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Enhancing Antifungal Activity of Itraconazole by Mesoporous Silica Nanoparticles

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Mesoporous silica nanoparticles (MCM-41) have an ability to increase the solubility of poorly soluble drugs. In this study, MCM-41 was synthesized. Afterward, Itraconazole (ITZ) was loaded into MCM-41 and then was wrapped by chitosan. The synthesized nanoparticles were characterized. Controlled release of ITZ from the MSN-ITZ and MSN-ITZ-CHI was evaluated in hydrochloride acid buffer (pH: 1.2) and phosphate buffer saline (pH: 7.4). Antifungal and cytotoxic activities of MSN-ITZ and MSN-ITZ-CHI were evaluated. Amount of loaded ITZ into MCM-41 was determined 85%. The properties of MCM-41 and loading procedure revealed high performance of the drug loading and release studies. The release profiles of MSN-ITZ were 60% in HCl medium and 40% in PBS medium. Data demonstrated more efficient and rapid release of ITZ from MSN-ITZ. Minimum inhibitory concentrations of MSN-ITZ and MSN-ITZ-CHI, on *Candida albicans* and *Aspergillus fumigatus* showed 0.25 and 0.5 μg/ml with low concentration and more inhibitory effects in comparison to the ITZ. MSN-ITZ and MSN-ITZ-CHI revealed lower toxicity in comparison to the pure drug on the cell viability of TC1 cell line ($P<0.05$). It could be concluded that the MSN-ITZ and MSN-ITZ-CHI nanoparticles have promising advantages for enhancing antifungal effects and drug delivery studies of itraconazole.

Key words*: Aspergillus fumigatus, Candida albicans*, Itraconazole, MCM-41, Mesoporous Silica Nanoparticles.

In the past decades, the incidence of invasive fungal infections that is caused by opportunistic fungal pathogens has increased considerably, especially in patients with severe immunodeficiency (Kuleta *et al*., 2009). With the advancements of medical sciences and increase the chance of survival in patients undergoing solid-organ transplantation, extensive surgeries and those patients with AIDS, immunosuppressive and cancer therapy, invasive fungal infections as the serious life-threatening infections are increasing (Pfaller and Diekema *et al*., 2004).

Most of commonly identified fungal infections are caused by pathogens species *Candida albicans* and *Aspergillus fumigatus*. These fungi are omnipresent that can be obtained from hosts or surroundings (Chakrabarti *et al*., 2005).

It must be noted that one of the reasons of increasing morbidity and mortality among infected patients in spite of intensive antifungal treatment, is fungal drug resistance and lack of effective antifungal drugs (Kuleta *et al*., 2009).

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Itraconazole (ITZ) is a kind of triazole member, which has a broader spectrum of activity in comparison to fluconazole against a number of fungal species. But it is a highly lipophilic drug with water solubility around 1 ng/ml at neutral pH and 4 µg/ml at pH 1 (Moazeni *et al*., 2012). Numerous reports on the drug resistance and reduced drug sensitivity of conventional Itraconazole have made the researchers in order to review and modify the drug formulation (Patil *et al*., 2011).

One of the methods for achieving complete dissolution of ITZ is large quantities of drugs that cause some disadvantage owing to the toxicity increased by high concentrations of ITZ. Scientists have recommended several strategies to achieve technologies which required lower amounts of materials for increasing the solubility of water insoluble drugs such as ITZ (Liu *et al*., 2015; Moazeni *et al*., 2012).

One of solution that progressively investigated in the earlier decade is use of nanoparticles as a drug delivery systems. Since the first report of MSNs (MCM-41 type) as a drug-delivery system in 2001 as yet, mesoporous silica nanoparticles (MSNs) have significant potential for biomedical applications and systemic delivery systems (Patil *et al*., 2011). In the past years have observed an increase in investigation on biomedical application of MSNs. It has become one of the most important field of study in nanomedicine for designing biocompatible and double functional MSNs-drug in microbial disease therapy over conventional drug nanocarriers (Slowing *et al*., 2008).

Mesoporous silica nanoparticles are solid materials with common types, MCM-41 and SBA-15 which have numerous of empty channels (mesopores), are able to absorb large amounts of molecules (Slowing *et al*., 2008; Hudson *et al*., 2008). The unique properties of mesoporous silica nanoparticles, such as large surface area of the pores, high pore volume and good thermal and chemical stability encouraged us to design MCM-41 and coat it with chitosan as a pH-sensetive, biocompatibile and nontoxic molecule with super-carrier properties for loading ITZ and controlled release applications against saprophytic fungi such as *C. albicans* and *A. fumigatus* in comparison to the pure crystalline ITZ (Hamman *et al*., 2010; Wang *et al*., 2011).

MATERIAL AND METHODS

CTAB (Cetyl trimethylammonium bromide), TEOS (Tetraethyl orthosilicate) were purchased from Merck. RPMI-1640 medium was prepared from Gibco. Chitosan and MTT were supplied from Sigma-Aldrich Co.Pure ITZ was gifted from Tehran Daru Pharmacutical Co. TC1 Cell line was provided by the Pasteur Institute, Tehran, Iran. *Candia albicans* and *Aspergillus fumigatus* were prepared from Tehran University.

Synthesis of Mesoporous Silica Nanoparticles (MSN)

1 g of CTAB was added into a solution that was containing 4 ml NaOH 2M and 480 ml of deionized water. After stirring with mild rotation at 80 °C for 2h, rotational speed increased in 1000 rpm for 5 min. Then 5 ml of Tetraethyl ortho silicate (TEOS) was added drop wise into the mixture. After 2h stirring at 80 °C the reaction resulting white sediment was washed with deionized water and dried overnight in desikator under vacuum condition at 25 °C in order to form MSN. Finally, CTAB was completely removed from MSN pores by calcination at 600 °C for 6h (Khoobi *et al*.2014).

Loading of Itraconazole into MSN (MSN-ITZ)

Loading of itraconazole (ITZ) was done by submersion method, in which 100 mg of the particles were submerged into 235.5 mg/ml itraconazole solution in 5ml dichloromethane. Then the mixture was dispersed for 30 min and suspension was stirred for 24 h at room temperature and protected from the light. After that it was centrifuged and washed by deionized water and at last the pure sediment was dried in desikator under vacuum condition at 25 °C overnight (Kinnari *et al*., 2011). The amount of loaded ITZ into MSN after storage in the nanopatrticles was measured three times by UV-Spectrophotometric method at λ=261 nm (1201, Shimadzu Co. Japan).

ITZ release experiments

The release of ITZ from MSN-ITZ and MSN-ITZ-CH was compared with the pure crystalline ITZ. The dissolution test methods were performed at 250 rpm with 500 ml hydrochloride acid buffer medium (pH= 1.2) and phosphate buffer

saline medium (pH=7.4) at 37 °C. The samples (100mg) were set in the gelatin capsules and sunk each capsule by standard basket. For each time 1 ml of every medium was collected and replaced with 1 ml of the same warm and fresh medium.

The experiences were done 120 min for three times and finally all collected samples were evaluated by UV-Spectrophotometric method at λ=261 nm (Kinnari *et al*., 2011).

Chitosan coating

0.6 g chitosan was ground an added into 10%v/v acetic acid aqueous solution, pH was adjusted at 6 with 1M NaOH, then 0.1 g MSN-ITZ was added into 20 ml solution that was resulted from the previous step, the mixture was stirred for 36h, afterward it was centrifuged and washed twice with deionized water and was dried in desikator under vacuum condition at 25 °C overnight (Chen et al., 2012).

Characterizing of nanoparticles:

Scanning Electron Microscopy (SEM) method

The micrographs of MSN-ITZ and MSN-ITZ-CHI nanoparticles were taken to notify information about shape, surface morphology, distribution and approximating size of nanoparticles by a BELSORP 28 Japan apparatus (Belsorp, Japan).

Transmission electron microscopy (TEM)

The exact size, shape of MSN-ITZ and coating form of MSN-ITZ-CHI nanoparticles were investigated using high resolution transmission electron microscopy (HRTEM, Philips, USA)

X-Ray Diffraction (XRD) method

The synthesized nanoparticles were characterized by XRD technique in order to identify the molecular structure and the state of MSN-ITZ and MSN-ITZ-CHI nanoparticle such as crystalline or amorphous. The XRD analysis was carried out by the device of XPERT MPD by advanced diffractometer (Philips, USA), using Cu Kα radiation with wavelength: 1.5406 Å in 2θ range of 1-10° at room temperature.

Fourier transform infrared spectrometer (FTIR)

Chemical structure of MSN, calcinated MSN, MSN-ITZ and MSN-ITZ-CHI were evaluated by FTIR method. The FTIR studies were performed by a Nicolet FT-IR Magna 550 spectrographs with KBr disks (Nicolet, USA).

Preparation of standard fungal cell suspension

In order to preparing the cell suspension after growing standard strains of *Candia albicans* (ATCC 10231) on sabouraud dextrose agar (SDA) medium at 35°C for 48h and *Aspergillus fumigatus* (ATCC 204305) on czapek dox agar medium at 35°C for 7 days, The fungal cells were harvested by scraping and washing agar surfaces in the sterile phosphate buffered saline (PBS). The inoculum suspention was adjusted at 2-5 × 10^5 cells/ml by hemocytometer counts and phase contrast microscopy (Olympus, Japan)

Antifungal susceptibility test

Microdilution test

The standard isolates of *Candida albicans* and *Aspergillus fumigatus* were applied for evaluation of susceptibility to MSN-ITZ, MSN-ITZ-CH and pure ITZ using the broth microdilution method according to the Clinical and Laboratory Standards Institute (CLSI), M38-A. For doing the test, RPMI 1640 medium was used containing buffered at pH 7.0 with 0.165 M MOPS as the culture medium. Samples were dissolved in dimethylsulphoxide (DMSO) to 1.6 mg/ml, and then serial dilution from 0.03 to 16 µg/ml were prepared with the RPMI 1640 medium, as recommended by the CLSI method. The effects of synthesized nanoparticles on the growth of isolates were assessed by 96-well microtiter plates.

At first, 100 µl of cell suspension added into a 96-well plates and diluted in culture medium with different concentrations of MSN-ITZ, MSN-ITZ-CH and ITZ. 10 µl of each sample was collected from all wells of standard microdilution plates and then dotted on to the sabouraud dextrose agar and czapek agar mediums.

After that the plates were incubated at 35°C for 48 h. At last the minimum inhibitory concentration (MIC) was determined as the lowest concentration of ITZ at which 50% (MIC 50) and 90% (MIC 90) of isolates were inhibited (Pfaller et al.2011; Ingroff et al.2005). All MIC determinations were repeated for three times and were compared with pure ITZ.

Disk diffusion test

A disk diffusion method for *Candida albicans* and *Aspergillus fumigatus* isolates against MSN-ITZ, MSN-ITZ-CH and ITZ, was carried out according to the CLSI guidelines. Suspensions of isolated colonies were adjusted to 10^5 cells/ml, after preparation of this turbidity, the

suspensions were placed on sabouraud dextrose agar and czapek agar medium using sterile swap, then MSN-ITZ, MSN-ITZ-CH and ITZ disks with 1.6 mg/ml concentration were placed slightly on the agar and finally, plates were incubated at 37°C and investigated after 48 h (Testore *et al*.2004; May *et al*.1997).

The quality and quantity of micro-colonies growth assessed within the zone. Zone sizes of micro-colonies were measured from the center of each disc to the edge of zone in millimeters scale with vernier calipers.

Cytotoxicity assay

Cell line and culture conditions

The MTT assay was applied in order to evaluate the in vitro cytotoxicity of nanoparticles base on conversion of blue tetrazolium bromide to the formazan in the mitochondria of viable cells. (Sgouras and Duncan, 1990). Murine tumor cell line (TC1) was obtained from the National Cell Bank of Iran (Pasteur Institute). Cells were cultured in DMEM medium complemented with 10% Fetal Calf Serum (FCS), 50 µg/ml of streptomycin, 50 units/ml of penicillin and 2mM L-glutamine then were kept at 37 °C in a 5% CO2 incubator.

The cytotoxicity of MSN-ITZ, MSN-ITZ-CHI and ITZ against TC1 cells were evaluated using MTT assay (the tetrazolium bromide dye 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyl based colorimetric assay). Approximately 1×10^4 cells/ml in complete medium were seeded in a flat-bottomed 96-well polystyrene plate. Stock solutions were prepared by dissolving samples in DMEM medium. Concentrations ranging from 0.05 to 500 µg/ml were exposed to TC1 cells and incubated 37°C in a 5% CO2. After 24h incubation, media containing nanoparticles were removed and then replaced with complete fresh media. 20 µl of 5 mg/ml in PBS of MTT was added to each well of the plates. After that plates were incubated for 5 h. Then the DMEM was removed and 100 µl DMSO was added and incubated for 30 min at 37°C.

Finally, the optical density (OD) was measured using ELISA reader at 570 nm. Viability cells of treated wells were compared with untreated wells (De-Simone *et al*., 2013; Vijayakumar *et al*., 2012).

Statistical analysis

All experiments were performed three times in triplicate. Data were collected and classified and then were analyzed by IBM SPSS Statistics version 22.0 software. For this purpose, the univariate ANOVA (Univariate Analysis of Variance) was used. After that, comparing pairs of groups using LSD (Least Significant Difference) was performed. Comparison among samples and control groups were performed using an independent sample t-test. All statistical tests were performed at a significance level of 0.05. A significant different of $P< 0.05$ was accepted.

RESULTS AND DISCUSSION

MSN, MSN-ITZ and MSN-ITZ-CHI synthesis and characterization

At first, MCM-41 nanoparticles were synthesized by using CTAB as a precursor. After that ITZ was loaded into MCM-41 porous by submersion method then a thin layer of chitosan was coated around of particles. CHI was coated around the MSN-ITZ via the hydrogen bonding between the amine of CHI and silanol MSN-ITZ (Chen *et al*., 2012).

The morphology of the prepared nanoparticles was evaluated by SEM, TEM investigations. Common morphologies of the MSN (Fig.1.A) and MSN-ITZ-CHI (Fig.1.B) were showed in Figure 1. The SEM micrographs revealed MSN and MSN-ITZ-CHI with relatively regular particle size and spherical shape; moreover, distribution around 98 nm. It can be seen in Figure 2 that nanoparticles were almost uniform nanosphere with rough surface and ordered array of channels with average diameter approximately 70-100 nm (Fig.2.A). Also, it can be observed a thin layer of chitosan was coated MSN (Fig.2.B).

The type of crystalline structure of the nanoparticles was considered by mean of the XRD. Figure 3 is shown XRD patterns of MSN (Fig.3.A) and MSN-ITZ-CHI (Fig.3.B). The Both XRD spectra showed that the synthesized MSN was the desired material and differences XRD patterns of MSN and MSN-ITZ-CHI showed crystalline form of MSN was converted to the anamorphic shape in MSN-ITZ-CHI.

According to the results, the synthesized nanoparticles were verified by FTIR. The spectrum of MSN (Fig.4.A) showed two distinctive peaks at 2800- 2950 cm^{-1}. These peaks were observed in MSN but they were absent in calcinated MSN that

indicate, CTAB was totally removed by calcination method at 550°C after 4 h (Fig.4.B).

Characteristic peaks of silica were observed in both synthesized MSN and calcinated MSN. The peaks at 467, 796 and 1079 cm^{-1} are related to the bending vibration of Si-O-Si, symmetry and dissymmetry, respectively. The sharp peaks at 1631 and 3410 cm^{-1} are result in bending vibration of OH and also stretching that indicate the existence of a great number of H2O molecules and hydroxyl groups on the surface of the particles.

The results were in good confirmative with Khoobi and colleagues studies (Khoobi *et al.*, 2014).

Some of the peaks characteristics of ITZ were differenced at 1700 and 1510 cm^{-1} (Wang *et al*.2007), which also appear in the FTIR spectra of the loaded form of MSN-ITZ nanoparticles (Fig.4.C) and also coating form of MSN-ITZ-CHI (Fig.4.D) nanoparticles. A similar finding was obtained by Kinnari (Kinnari *et al*. 2011).

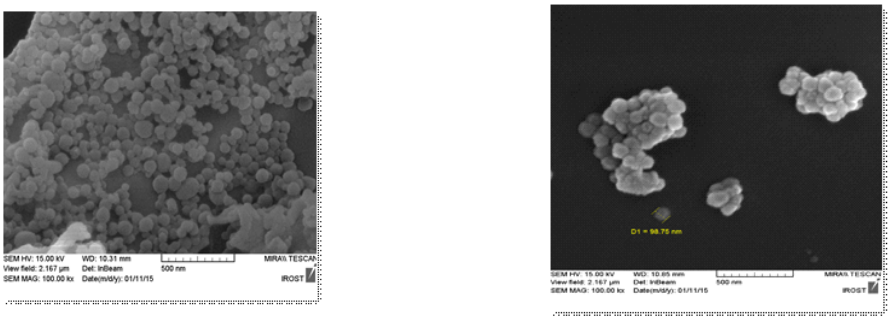

Fig. 1. Scanning electron microscopy (SEM) images of the MSN (A) and MSN-ITZ-CHI (B)

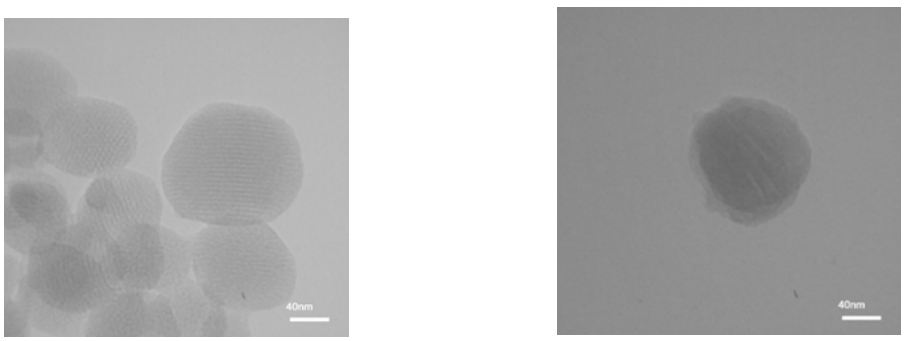

Fig. 2. Transmission electron microscopy (TEM) images of the MSN (A) and MSN-ITZ-CHI (B)

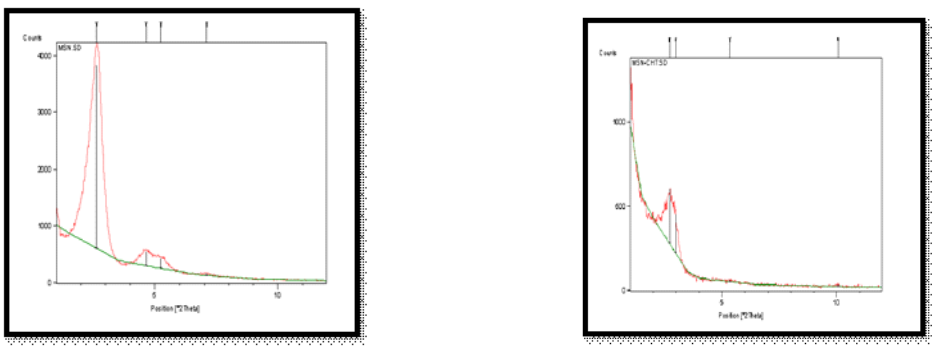

Fig. 3. X-ray diffraction (XRD) of MSN (A) and MSN-ITZ-CHI (B)

Loading and release studies

The total quantity of loaded ITZ into MCM-41 was determined by UV-Spectrophotometric with average amount of 85%. MSNs due to the huge amounts of silanol groups that interact with ITZ through hydrogen bonds plays an important role to obtain higher drug loading (Horcajada *et al*.2006; Song *et al*. 2005).

The release potential of ITZ from MSN and MSN-CHI was evaluated in different neutral and acidic mediums. Fig .5 and 6 display the in vitro pH sensetive release of ITZ from MSN-ITZ

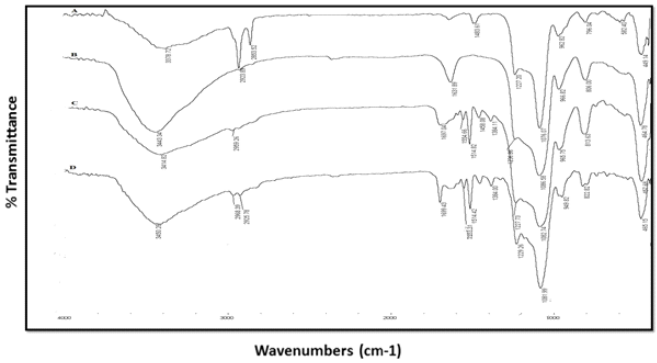

Fig. 4. Fourier transform infrared spectroscopy (FTIR) of MSN (A), calcinated MSN (B), MSN-ITZ (C) and MSN-ITZ-CHI (D)

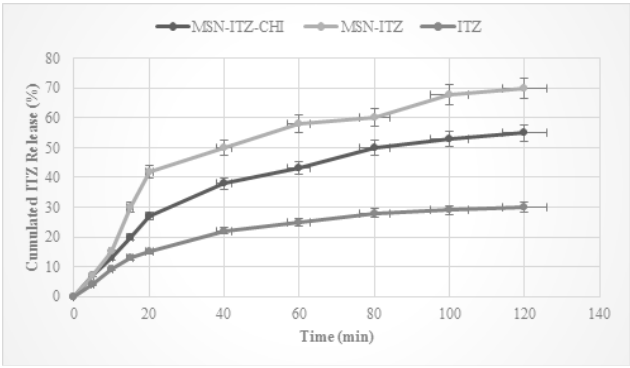

Fig. 5. Comparison of the release profiles of ITZ from MSN and MSN-CHI in phosphate buffer saline (PBS) at pH: 7.4 and 37°C with pure ITZ

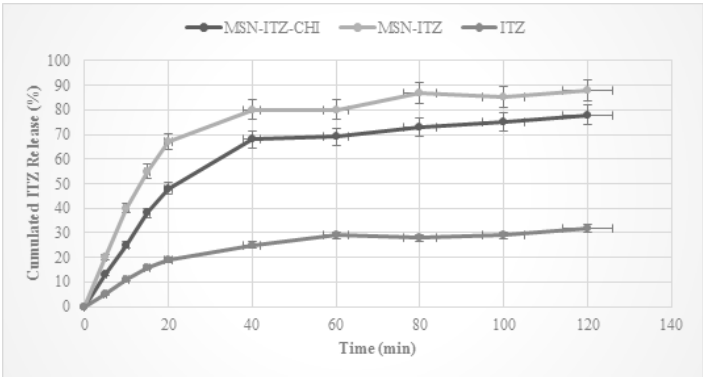

Fig. 6. Comparison of the release profiles of ITZ from MSN and MSN-CHI in Hydrogen Chloride (HCl) medium at pH: 1.2 and 37°C with pure ITZ

and MSN-ITZ-CHI in two types of pH 1.2 (HCl) and pH 7.4 (PBS) solutions respectively. Results showed that the amount of released ITZ from MSN-ITZ in pH 1.2 solution was about 68 % for 20 min and 89 % for 120min whereas, the released ITZ from MSN-ITZ in pH 7.4 solution was around 42% for 20min and 72 % for 120min. The amount of released ITZ in pH 1.2 was slightly higher than in pH 7.4. The amounts of released ITZ from MSN-ITZ-CHI in pH 7.4 release medium was about 28% for 20min and 53% for 120min, while released ITZ from MSN-ITZ-CHI in pH 1.2 release medium was about 49 % for 20min and 78% for 120min. Comparing to MSN-ITZ, ITZ released from MSN-ITZ-CHI in pH 1.2 after 120min was about 20% more than in pH 7.4. This system provides the intelligent pH sensitive controlled release system for treatment of infected tissues especially in the

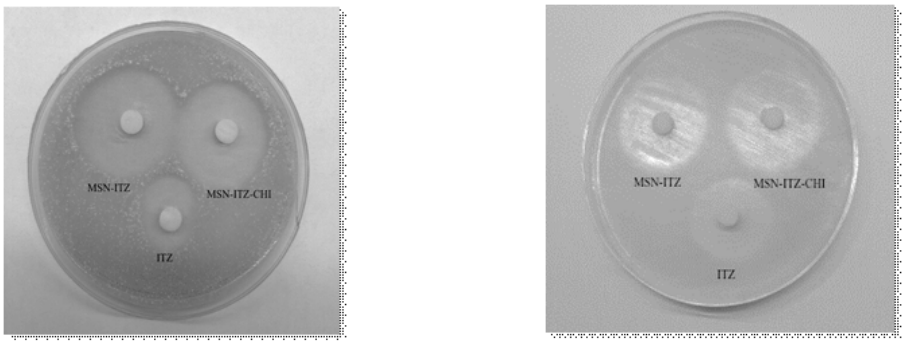

Fig. 7. Agar disk diffusion assay showing inhibition zone and antifungal activities of MSN-ITZ, MSN-ITZ-CHI in comparison with standard ITZ against *Candida albicans* (A) and *Aspergillus fumigatus* (B)

Table 1. MIC50 and MIC90 results against ATCC reference strains *Candida albicans* and strains *Aspergillus fumigatus* after 48h

		MIC 50 (µg/ml)	MIC 90 (µg/ml)
Candida albicans	MSN +ITZ	0.125	0.25
	MSN + ITZ +Chitosan	0.125	0.25
	ITZ	0.25	0.5
Aspergillus fumigatus	MSN +ITZ	0.25	0.5
	MSN + ITZ +Chitosan	0.25	0.5
	ITZ	0.5	1

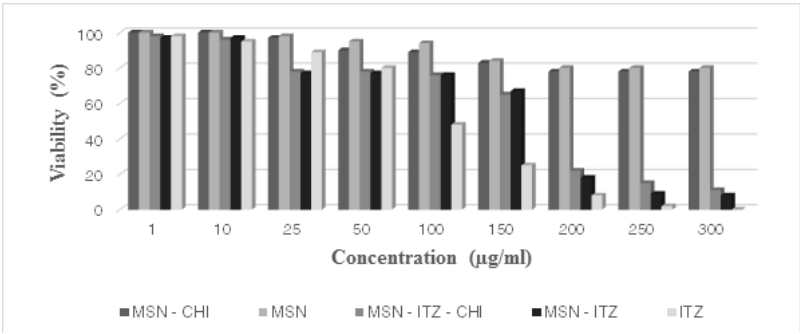

Fig. 8. MTT assay results of ITZ pure in comparison with MSN, MSN-ITZ, MSN-CHI and MSN-ITZ-CHI. At lower concentrations samples showed similar almost effect on cell growth. But the differences were apparent at higher concentrations. Data are means ± standard deviations of three independent experiments performed in triplicate ($p < 0.05$)

case of pulmonary aspergillosis disease that the pH is somewhat lower than normal tissue (Burtuzzi *et al*., 2014).

According to the mentioned results, encapsulation of ITZ within hexagonal cylindrical porous of MCM-41 increased dissolution rate.

When CHI is immerged into PBS solution with pH 7.4, the hydrogen bonding is strong and the amine groups do not possess any charge, so electrostatic repulsion does not exist between CHI molecules, while CHI is immerged into pH 1.2 medium, the hydrogen bonding is weaker so the pores release a large amount of ITZ into the medium than in neutral environment (Chen *et al*., 2012; Patil *et al*., 2011; Xiao *et al*.2009).

Nevertheless, in overall, comparison of results revealed that the release of ITZ from MSN-ITZ was better and faster than MSN-ITZ-CHI in both pH 1.2 and 7.4 mediums. This fact may be due to the preventing effects of the coating layer of chitosan on complete release of the molecules (Chen *et al*., 2012; Xiao *et al*.2009).

In current study, we report antifungal properties of loaded ITZ into MCM-41 for the first time due to the several experiments. At first, antifungal activities of MSN-ITZ and MSN-ITZ-CHI were evaluated by disk diffusion method. The mean inhibition zones of MSN-ITZ, MSN-ITZ-CHI and ITZ were 26, 26 and 14 mm in *C. albicans* samples (Fig.7.A) 24, 24 and 16 mm in *A. fumigatus* samples (Fig.7.B) respectively. As were shown in Figure 7 disks with concentration 1.6 mg/ml MSN-ITZ and MSN-ITZ-CHI had significant more inhibitory effects than pure ITZ as the control group against both of fungi. Results of disk diffusion indicated antifungal activities of MSN-ITZ and MSN-ITZ-CHI at 1.6 mg/ml were in the same amount with the mean diameter zones of inhibition 26 and 24 mm against *C.albicans* and *A. fumigatus* respectively. Each experiment was performed three separate times.

In order to examine antifungal efficacy of the MSN-ITZ and MSN-ITZ-CHI the microbroth dilution was performed (Table). MIC concentrations ranges 0.03–16 µg/ml compared to ITZ against both groups (Pfaller *et al*.2011; Ingroff *et al*.2005; Li *et al*.2000). MSN-ITZ and MSN-ITZ-CHI showed good activity against both fungal strains, *C.albicans* and *A. fumigatus*.

The MIC 90 of MSN-ITZ, MSN-ITZ-CHI on *C.albicans* were 0.25 µg/ml and 0.5 µg/ml for pure ITZ. Also antifungal activities of MSN-ITZ-CHI and MSN-ITZ on *A. fumigatus* MIC 90 were 0.5 µg/ml and about ITZ was 1 µg/ml (Table).

MIC of the synthesized samples revealed nearly two times more activity than the pure ITZ. These MIC reductions in MSN-ITZ and MSN-ITZ-CHI could be due to the increase ITZ release in the media and improving the drug effects on fungal cells. Cunha-Azevedo and colleagues group acquired similar results about MIC of loaded ITZ into PLGA (poly lactic-co-glycolic acid) nanoparticles (Cunha-Azevedo *et al*.2011).

However antifungal activity of chitosan has been proved in several articles (Pu *et al*., 2014; Tayel *et al*., 2010; Kong *et al*.,2010) but in this study chitosan had not antifungal effects on both strains and results were same as MSN-ITZ group.

Cell cytotoxicity

Silica nanoparticles have attracted the consideration of scientists in the last decade owing to the fact that they can use for different biopharmaceutical applications but a number studies have been reported to induce cytotoxicity in some cell lines (Patil *et al*.2011). In our study, the cytotoxicity of the nanoparticles was measured using MTT assay on TC1 cells.

In this study, results demonstrated that cell cytotoxicity relies on the dose of nanoparticles. The amounts of optical density for the MSN, MSN-ITZ, MSN-ITZ-CHI and ITZ as a control group are shown in histogram of Figure 4. In our study, at the low concentration (1, 10 ìg/mL) of samples cell viability were same as each other and not distinguishable. However at higher concentrations (100 -300 ìg/ml) some significant difference in results were found. Cell death was distinguishable with each other at 200-300 µg/ml concentrations, MSN-ITZ, MSN-ITZ-CHI and ITZ at the highest concentration (300 ìg/ml) were more cytotoxic; however, MSN-ITZ and MSN-ITZ-CHI revealed lower cytotoxicity than pure ITZ. Results were in agreement to previous studies Mirza and colleagues study, (Mirza *et al*.2012).

In this study all experiments were done in triplicate and three independent experiments ($p < 0.05$). According to the former studies, itraconazole interacts with cytochrome P-450 and can lead to changes in cells and also inhibition of the enzymatic system of cells. Therefore, a higher

toxicity in TC1 cells would be estimated. Nevertheless, the results revealed that the combination of drugs in MSN-ITZ and MSN-ITZ-CHI nanoparticles enhanced a definite protection against cytotoxicity (Mirza *et al*.2012; Cunha-Azevedo *et al*.2011; Catalan *et al*.2006).

Although further studies are needed in order to prove safeness of MCM-41 nanopartilces; however, recent study indicated that synthesized nanoparticles MSN-ITZ and MSN-ITZ-CHI have low in vitro cytotoxicity at low concentration and have potential for systemic drug delivery systems of ITZ.

CONCLUSION

In this study, MCM-41 was synthesized with the tiny size. pH-sensitive release system of MSN-ITZ-CHI had a significant difference in comparison with MSN-ITZ. New formulations of ITZ, MSN-ITZ and MSN-ITZ-CHI, showed more effective release of ITZ and inhibitory activities on the growth of *C. albicans* and *A. fumigatus*, as two important pathogens, than the Pure ITZ. According to the MTT assay results, toxicity of MSN-ITZ and MSN-ITZ-CHI on TC1 cells was dose dependent and cell viability of synthesized nanoparticles samples were more than the commercially available ITZ. However, further studies need especially in vivo phase, MSN could be a very recommendable nanoparticle for enhancing therapeutic effects and localized drug delivery designing studies of Itraconazole.

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