts on the liver and the kidney like imidazole anti-fungal drugs [1].

Fortunately, mucoadhesive microspheres sustain the release of the drugs, enhance the bioavailability and increase the absorption due to the intimate contact with the vaginal mucosa [2]. Tioconazole is (RS)-1-[2-(2-Chloro-3-thienyl) methoxy]-2-(2, 4-dichlorophenyl) ethyl]-1H-imidazole. It adversely affects both the kidney and the liver and suffers from poor bioavailability.

Hani et al. formulated clotrimazole into intravaginal microspheres by the spray drying technique, which was inserted into a mucoadhesive gel for the local treatment of vaginal candidiasis [3]. Kalita et al. increased the vaginal residence time of metronidazole through the preparation of PLC microspheres, which were incorporated into carbopol 934p and HPMC K4 M bioadhesive gel [4]. Khan and Thakur developed novel chitosan mucoadhesive microspheres for vaginal administration of tenofovir disoproxil fumarate [5]. The principal intention of this research study was to formulate vaginal mucoadhesive microspheres loaded with tioconazole to avoid the harmful impacts of the oral administration of the drug on the kidney and the liver and enhancing its poor oral bioavailability through increasing the vaginal retention time of the drug.

MATERIALS AND METHODS

Materials

Tioconazole was kindly supplied by Pfizer Company, Egypt. Ethyl cellulose N14, carbopol 940 (Cbp 940) and HPMC K100 M were kindly supplied by EIPICO Company, Egypt. Tween80, methylene chloride, methanol and potassium dihydrogen orthophosphate were purchased from El-Nasr Company, Egypt. All other chemicals were of analytical grade.

Table 1: Composition of different tioconazole microspheres formulations

| Formulation code | Drug: ethylcellulose N14 ratio | Drug: HPMCK100M ratio | Cbp 940 (%W/W) | Tween 80 (%W/V) | Time (h) | RPM |
|------------------|-------------------------------|-----------------------|----------------|----------------|----------|-----|
| F1               | 1:1                           |                       | 0.5            | 1              | 1000     |
| F2               | 1:3                           |                       | 0.5            | 1              | 1000     |
| F3               | 1:5                           |                       | 0.5            | 1              | 1000     |
| F4               | 1:1                           |                       | 0.5            | 1              | 1000     |
| F5               | 1:3                           |                       | 0.5            | 1              | 1000     |
| F6               | 1:5                           |                       | 0.5            | 1              | 1000     |
| F7               | 1:1                           |                       | 0.5            | 1              | 1000     |
| F8               | 1:3                           |                       | 0.5            | 1              | 1000     |
| F9               | 1:5                           |                       | 0.5            | 1              | 1000     |
| F10              | 1:5                           |                       | 0.5            | 1              | 1000     |

Cbp 940: Carbopol 940, HPMC: Hydroxy propyl methyl cellulose
Methods

Preparation of vaginal mucoadhesive microspheres

Microspheres were prepared by the emulsion solvent evaporation method. The drug and the polymer were accurately weighed by electron digital balance (Metter-Toledo, Ag, CH 8606, Greifensee, Switzerland). The components were dissolved into methylene chloride at ambient room temperature. The organic phase was slowly added to 100 ml distilled water containing 0.5 % (W/V) Tween 80 and emulsified by stirring at 1000 RPM using a mechanical stirrer (Heidolph PZP-2000, Germany) for 1 h. The solution was filtered, washed and dried overnight at ambient room temperature [6].

The prepared microspheres were collected, dried and weighed. The actual weight was divided by the total weight of the starting material to determine the yield percentage.

Yield percentage

\[
\text{Yield percentage} = \frac{\text{Actual weight of the product}}{\text{Total weight of the drug and excipient}} \times 100 \quad [8]
\]

Drug entrapment efficiency

Microspheres equivalent to 10 mg of the drug were crushed, suspended in 20 ml phosphate buffer pH4.5 and stirred at 100 RPM at ambient room temperature in a thermostatic shaker water bath (Julabo SW-20 C, Germany) till equilibrium. Then, the solution was filtered, suitably diluted and the drug content was determined by RP-C18 HPLC method using methanol: phosphate buffer (70:30) adjusted to pH 4 by orthophosphoric acid as a mobile phase at a flow rate of 1.5 ml/min and UV detection at 260 nm. The experiment was done in triplicate.

Entrapment efficiency

\[
\text{Entrapment efficiency} = \frac{\text{Actual drug content}}{\text{Theoretical drug content}} \times 100 \quad [4]
\]

Swelling index

The extent of the swelling was expressed as the percentage weight gained by the microspheres. 20 mg of each formula was kept in a petri dish containing phosphate buffer pH 4.5 at ambient room temperature for 4 h. At the end of the experiment, the microspheres were withdrawn and weighed. The swelling index was determined according to the following equation:

\[
\text{Swelling} \% = \frac{(W_t - W_0)}{W_0} \times 100
\]

Where \( W_0 \) is the weight of the dried microspheres and \( W_t \) is the weight of the swollen microspheres after 4 h. The experiment was done in triplicate [9].

Mucoadhesion strength measurement

The mucoadhesion strength was estimated by measuring the force required to detach the microspheres from the mucin disc in between two vials using a modified balance. The modified apparatus comprised of a two-arm balance, one side of which contained a plastic jar, and the other side contained two glass vials; one of the vials was attached permanently to the base of the stage, and the other was attached to the arm of the balance by a thick strong thread. Two mucin discs (E) were secured to the two glass vials (C) separately using a cyanoacrylate adhesive and a rubber band (fig.1). The microspheres (0.5 g) were applied to the mucin disc between the two vials. The height of the vial adjusted so that the spheres could adhere to the surface of both vials then, a constant weight was applied on the upper vial for 2 min, after which, it was removed and the upper vial was connected to the balance. Water was added slowly at a constant rate to a plastic jar (13–15 drops per min), until both vials were separated. The mucoadhesion strength, expressed as the detachment stress in dynes/cm² was determined using the following equation:

\[
\text{Detachment stress (dynes/cm}^2) = \frac{m}{A} \quad [10]
\]

Where \( m \) is the weight of water (g) that detached the two vials, \( gr \) is the acceleration due to gravity taken as 980 cm/s², \( A \) (cm²) is the area of the mucin exposed and is equal to \( \pi r^2 \) (r is the radius of the exposed mucin). The experiment was done in triplicate.

\[\text{Fig. 1: Modified balance for measuring mucoadhesion strength: (A) Modified balance; (B) Plastic jar; (C) Glass vial; (D) Microspheres formulation; (E) Mucin; (F) Height-adjustable pan [11]}\]

PH determination

10 mg of each formula was allowed to swell for 3 h in 25 ml distilled water. The pH of the solution was determined using a pH meter (JENCO Model-5005 USP). The experiment was done in triplicate.

In vitro release study

The cumulative release percentage of tioconazole microspheres were carried out in phosphate buffer pH4.5 for 8 h in USP-type II dissolution apparatus at 372±0.5 °C and 100 RPM. Microspheres equivalent to 10 mg drug were used in this study. The samples were taken hourly and replaced with an equal volume of fresh release medium to maintain sink condition. After suitable dilution, samples were analyzed using HPLC-UV detection at 260 nm [12].

In vitro antifungal activity

In vitro antifungal activity of tioconazole loaded microspheres was investigated by the agar diffusion disc method [13]. Exactly four cups of 10 mm diameter were made in sabourated dextrose agar and inoculated with Candida Albicans. Two cups were filled with a calculated amount of selected microspheres and 0.5 ml distilled sterilized water was added for microspheres wettability. The other two cups were filled with tioconazole solution as a positive control and the drug-free spheres as a negative control. The plates then were incubated at 25 ° C for 72 h. The radius of the inhibition zones was calculated and compared [14].

Kinetic analysis of the data

The data of the drug release from the tested microspheres formulations were subjected to theoretical analysis, to determine the order of kinetic release according to the following equations:

- Zero-order kinetic: \( CT = Co – Kt \)
- First order kinetic: \( \log CT = \log Co – Kt \frac{2.303}{2.303} \)
- Diffusion control model: \( QA = 2Co (A/r)^{0.49} \)

Statistical analysis of the data

Experimental results were expressed as the mean±SD (standard deviation of the mean). One-way analysis of variance (ANOVA) was done in triplicate.
RESULTS AND DISCUSSION

Spherical, uniform and smooth surface microspheres were obtained as demonstrated in fig. 2.

Fig. 2: Photomicrograph of 1:5 HPMC K100M (1:5, 0.5% Tween 80, 1000 RPM, 640 x 320 pixels)

Particle size

The mean particle size of the prepared tioconazole microspheres is given in tables 2, 4, and 5. It is greatly obvious that microspheres which were prepared with ethyl cellulose N14 (F1, F2, F3) were smaller than those prepared with HPMC K100 M (F4, F5, F6), and this could be attributed to the impact of the polymer solution viscosity.

As a drug to polymer ratio was increased from 1:1 to 1:5, there was a comparable and significant increase in the mean particle size from 267±4.2 µm (F1) to 400±3.5 µm (F3) and from 297±6.1 µm (F4) to 435±7.8 µm (F6).

This was in a good agreement with the results of Sabry et al. [16] who found that as a drug to polymer ratio was increased from 1:1 to 1:5, the size of nizatidine micro balloons significantly increased from 230±1.9 to 324±2.6 respectively.

As the stirring rate was increased from 1000 to 1600 RPM, the particle size significantly decreased from 400±3.5 µm to 315±5.9 µm and from 435±7.8 µm to 335±6.3 µm, for F3 and F6 respectively. This may be due to the high shear force associated with the high speed which splits the larger droplets into smaller ones [16, 18].

The increase in the polymer concentration resulted in an increase in the viscosity of the polymer solution and consequently larger particles were formed.[17]

Entrapment efficiency

The results in table 2 show that the mean entrapment efficiency for F1, F2, F3, F4, F5, and F6 were 55.0±1.5, 75.2±0.8, 80.4±2.5, 69.2±2.1, 85.1±3.1, and 97.8±1.6 respectively. It is clear that there was a significant increase in the drug entrapment with an increase in the polymer concentration.

This was in great accordnace with Kalita et al. who found that metronidazole entrapment increased with an increase in HPMC K4 M concentration [4].

The increase in the polymer concentration resulted in an increase in the viscosity of the polymer solution which produced larger particles and consequently much drug was entrapped [8].

There was a reduction in the entrapment efficiency from 88.4±2.5 to 50.1±2.1, and from 97.8±1.6 to 63.6±2.6, for F3 and F6 respectively (table 4) with an increase in the stirring rate from 1000 to 1600 RPM. Higher stirring rate resulted in smaller particle size microspheres which entrapped less drug.

As surfactant concentration increased from 0.5% to 1.5%, there was a significant decrease in the entrapment efficiency from 88.4±2.5 to 65.1±1.9, and from 97.8±1.6 to 73.1±2.1, for F3 and F6 respectively (table 5). The intact and smooth microspheres will be formed at a low surfactant concentration, but as the concentration increased, the microspheres become more brittle and much drug will be lost during washing [19]. The addition of Cbp940 had a non-significant effect on the drug entrapment.

Yield percentage

Table 2 demonstrates the effect of the polymer concentration on the production yield percentage. When the drug to polymer ratio was increased from 1:1 to 1:5, there was a significant increase in the yield percentage from 59.1±1.3 (F1) to 85.0±2.3 (F3), and from 65.1±1.3 (F4) to 95.2±1.2 (F6). A significant reduction in the yield percentage with the increase of either stirring rate [18] or surfactant concentration [20] was observed (tables 4 and 5). This could be ascribed to the formation of smaller particles which were lost during the filtration and the collection of the microspheres. It was found that the addition of Cbp940 had a non-significant effect on the yield percentage.

Mucoadhesion strength

Table 3 demonstrates mucocoidal strength of the prepared microspheres formulae. The results showed that the microspheres which were prepared with HPMC K100 M (F4, F5, and F6), showed mucocoidal strength of 32.5±1.2, 38.1±1.6, and 45.6±1.2 respectively. These results were greater than those of ethyl cellulose N14 microspheres (F1, F2, F3), which were 20.6±0.1, 26.7±1.2, and 31.5±1.6 respectively. This could be attributed to several factors. The hydroxy groups’ content of HPMC K100 M is greater than that of ethyl cellulose N14 which allows more swelling, resulting in an increase in the flexibility and consequently has more distance between its chains, which gives more hydrogen bonding with the substrate [21]. Also, HPMC K100 M has a molecular weight higher than that of ethyl cellulose, and this could be ascribed to the higher mucoadhesion strength [24].

The addition of Cbp940 (F7, F8, F9, F10) produced a significant increase in the mucocoidal and this could be ascribed to either the increase hydrogen bonding or the molecular weight or both.

Swelling percentage

The results in table 3 declare that the swelling percentages of ethyl cellulose microspheres F1, F2 and F3 were 120.5±1.5, 131.6±0.9, and 143.1±1.3 respectively and those of HPMC microspheres were 133.1±2.3, 141.6±1.6, and 154.2±0.8 respectively. The obtained results inferred that the swelling of HPMC is higher than that of ethyl cellulose, and this could be ascribed to the higher hydrophilicity of the former than the later [21]. Also, the swelling percentage significantly increased with an increase in the polymer concentration [25]. Iswaririya et al. reported an increase in the swelling of ranolazine micro balloons with an increase in sodium alginate concentration [26].

The addition of 1% Cbp940 increased the swelling from 154.2±0.8 (F6) to 160.2±1.9 (F7) and from 143.1±1.3 (F3) to 150.6±0.9 (F9). Further increase in Cbp940 concentration to 2% resulted in a significant increase to 173.2±2.1 and 159.6±2.1 for F8 and F10 respectively.

PH

The pH of the vagina falls within the range of 4 to 5 and that of all prepared formulae was within the range of 4.6 to 5.9, which ensures non-irritability to the vaginal mucosa.
of Cbp940. It formed a gelatinous mass when came in to contact. The hydration capacity of the polymer increased with the addition of the polymer matrix and the diffusional path length, which might delay the release [27-29].

The increase of the polymer concentration increases the swelling of the polymer ratio from 1:1 to 1:5, resulted in a significant decrease in the percentage of tioconazole which released after 8 h from 74.1±0.6 (F4) to 58.4±0.3 (F6), and from 88.2±0.5 (F1) to 72.8±0.3 (F3).

The release of tioconazole from all prepared microspheres formulations followed Higuchi diffusion model. The effect of the polymer type and concentration on the release study of tioconazole was studied. The results demonstrated that the percentage of tioconazole released from the microspheres increased with an increase in the polymer concentration. The increase of the stirring rate from 1000 to 1600, resulting in an increase in the cumulative release percentage from 58.4±0.3 to 79.1±0.8 and from 72.8±0.5 to 90.2±0.9, for F6 and F3 respectively (fig. 7 and 8). Smaller microspheres with the larger surfactant concentration to 1.5% increased the cumulative release percentage upon adding Cbp940 (fig. 5 and 6). Sarfraz reported a decrease in rifampicin release from 91.2% to 81.7% with the increase of the dissolution medium and retarded the release of the drug [30]. This explains the decrease in the cumulative release percentage upon adding Cbp940 (fig. 5 and 6). Sarfraz reported a decrease in rifampicin release from 91.2% to 81.7% with the increase of the dissolution medium and retarded the release of the drug [30].

### Table 2: Particle size, yield percentage and entrapment efficiency of mucoadhesive microspheres

| Formulation code | Mean particle size (µm)±SD* | Mean yield percentage±SD* | Mean entrapment efficiency (%)±SD* |
|------------------|----------------------------|---------------------------|----------------------------------|
| F1               | 267±4.2                    | 59.1±1.3                  | 55.0±1.5                         |
| F2               | 334±5.6                    | 70.2±2.1                  | 87.0±1.2                         |
| F3               | 400±3.5                    | 85.0±2.3                  | 88.4±2.5                         |
| F4               | 297±6.1                    | 65.1±1.3                  | 69.2±2.1                         |
| F5               | 352±3.9                    | 83.1±2.6                  | 85.±1.8                          |
| F6               | 435±7.8                    | 95.2±1.2                  | 97.8±1.6                         |
| F7               | 415±6.6                    | 96.6±2.7                  | 96.2±1.3                         |
| F8               | 425±6.2                    | 95.6±1.9                  | 98.1±1.5                         |
| F9               | 395±5.1                    | 86.7±1.9                  | 85.6±1.5                         |
| F10              | 405±7.1                    | 88.6±2.1                  | 89.1±2.5                         |

*Mean of three determinations±standard deviations of the mean.

### Table 3: Mucoadhesion strength, swelling after 4 h and PH of mucoadhesive microspheres

| Formulation code | Mucoadhesion strength (*10^5dyne/cm²)±SD* | Swelling (%) after 4 h±SD* | pH±SD* |
|------------------|------------------------------------------|---------------------------|--------|
| F1               | 20.6±0.1                                 | 120.5±1.5                 | 4.7±0.2|
| F2               | 26.7±1.2                                 | 131.6±0.9                 | 4.9±0.8|
| F3               | 31.5±1.6                                 | 143.1±1.3                 | 4.7±0.1|
| F4               | 26.8±0.9                                 | 133.1±2.3                 | 4.9±0.5|
| F5               | 32.5±1.2                                 | 141.6±1.6                 | 5.3±0.1|
| F6               | 38.1±1.6                                 | 153.8±0.8                 | 4.6±0.5|
| F7               | 45.6±1.2                                 | 160.2±1.9                 | 4.7±0.8|
| F8               | 51.6±0.9                                 | 173.2±2.1                 | 4.6±0.1|
| F9               | 36.2±1.2                                 | 150.6±0.9                 | 5.1±0.1|
| F10              | 43.5±0.8                                 | 159.6±2.1                 | 4.9±0.8|

*Mean of three determinations±standard deviations of the mean.

### Table 4: Effect of stirring rate on particle size, yield percentage and entrapment efficiency

| Formulation code | RPM | Mean particle size (µm)±SD* | Mean yield percentage±SD* | Mean entrapment efficiency (%)±SD* |
|------------------|-----|-----------------------------|---------------------------|----------------------------------|
| F3               | 1000| 400±3.5                     | 85.0±2.3                  | 88.4±2.5                         |
| F2               | 1200| 355±6.5                     | 72.0±1.6                  | 73.5±1.1                         |
| F3               | 1600| 315±5.9                     | 59.2±2.1                  | 50.1±2.1                         |
| F6               | 1000| 453±7.8                     | 95.2±1.2                  | 97.8±1.6                         |
| F2               | 1200| 305±7.2                     | 83.1±2.1                  | 81.6±2.1                         |
| F3               | 1600| 335±6.3                     | 70.5±1.5                  | 63.6±2.6                         |

*Mean of three determinations±standard deviations of the mean.

### Table 5: Effect of Tween 80 concentration on particle size, yield percentage and entrapment efficiency

| Formulation code | Tween80 (%W/V) | Mean particle size (µm)±SD* | Mean yield percentage±SD* | Mean entrapment efficiency (%)±SD* |
|------------------|----------------|----------------------------|---------------------------|----------------------------------|
| F3               | 0.5            | 400±3.5                    | 85.0±2.3                  | 88.4±2.5                         |
|                  | 1.0            | 352±6.1                    | 74.6±1.7                  | 76.3±1.6                         |
|                  | 1.5            | 310±6.7                    | 61.6±2.1                  | 65.1±1.9                         |
| F6               | 0.5            | 435±7.8                    | 95.2±1.2                  | 97.8±1.6                         |
|                  | 1.0            | 385±5.3                    | 86.2±1.6                  | 85.1±2.5                         |
|                  | 1.5            | 332±5.3                    | 76.4±1.5                  | 73.1±2.1                         |

*Mean of three determinations±standard deviations of the mean.

### In vitro release study

The release of tioconazole from all prepared microspheres formulations followed Higuchi diffusion model. The effect of the polymer type and concentration is shown in fig. 3 and 4. It is obvious that the cumulative release percentages of the drug from F1, F2 and F3 were greater than those from F4, F5 and F6. The increase of the drug to polymer ratio from 1:1 to 1:5, resulted in a significant decrease in the swelling of tioconazole which released after 8 h from 74.1±0.6 (F4) to 58.4±0.3 (F6), and from 88.2±0.5 (F1) to 72.8±0.3 (F3). The increase of the polymer concentration increases the swelling of the polymer matrix and the diffusional path length, which might delay the release [27-29].

The hydration capacity of the polymer increased with the addition of Cbp940. It formed a gelatinous mass when came into contact with the dissolution medium and retarded the release of the drug [30]. This explains the decrease in the cumulative release percentage upon adding Cbp940 (fig. 5 and 6). Sarfraz reported a decrease in rifampicin release from 91.2% to 81.7% with the increase of drug to Cbp974 ratio from 1:1 to 1:2 [31]. The increase of the stirring rate from 1000 to 1600, resulting in an increase in the cumulative release percentages from 58.4±0.3 to 79.1±0.8 and from 72.8±0.5 to 90.2±0.9, for F6 and F3 respectively (fig. 7 and 8). Smaller microspheres with the larger surface area were formed at a higher stirring rate, which suggested the increase in the cumulative release percentage after 8 h [20]. Fig. 9 and 10 show that an increase in the surfactant concentration to 1.5% increased the cumulative release to 78.1±and 87.6±0.5, for F3 and F6 respectively. The increased surfactant concentration gave porous microspheres which resulted in higher release percentages [19, 32].
Fig. 3: Effect of tioconazole: ethyl cellulose ratio on the cumulative release of tioconazole, Number of experiments=3

Fig. 4: Effect of tioconazole: HPMC ratio on the cumulative release of tioconazole, number of experiments=3

Fig. 5: Effect of Chp940 on the cumulative release of tioconazole from ethyl cellulose microspheres, number of experiments=3
Fig. 6: Effect of Cbp940 on the cumulative release of tioconazole from HPMC microspheres, number of experiments=3

Fig. 7: Effect of stirring rate on the cumulative release of tioconazole from ethyl cellulose microspheres, number of experiments=3

Fig. 8: Effect of stirring rate on the cumulative release of tioconazole from HPMC microspheres, number of experiments=3
In vitro antifungal activity

The results in Table 6, Fig. 11 and 12 exhibit the inhibition zones of the tested formulae F3 and F6 respectively. Tioconazole free microspheres showed zero inhibition zones, which indicated non-antifungal activity.

Tioconazole solution showed an inhibition zone of 43±2.6 mm. For F3 and F6, the inhibition zones were 47.6±0.6 and 52.3±1.5 respectively.

From the previous results, it can be concluded that both ethyl cellulose and HPMC loaded tioconazole mucoadhesion microspheres produced a significant antifungal activity against Candida Albicans.
CONCLUSION

Both ethyl cellulose and HPMC mucoadhesive microspheres had achieved the goal of this study through an increase in the retention time of the drug inside the vagina, which reflected by the high mucoadhesion strength, the delayed release and the significant anti-fungal activity against Candida Albicans. It can be concluded that both ethyl cellulose and HPMC mucoadhesive microspheres are considered promising tools to enhance both the vaginal availability and bioavailability.

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AUTHOR CONTRIBUTION

The corresponding author Sheeren Ahmed Sabry had developed the experimental section of the work, writing up and correction of the manuscript. Micronalynical center, faculty of science, Cairo University is responsible for the conduction of the anti-fungal studies.

CONFLICT OF INTERESTS

The author reports no conflict of interest.

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Table 6: In vitro antifungal activity of tinidazole formulations

| Formula                                      | Zone of inhibition (mm)±S.D.* |
|----------------------------------------------|-------------------------------|
| Tinidazole solution                          | 43±2.6                        |
| Tinidazole free ethyl cellulose microspheres | 0                             |
| Tinidazole free HPMC microspheres           | 0                             |
| F3                                           | 47.6±0.5                      |
| F6                                           | 52.3±1.5                      |

*Mean of three determinations±standard deviations of the mean.