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ORIGINAL RESEARCH ARTICLE

Comparative analysis of potency of Azole derivatives to target ASL and GPI proteins responsible for pathogenesis of *Candida albicans* using *in silico* approach

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ABSTRACT

Candida albicans, a dimorphic fungus which is commensal organism of human gut flora, but due to overgrowth or immune-compromised conditions become pathogenic causing vulvovaginal candidiasis infection which is most common in females. The present study was conducted to determine an effective treatment strategy by making a comparative analysis of potency of azole derivatives such as itraconazole, fluconazole and ketoconazole to target ASL and GPI proteins responsible for pathogenesis of *C. albicans* using *in silico* approach. By comparing Gibbs free energy, it becomes clear that itraconazole possess more potency in comparison to fluconazole and ketoconazole to target ASL and GPI macromolecule. The present study clearly indicated that itraconazole can overcome complications of pathogenesis induced by *C. albicans* inside the host thereby acting as a major drug of interest in comparison to other azole derivatives to treat vulvovaginal candidiasis. In conclusion, itraconazole exhibits better potential than fluconazole and ketoconazole to target GPI and ALS. This clearly reveals the potency of itracaonazole to target *C. albicans*, but more research is needed to be carried out to determine the mechanistic approach involved in exhibiting this effect.

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INTRODUCTION

Pathogenesis is a biological mechanism that causes the diseased state. It describes the origin of adhesion, invasion, development and virulence of the disease (Arora *et al*., 2016). The vulvovaginal candidiasis occurs due to the fungal infection caused by *Candida albicans*. For the study of infectious disease, microbial adhesion plays an important role which implies the interaction of the host tissue with the microorganism which is pathogenic in nature. In order to cause infection, microorganism has to first adhere to cell surface of the host organism which require some kind of specific protein or receptors named integrin for their attachment to the cell surface. In order to cause infection, micro-organisms have to 1st adhered to the cell surfaces of the host cell wall which require some kind of specific proteins or receptors (Pérez-Martín *et al*., 1999; Raperia *et al*., 2017). In a process of adhesion to the host tissue special type of proteins are required, these proteins are glycoproteins required by microorganism or fungus in order to cause infection.

The glycosylphosphatidylinositol (GPI) linked cell surface glycoproteins are the proteins which are responsible for adhesion in *C. albicans* (Kaur *et al*., 2016). Among all these genes, ALS3 was found to play an important role in adhesion as it is found to be upregulated during infection in case of *C. albicans*. Potency of fluconazole, Itraconazole and ketoconazole needs to be evaluated to target GPI and ALS. Furthermore, target site those residues which are specifically getting targeted by them also needs to be checked to determine the amino acid residues which facilitates the attachment of *C. albicans* to the host and which can be efficiently targeted by azole derivatives (Rustchenko-Bulgac *et al*., 1991). Binding potency of various drugs to these macromolecules needs to be evaluated. A comparison between their binding affinities also needs to be carried out to reveal that amongst these three metabolites

which can be proposed as a strong drug candidate for the future against *C. albicans* (Ankitha *et al*., 2017). Keeping in view, the mechanistic approach of *Candida albicans* causing vulvovaginitis, the present study was conducted, in order to identify the potent receptor-ligand interaction through bio-informatics approach so that a better understanding to the pathogenesis and hence the solution of the disease could be gained.

MATERIALS AND METHODS

Identification of proteins responsible for adhesion, invasion and biofilm formation of *C. albicans*

In order to cause infection, micro-organisms have to 1st adhered to the cell surfaces of the host cell wall which require some kind of specific proteins or receptors. The genes responsible for adherence were identified from literature surveys. After the identification of target, their protein structure was fetched from RCSB protein database. Their complex structures, single chains were prepared by using Chimera software and were made ready to check their interaction with the drugs chosen for the study by using molecular docking approach.

Preparation of 3D structures of ligands (fluconazole, ketoconazole and Itraconazole)

3D structure of fluconazole, ketoconazole and Itraconazole were prepared by using Frog server from canonical smile obtained from Pubchem with Pubchem Id 3365, 456201 and 55283, respectively.

Determining the potency of fluconazole, ketoconazole and Itraconazole to target receptors by using molecular docking study

To assess the potency of fluconazole, ketoconazole and Itraconazole to target ALS and GPI proteins. Virtual docking study was carried out at their respective catalytic domains. 3D structure of ALS and GPI were obtained from RCSB protein database. Ligands were docked to the receptor chosen for the study by using Autodock tool of Autodock 4.2.6 package. Grid map of 40×40×40 grid point with 0.375 Å spacing were generated using Autogrid program. According to Lamarckian Genetic Algorithm (LGA) with maximum 250000 energy, binding efficiency of ligands to various receptors was determined (Trott and Olson, 2010; Mittal *et al*., 2018; Ferreira *et al*., 2015 and Morris *et al*., 2009).

RESULTS AND DISCUSSION

Identification of proteins responsible for adhesion, invasion and biofilm formation of *C. albicans*

Akhtar *et al.* (2012) in their clinical trial study mentioned that itraconazole is more effective for the treatment of vulvovaginal candidiasis in comparison to fluconazole but they couldn't explain the mechanistic approach exhibited by itraconazole in exhibiting this effect (Akhtar *et al*., 2012). In this phase of study we identified that glycosylphosphatidylinositol (GPI) linked cell surface glycoproteins are the proteins which are responsible for adhesion in *C. albicans*, they are encoded by 8 sets of Agglutinin like sequences genes (ALS) which are: ALS1, ALS2, ALS3, ALS4, ALS5, ALS6, ALS7 and ALS9. Among all these genes, ALS3 was found to play an important role in adhesion as it is found to be upregulated during infection in case of *C. albicans*. ALS3 is most commonly seen in oral and vaginal epithelial cell infection. In order to provide a permanent cure from the disease, adhesion of *C. albicans* to the host needs to be controlled. Potency of fluconazole, Itraconazole and ketoconazole needs to be evaluated to target GPI and ALS. To carry out *in silico* study different chains using Chimera software were prepared: ALS complex (Figure 1A), Chain A (Figure 1B), Chain B (Figure 1C), Chain C (Figure 1D) and Chain D (Figure 1E) and GPI complex (Figure 2A), Chain A (Figure 2B), Chain B (Figure 2C), Chain C (Figure 2D) and Chain D (Figure 2E).

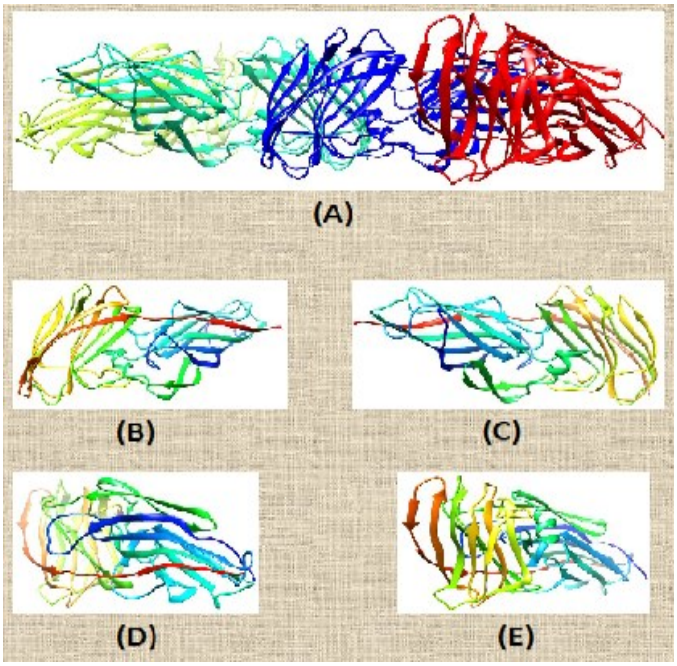

Figure 1. *3-D complex structure of ALS protein (A), chain A (B), chain B (C), chain C (D) and chain D (E).*

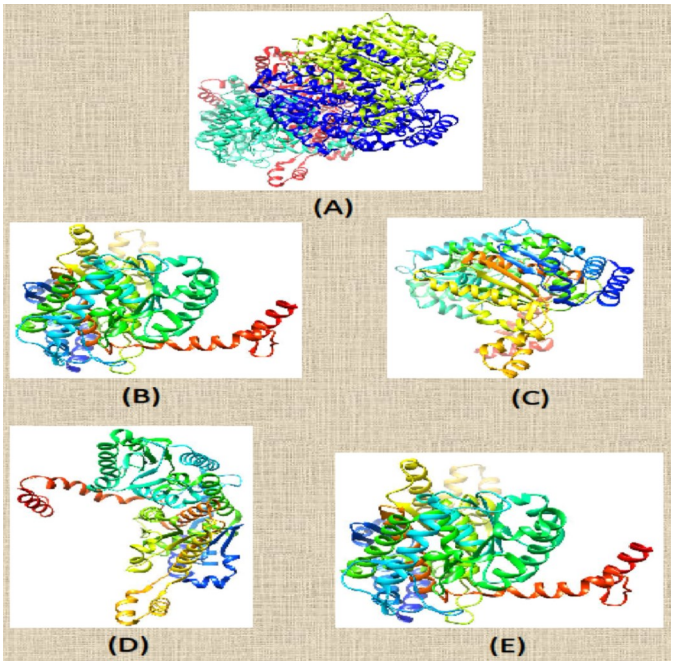

Figure 2. *3-D complex structure of GPI protein (A), chain A (B), chain B (C), chain C (D) and chain D (E).*

Preparation of 3D structures of ligands (fluconazole, ketoconazole and Itraconazole)

Canonical smiles were fetched from PubChem. 3D structures of ligands were prepared by using canonical smiles from FROG server. 3D structures were downloaded. Single isomeric structures were prepared from complex structures by using Chimera. Chemistry of ligands was studied, to check location of different atoms. Structures of fluconazole (Figure 3A), fluconazole isomer (Figure 3B), Itraconazole (Figure 3C), Itraconazole isomer (Figure 3D), ketoconazole (Figure 3E) and ketoconazole isomer (Figure 3F) were prepared by using Chimera software.

Determination of the potency of fluconazole, ketoconazole and Itraconazole to target receptors by using molecular docking study

In order to assess the potential of various azole derivatives namely fluconazole, Itraconazole and ketoconazole; molecular docking study was carried out at the catalytic domain of ALS and GPI proteins. Fluconazole, Itraconazole and ketoconazole were docked with the respective chain of ALS and GPI and it was found that Itraconazole exhibits highest potential to target ALS and GPI. Binding with all the chains respectively exhibits equivalent binding affinity to and same affinity of fluconazole, Itraconazole and ketoconazole to target all the chains with equal potential. When a comparison was made among the different chains of ALs and GPI, it was observed that all these three chemical moieties are targeting these receptors with equal potency. Itracaonazole was found to exhibit highest potency to target ALS and GPI, binding affinity was found to be higher than ketoconazole and fluconazole.

Assessment of potency of fluconazole, Itraconazole and ketoconazole to target ALS

Molecular docking study was carried out to determine potency of fluconazole, Itraconazole and ketoconazole to target ALS. It was observed that ALS macromolecule forms the binding site with fluconazole comprises of SER124, THR122, PHE183, VAL184 residues and displays binding affinity of – 5.0 Kcal/mol (Figure 4A) on the other hand when binding affinity of Itraconazole with ALs macromolecule was assessed it was observed that Itraconazole exhibits binding affinity of – 7.1 Kcal/mol at site comprises of THR62, PHE60, SER124, GLY123 residues (Figure 4B). When binding affinity of ketoconazole with ALS macromolecule was assessed it was observed that ketoconazole exhibits binding affinity of - 6.0 Kcal/mol at site comprises of SER124, MET59, PHE60, GLY304 residues (Figure 4C). By comparing Gibbs free energy of binding of all three molecules, it becomes clear that Itraconazole possess more potency in comparison to fluconazole and ketoconazole to target ALS macromolecule. Chain A, B, C and D were docked separately with fluconazole, Itraconazole and ketoconazole but the binding affinity as revealed from Gibbs free energy level shows that all three compounds exhibited equivalent binding affinity at different chains but with different binding sites.

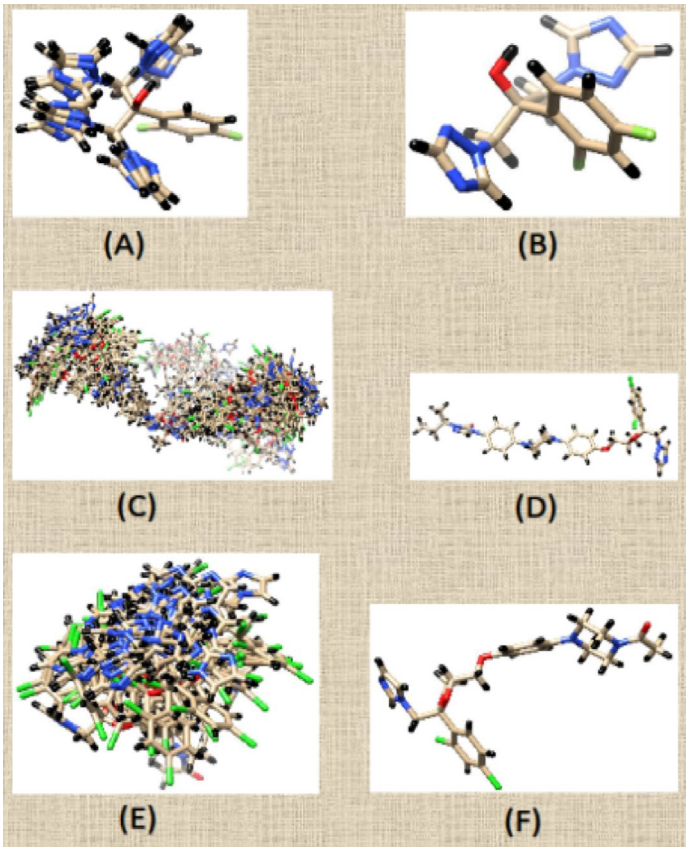

Figure 3. *Fluconazole (A), fluconazole isomer (B), itraconazole (C), itraconazole isomer (D), ketoconazole (E) and ketoconazole isomer (F).*

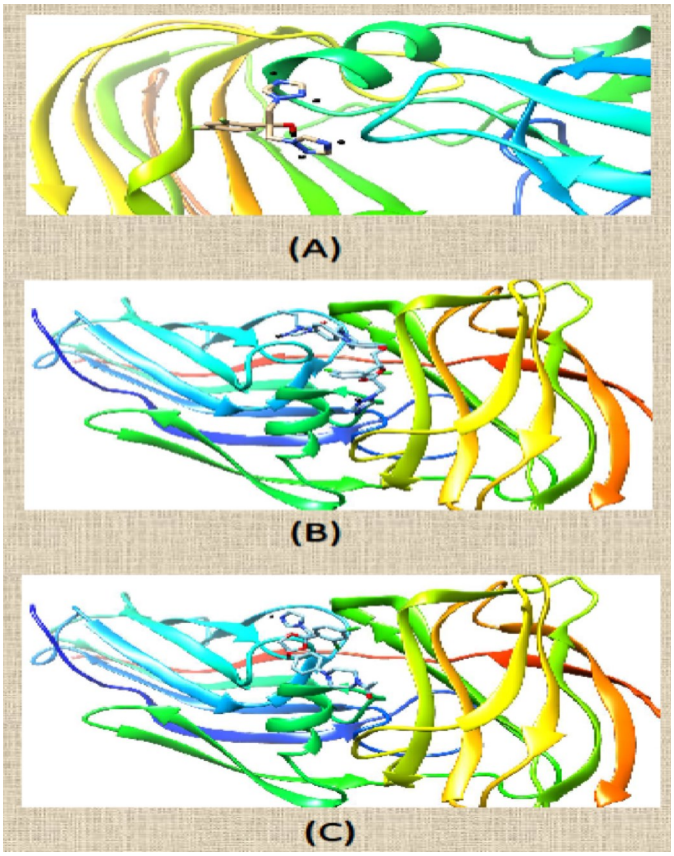

Figure 4. *Docked structure of ALS with fluconazole (A), itraconazole (B) and ketoconazole (C).*

Assessment of potency of fluconazole, Itraconazole and ketoconazole to target GPIs

In a process of adhesion to the host tissue special type of proteins are required, these proteins are glycoproteins required by microorganism in order to cause infection. The glycosylphosphatidylinositol (GPI) linked cell surface glycoproteins are the proteins which are responsible for adhesion of *C. albicans* to the host which further plays a prominent role in enhancing complexity of the disease. In order to evaluate potency of fluconazole, Itraconazole and ketoconazole to target GPI virtual docking study was carried out individually at all the 4 different chains of GPI. It was observed that GPI macromolecule forms the binding site with fluconazole comprises of VAL115, ASP158, VAL162, ILE275 residues and displays binding affinity of – 5.3 Kcal/mol (Figure 5A) on the other hand when binding affinity of Itraconazole with GPI macromolecule was assessed it was observed that Itraconazole exhibits binding affinity of – 7.5 Kcal/mol at site comprises of GLN349, LEU487, VAL162, ALA189 residues (Figure 5B). When binding affinity of ketoconazole with GPI macromolecule was assessed it was observed that ketoconazole exhibits binding affinity of -6.5 Kcal/mol at site comprises of GLU353, LYS548, ALA496, LYS357 residues (Figure 5C). By comparing Gibbs free energy of binding of all three molecules, it becomes clear that Itraconazole possess more potency in comparison to fluconazole and ketoconazole to target ALS macromolecule. Chain A, B, C and D were docked separately with fluconazole, Itraconazole and ketoconazole but the binding affinity as revealed from Gibbs free energy level shows that all three compounds exhibited equivalent binding affinity at different chains but with different binding sites and amongst all the three different moieties Itraconazole exhibits better potential than fluconazole and ketoconazole to target GPI (Spacek and Buchta, 2005). This clearly reveals that more research needs to be carried out on Itracaonazole to further elucidate its potency to target *C. albicans*.

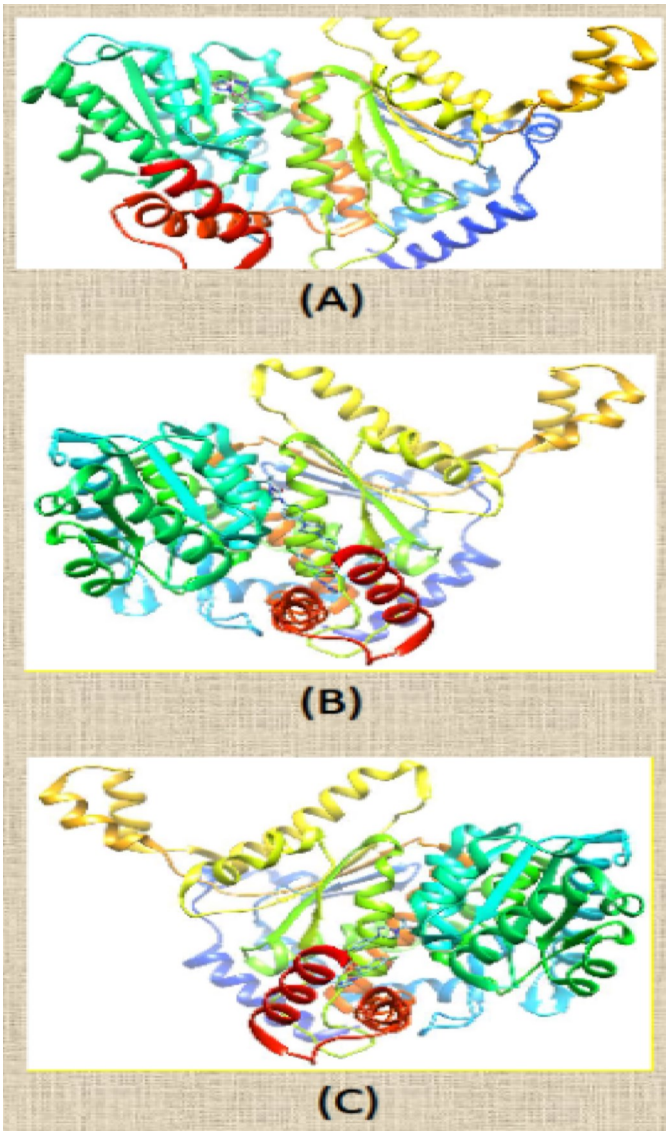

Figure 5. *Docked structure of GPIs with fluconazole (A), itraconazole (B) and ketoconazole (C).*

Conclusion

Vulvovaginal candidiasis has become a major havoc as currently 70-80% of women are affected from it. Overgrowth of *C. albicans* is considered to be a major causative agent of disease. Azole derivatives are considered to be potential drug of interest against it but the infection rate is increasing rapidly. Lack of identification of molecular pathways involved in complicating the disease further reduces the chances of providing an effective treatment strategy. The present study has identified the molecular target involved in enhancing the disease by inducing pathogenesis along with an effective therapeutic approach against it. Itraconazole can efficiently target GPIs and ALS which predominantly contributes to event of pathogenesis even better than fluconazole and ketoconazole. Researchers need to focus more on the molecular targets and the best suited approach to fight against the disease.

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Conflicts of interest

The author(s) declare that there no any conflicts of interest regarding publication of this manuscript.

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