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Investigation the effect of [Met-Hcl] [Pys] ionic liquid on *Candida albicans* standard strain and evaluation of its toxicity on mammalian cells

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ABSTRACT

Recently, fungal drug resistance has significantly increased especially in opportunistic fungus like *Candida albicans.* Accordingly, it is necessary to use more effective drugs with less toxicity and high influences. Among a large of investigations, ionic liquids showed biological influence and antimicrobial activities. The aim of this study was in vitro investigation of antifungal activity of pyridine –based ionic liquid on a standard strain of *Candida albicans* and evaluate its toxicity on host cells. A standard strain of *Candida albicans* was re- cultured on Sabouraud Dextrose Agar (SDA) containing chloramphenicol, incubated at 37°C for 24 h. Antifungal effect of a novel ionic liquid ([Met-Hcl] [Pys]), was evaluated using inhibitory zone diameter. The minimum inhibitory concentration (MIC) and minimum fungal concentration (MFC) were performed using micro dilution method. In continue MTT test were done to evaluate the toxicity of the liquid on host cells. The ionic liquid showed a good effect to prevent of *Candida albicans* growth. Inhibitory zone diameter was between 34 ± 1 mm. The MIC evaluation was 708.4 ppm. Also the results of MTT test showed the viability of host cells at the 16 dilution. In conclusion, the results of this study manifested that the novel ionic liquid has a good antifungal activity against *Candida albicans* standard strain with low toxicity to human cells and probably can use as a good novel drug in treatment of candidiasis. Although the need for more studies is crystal clear.

1. Introduction

Fungi are a group of eukaryotic species on Earth that only a few of them are human pathogens. Among the opportunistic fungus, *Candida* spp are one of the most common cause of systemic infections in the world (Yenisehili et al., 2015). *Candida albicans (C. albicans)* is the polymorphic fungus and a member of the human gut microflora. It can cause two major types of infections in hosts including superficial candidiasis (oral and vaginal infections) and life-threatening infections. *C. albicans* has the ability to infect especially immunocompromised people, using a wide range of virulence factors including morphological transition between yeast and hyphal forms, formation of biofilms, expression of genes involve in secretion of hydrolytic enzymes and phenotypic switching (Brown. *C. albicans* cells grow at low PH (< 6) in the yeast form while at the PH over 7 (> 7) , it can be seen in the form of hyphae. This transition is turned dimorphism and both of the two forms are important for pathogenicity (Li et

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al., 2002). More over *C. albicans* has a set of proteins which mediate it cells to adhere other cells / microorganisms, abiotic surface and host cells. *C. albicans* can utilize two mechanisms to invade in to host cells: active penetration and inducing endocytosis with expression of specialized proteins on the cell surface (Henriques and Silva, 2021). Azoles consist of imidazole and triazoles are common drugs with which activity against most yeast and filamentous fungi. These agents inhibit sterol biosynthesis (Yoshida, 1998). Recently, azoles resistant strains of *C. albicans* has been increased (Joseph-Horne & W. Hollomon, 1997). Appearance of high multidrug resistant clinical isolates of the fungi has amplified the need for novel drugs. Ionic liquids (ILs) are a class of organic salts with melting points below 100 °C. The cation and anion pair may be composed of organic ions or a combination of organic and inorganic ions (Clare et al., 2010). In most cases, the cation is an organic compound with positively charged nitrogen or phosphorous. Theory and experiment have moved forward together to grow our understanding of ionic liquid structures (Russina et al., 2015).

Some studies manifested the importance of ionic liquids (IL) in treatment of fungal infections. Bergamo manifested in their work the effect of hexadecyl side chain containing imidazolium ionic liquid against *candida* species (Bergamo et al., 2016). The ILs based on imidazole showed strong antifungal activity. Imidazole-based ionic liquids have the ability in reducing the content of ergosterol (Schrekker et al., 2013). It has been reported that *C. albicans* ability to form biofilms is a major factor that contributes to its virulence. 1-alkylquinolinium ionic liquids possess broad spectrum against biofilm formation in both Gram positive and Gram negative bacteria as well as fungi (Busetti, 2010).

In spite of the researches, the study of these liquids is still a young field. By changing the component of ionic liquids (cations and anions), their chemical and physical properties can be altered to suit a specific function (Ortega vega, 2017), so this study aimed to investigate the effect of a new ionic liquid with methionine amino acid ([Met-Hcl] [Pys]), on *C. albicans* growth as well as its effect on mamalian cells.

2. Materials and Methods

Candida strain: The standard strain of *C. albicans* were received kindly from the Dr. Amini laboratory and were used in all experiments in this study. The strain was re-cultured on Sabouraud Dextrose Agar (SDA) containing chloramphenicol, incubated at 37℃ for 24 h to obtain a fresh strain.

2.1. Determination of MICs and MFC

To determine the minimum inhibitory concentration (MICs) of the ionic liquid, micro dilution method was used according to the Clinical and Laboratory Standard Institute (CLSI) guideline.

The strain were adjusted to 2×10^2 CFU/ ml in 100μl RPMI medium. The suspension was added to 100 μl of the ionic liquid and incubated at 37 ℃ for 48 h. The ionic liquid concentration was two- fold diluted serially (2, 4, 8, 16, 32, 64, 128, 256, 1024 $\mu g/ml$).A negative control was considered with culture medium and *C. albicans*. A positive control sample was prepared including 100 μl of the ionic liquid and medium culture. To estimate MFC, aliquots of suspension from wells with no growth of *C. albicans* was selected and became homogenous via a micro –pipette. The selected wells were re-cultivated by five potato dextrose agar (PDA) plates and incubated at 37℃ for 48 h. The lowest concentration at which no colonies of *C. albicans* was recorded as MFC.

2.2. Statistical analysis

The data analysis using One-way ANOVA and Tukey test, in which $p\leq 0.05$ were considered as statistically significant. All experiments results were reported as mean$\pm$ standard deviation (n=3).

2.3. MTT performance

MTT assay is a colorimetric test. In this method, NAD (p)H dependent cellular oxidoreductase enzymes reflected the number of viable cell present. MTT test are used to measure cytotoxicity or loss of viable cells. Firstly 10×10^4 cells suspended in 100 μl of the medium and seeded in 96-well plate and incubated for 24h at 37℃ in order to adhere the

cells at the bottom of the plate. The cells was washed twice in PBS, then 100 μl of 0.05 mg/ml MTT in serum free medium was added in to each cell, incubated for 3h at 37℃ to MTT metabolisation. Isopropanol was added to cells to solubilize the colored crystals. Colorimetric detection was done at a wavelength of 570 nm. The wells with the more viable cells showed higher OD compare to the wells with low viable cells.

3. Results

In this study we examined a novel IL([Met-Hcl] [Pys]), for its antifungal activity against *C. albicans* standard strain. The minimum inhibitory concentration and the diameter zone of inhibition were measured by micro dilution and disk diffusion methods respectively. As it was shown in Fig.1, the diameter zone of the IL with methionine amino acid showed the best inhibition of growth of *C.albicans* ($34 \pm 1\ mm$). This result (Fig.1) manifested that the effect of IL with methionine amino acid was superior on inhibiting the strain growth compare to pyridine,SO3H and the IL with proline amino acid ($\rho < 0.0001$).

The results of MIC values showed that the IL can inhibit the growth of the strain at 708.4 ppm concentration (Fig.2).

3.1. MTT

Treatment with the novel IL used in this study on the *C. albicans* standard strain, exhibited that the IL can inhibit the fungal growth. The minimum concentration of the IL showed lethality of 708.4 ppm. The same treatment which was given to human cells were assayed for viability. The results showed the amount of the IL required to reach 50% lethality in the *C. albicans* and at the same time little increase toxicity to human cells was at the $32 \mu m$ dilution where 90% of the human cells survived (Fig 3).

The viability of human cells after treatment with the IL was shown in Tale 1. As it exhibited here, at a dilution of 16 $\mu m, a$ pproximately 50% of the cells survived but the viability of cells at a dilution of 32 μm (708.4 ppm), was about 90% (87.178%).

Table 1. the effect of the IL on human cells at different dilutions

	MEAN	SD	N
CONTROL	100	3.0789	5
2µM	3.0289	0.4552	5
4µM	4.0227	0.6899	5
8µM	15.5845	0.9842	5
16µM	57.1936	1.7597	5
32µM	87.1784	2.7842	5

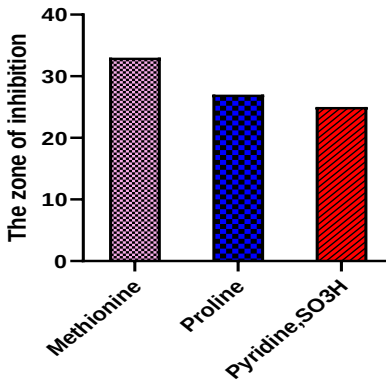

Fig.1.Mean of inhibition of growth of the ILA (the IL with methionine amino acid) on *C. albicans* standard strain

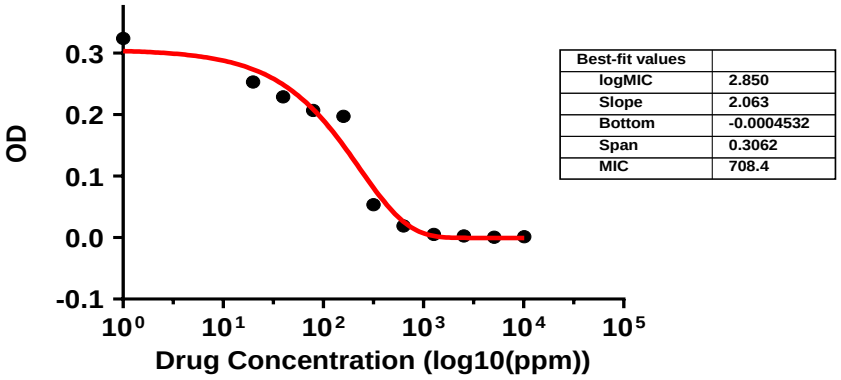

Fig 2. Mean of [Met-Hcl] [Pys] IL minimum inhibitory concentration (MIC) values for *C. albicans* standard strain.

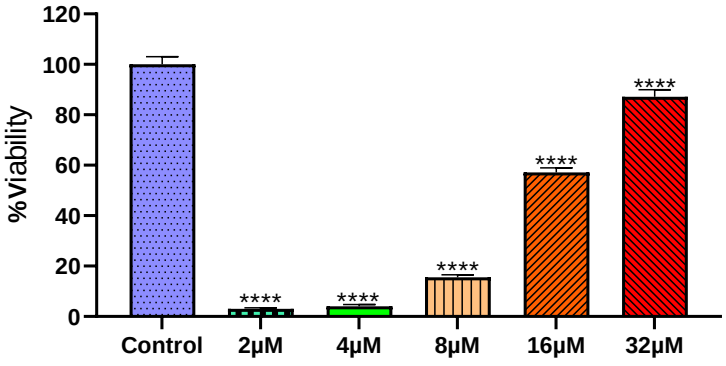

Fig 3. The results of the IL([Met-Hcl] [Pys]), on human cells viability

4. Discussion

C. albicans is one of the most fungal pathogens that cause more than 400000 invasive fungal infections annually. The fungus species are a leading cause of mucosal disease, including oral and vaginal candidiasis (Luna-Tapia et al., 2018). Azoles are the most widely agents that uses for therapies, inhibit the lanosterol depletion of cellular ergosterol. Recently, increasing azoles- resistant species of *C. albicans*, leads attention to design and manufacture new drugs.

IL exhibit chemical properties such as low melting point and negligible vapor pressure. Their ability to reduce the microbes, make them important as antimicrobial agents (Welton, 2018).

The results of MIC and MFC of our studied IL showed better effect of our IL on *C. albicans* (MIC $< 1000\ mg/ml$) than the one (dodecyl substituted ionic liquid) examined (Navale et al.,2015) with *C.albicans* strain NCIM 3628 (MIC $< 2000\ mg/ml$)

In a study, ILs with pyridine- based cations were successful in permeating the membranes of microorganisms (Cook et al., 2019). The results of this study also manifested that the novel IL ([Met-Hcl] [Pys]), with pyridine- based cation and amino acid methionine has the ability to permeate the cell membrane of *C. albicans* standard strain and inhibit its growth.

Previous studies manifested toxicity of ILs against many microorganisms. In a study, [C4 MIM] [Br], [C6 MIM] [Br] and [C8 MIM] [Br] ILs had antimicrobial effect on *Chlamydomonas reinhardtii* and showed that the toxicity of these ILs are more than acetone, benzene and phenol (Kulacki and Laberti, 2008). In present study, the novel IL([Met-Hcl] [Pys]), showed toxicity against *C. albicans* more than pyridine and IL with proline amino acid. The study of Yang, showed the antimicrobial agents imidazolium chloride- based ILs enhanced toxic activity against a subset of microorganisms and can reduce the concentration of antimicrobial compound necessary to inhibit microbial growth (Yang et al., 2021). Similarly, the results of our study manifested the effect of the novel IL([Met-Hcl] [Pys]), in reducing the concentration for inhibiting microbial growth.

MTT assay or mammalian cell cytotoxicity test is important to assess cellular damage that may arise after treatment with different chemical agents (Amde et al, 2015). In present study the effect of the novel IL([Met-Hcl] [Pys]), was investigated against mammalian cells. After treatment with the IL, the results showed little increase in toxicity to human cells that is not considerable.

Conclusion

The results of this study showed effective antifungal activity of the ([Met-Hcl] [Pys]) IL against *C. albicans* standard strain and manifested that the IL can be used as candidate in treating candidiasis with low cytotoxicity to mammalian cells.

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Conflict of interest

There is no conflict of interest

Author contribution

Acquisition of data: Mona Eslami,
Analysis and interpretation of data: Dr. Ali Ezabadi, Mona Eslami.
Drafting of the manuscript: Dr. Pejman Mortazavi, Dr. Mansour Bayat and Mona Eslami
Critical revision of the manuscript for important intellectual content: Dr. Ali Ezabadi, Dr. Behin Omidi and Dr. Mansour Bayat.
Statistical analysis: Mona Eslami
Administrative, technical, l, and material support: Dr. Ali Ezabadi, Mona Eslami

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