Title Page

Exploring the binding of Ajmalicine, Dexamethasone and Acetaminophen to *Enterococcus faecalis* Homoserine dehydrogenase

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ABSTRACT

The rise in the resistance to commonly used antibiotics has reignited interest in the development of effective antibacterial drugs. *Enterococcus faecalis* (*E. faecalis*) is a major opportunistic pathogen with a diverse host range in hospital settings. They have an extraordinary ability to adapt according to the surroundings and acquire antibiotic resistance determinants. Due to its multi-drug resistance (MDR) nature against numerous antibiotics, there is an urgent need to develop therapeutic approaches to inhibit *E. faecalis*-associated infections. The protein homoserine dehydrogenase, a crucial regulatory enzyme in the aspartate pathway, is the target of our current study. This enzyme is responsible for the aspartate-mediated synthesis of methionine, threonine, and isoleucine from aspartate. Since this enzyme is essential for the survival of the pathogen, inhibition of Homoserine dehydrogenase will in turn obstruct the *E. faecalis* infections. So, considering the important role of Homoserine dehydrogenase in *E. faecalis* in the present work, we have reported data priming, molecular docking, and pharmacokinetics studies. We have also reported molecular dynamics simulation (MD simulation) studies at 50ns simulation run. It was observed that the lead molecules bind to the enzyme with significant interactions. In order to comprehend how Ajmalicine, Dexamethasone, and Acetaminophen bind to *Enterococcus faecalis* Homoserine dehydrogenase, these findings provide a fresh insight.

Keywords: Homoserine dehydrogenase; *Enterococcus faecalis*; Inhibitors; cysteine; Threonine

Introduction

One of the major concerns of healthcare system is the increasing prevalence of infections caused by multidrug-resistant bacteria. Patients infected with MDR pathogen shows worse outcome than patients infected with more suspicious organisms. *E. faecalis* is an opportunistic MDR pathogen associated with nosocomial infections in hospital settings. (Beganovic et al., 2018). They are presently ranked amongst the most prevalent MDR hospital pathogens. *E. faecalis* can cause a wide range of infections including, sepsis, surgical wound infections, and urinary tract infections especially in the nosocomial environment (Rôças et al., 2004; Beganovic et al., 2018). They are critically responsible for increased mortality, mobility, and health care cost. There are several proteins that are critically involved in the survival of the bacterium (Rôças et al., 2004). One such protein is homoserine dehydrogenase, which belongs to the family of oxidoreductases and works on the CH-OH group of the donor when NAD+ or NADP+ is the acceptor. This enzyme class is referred to by its scientific name, L-homoserine:NAD(P)+ oxidoreductase(Cami et al., 1993; Thomas et al., 1993). Other terms that are frequently used are HSDH and HSD. The highly branching biosynthetic route in bacteria produces four amino acids from aspartic acid: lysine, threonine, isoleucine, and methionine(Tomonaga et al., 2015). Aspartokinase, a first step in the conversion of aspartate, is followed by aspartic semialdehyde dehydrogenase, a second step in the process that results in aspartic semialdehyde, a common intermediate in the biosynthesis of lysine and threonine, isoleucine, and methionine(Ogata et al., 2018). The intermediate of the threonine/methionine biosynthetic branch, homoserine, is created by a further reduction of semialdehyde (homoserine dehydrogenase)(Ogata et al., 2018). Homoserine dehydrogenase enzyme found in a class of bacteria with similar function and activity. NADPH was discovered in the coenzyme binding site in the crystal structure, and NADP functions as a potent dead end inhibitor of NAD-dependent activity(Tomonaga et al., 2015). It has been suggested that the disulfide bond formed between cysteine residues (Cys304) in the C-terminal portions of the homodimer subunits is what activates the HSD from the hyperthermophilic archaeon Sulfolobus tokodaii (StHSD)(Cami et al., 1993; Ogata et al., 2018; Thomas et al., 1993, 1993). In the current work, we have focused on *E. faecalis*, a gram-positive, non-sporulating rumen bacterium, and main aspartate metabolism enzymes. Therefore, creating an effective treatment for E. faecalis-mediated enterococcal infections is a challenging undertaking. So, looking the significance role of Homoserine dehydrogenase in *E. faecalis*, the structure-function relationship of *E. faecalis* Homoserine dehydrogenase was elucidated, and the binding of lead molecules were identified.

Materials and Methods

Data collection

We downloaded the Homoserine dehydrogenase amino acid sequence from *E. faecalis* in FASTA format from the NCBI server (https://www.ncbi.nlm.nih.gov/protein/EPI21277.1?report=fasta), and then we excess HHpred to search for templates. We discovered a perfect match with PDB ID 3MTJ of *Thiobacillus denitrificans*, so we performed modelling by using 3MTJ as a template. Similarly we have downloaded the ligands for docking from PubChem (https://pubchem.ncbi.nlm.nih.gov/#query=dexamethasone) data base(Berman, 2000; Hildebrand et al., 2009).

Data priming

Homoserine dehydrogenase was modelled in HHpred, examined with Pymol's assistance, and prepared with UCSF Chimera, to which we added side chains and charges. Similar to how ligand was manufactured, charges and H-bonds were added for better outcomes(Hildebrand et al., 2009; Pettersen et al., 2004; Yuan et al., 2004).

Molecular docking

As we don't know about the active site of homoserine dehydrogenase of *E. faecalis* and we have modeled the protein structure, so we perform blind docking for collected ligands and modeled protein. For this purpose, we use CB-Dock server, which is cited in the nature article for its accuracy. By using this server, we have observed the binding energy, cavity size, center for docking and size of the grid. We employ the CB-Dock server, which is cited in the Nature paper for its correctness, for this purpose. We have measured the binding energy, cavity size, docking centre, and grid size using this server(Liu et al., 2016).

Pharmacokinetics

After blind docking, we check the ADME properties of these molecules in the Swiss ADME server (http://www.swissadme.ch/index.php#top), where we have examined the pharmacokinetics, medicinal chemistry, and druglikeness of these compounds as well as their ability to cross the blood-brain barrier and other physical and chemical properties (Daina et al., 2017).

Molecular dynamic simulation

Desmond used to simulate explicit solvents with periodic boundary conditions using cubic, orthorhombic, truncated octahedron, rhombic dodecahedron, and arbitrary triclinic simulation boxes. He also used to model explicit membrane systems under varied situations. We used default

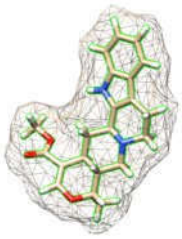
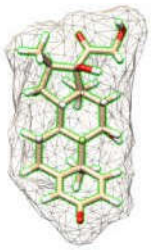
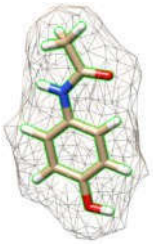
parameters to perform molecular dynamic simulation of 50ns. We ran a 50ns molecular dynamic simulation using the default parameters. We have set the number of frames at 300 K and the recording interval trajectory at 10.0. (bar).

Result

Data collection and Homology modeling

All three compounds have with their compound ID, IUPAC, and canonical smiles shown in the table1 utilize for the pharmacokinetics and validation of the data. 3D

Table1: Characteristics features of selected compounds.

CID	Common name	IUPAC	Canonical smiles	3D structure
441975	Ajmalicine	methyl (1S,15R,16S,20S)-16-methyl-17-oxa-3,13-diazapentacyclo[11.8.0.02,10.04,9.015,20]henicosa-2(10),4,6,8,18-pentaene-19-carboxylate	CC1C2CN3CCC4=C(C3CC2C(=CO1)C(=O)OC)NC5=CC=CC=C45	
5743	Dexamethason	(8S,9R,10S,11S,13S,14S,16R,17R)-9-fluoro-11,17-dihydroxy-17-(2-hydroxyacetyl)-10,13,16-trimethyl-6,7,8,11,12,14,15,16-octahydrocyclopenta[a]phenanthren-3-one	CC1CC2C3CCC4=CC(=O)C=CC4(C3(C(CC2(C1(C(=O)CO)O)C)O)F)C	
1983	Acetaminophen	N-(4-hydroxyphenyl)acetamide	CC(=O)NC1=CC=C(C=C1)O	

Molecular Docking

The use of molecular docking techniques for chemical compound analysis by repurposing against the target molecule is very common. The molecular docking method was also used in this investigation to determine ligand potentials against the inhibition of the homoserine

dehydrogenase protein in *E. faecalis*. All three compounds were docked in the anticipated active pocket and displayed binding energies more than -5.5 Kcal/mol at least RMSD. Homoserine dehydrogenase-docked Ajmalicine's complex made bonds with L54, L57, G58, M62, Y67, Q72, H73, V75, V93, K94, H96, and Y100 and had a substantial binding energy of -8.3 Kcal/mol. The homoserine dehydrogenase-Dexamethasone complex that was similarly docked showed a good binding affinity of -7.5 Kcal/mol and displayed bonds with the amino acids E196, A195, G193, F194, E286, G308, A307, K211, K111, D112, D207, N165, G166, D202, and N201. Furthermore, the homoserine dehydrogenase-acetaminophen complex displayed a high binding energy of -5.9 Kcal/mol with residues Q126, H129, C130, D131, H105, N326, M335, A330, and G333. All three compounds interact with *E. faecalis* -homoserine dehydrogenase with good binding energy at the location residues shown in figure 1.

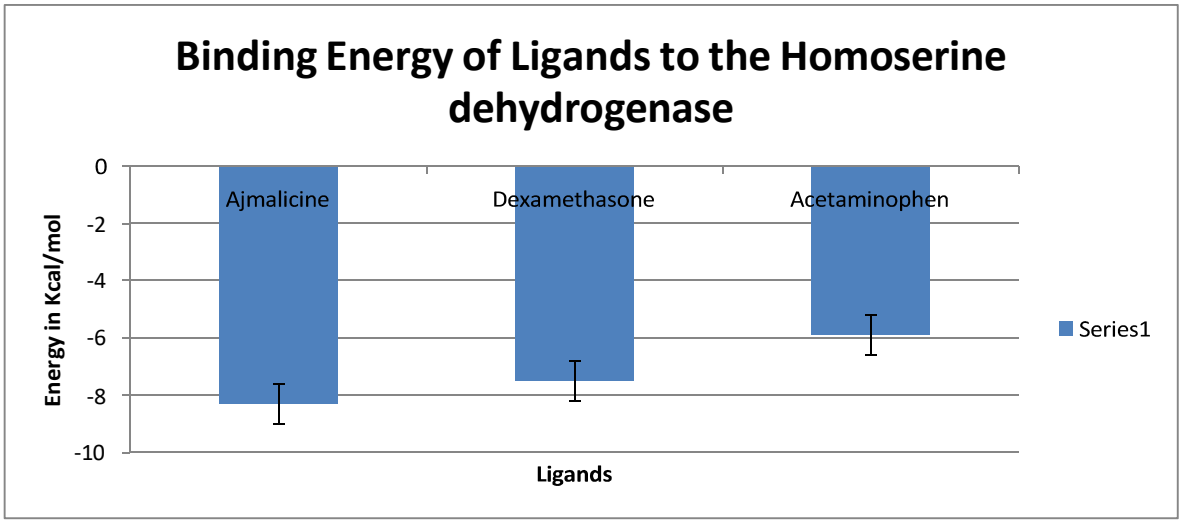
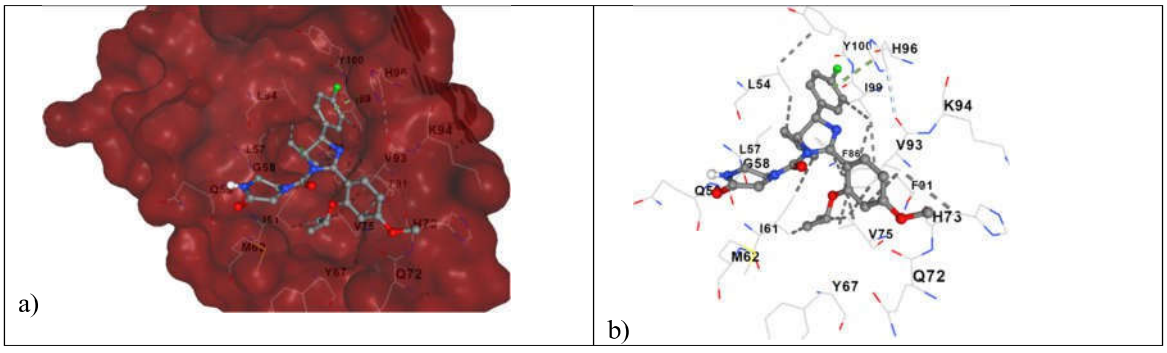


Figure1: Ajmalicine, Dexamethasone and Acetaminophen show binding energy with *E. faecalis* Homoserine dehydrogenase.



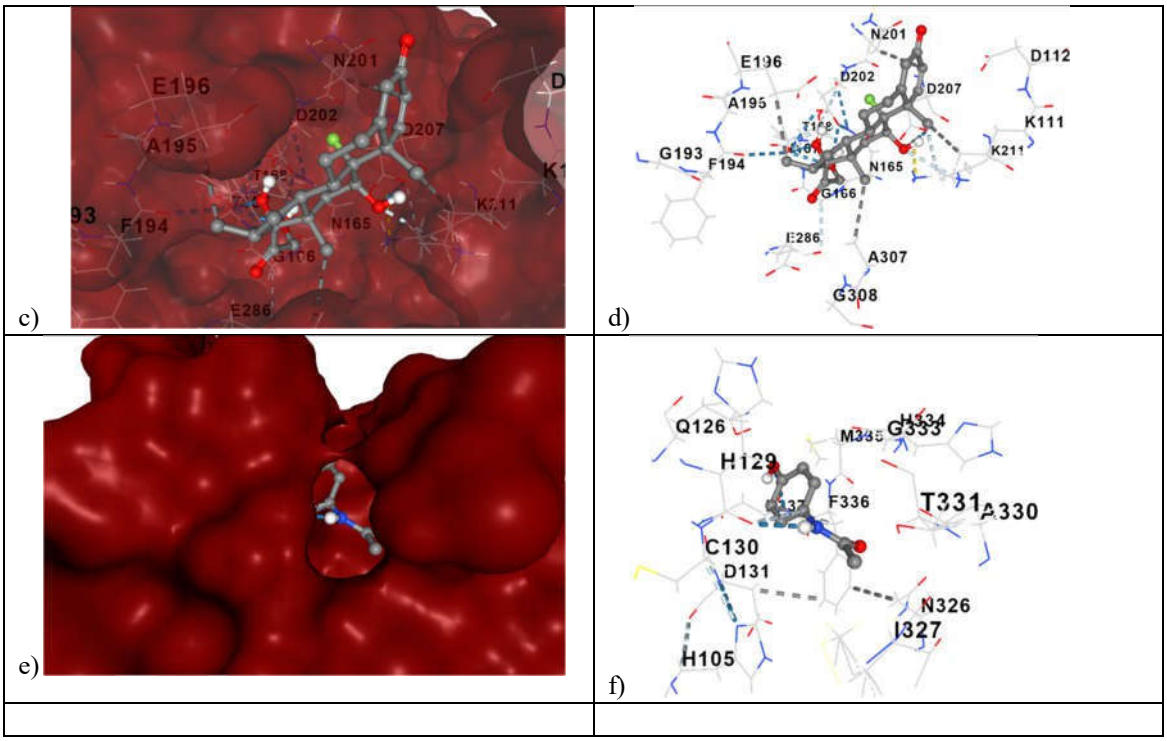


Figure 2: Ligand binding in active pocket and residues of *E. faecalis* Homoserine dehydrogenase.

Pharmacokinetics

Ajmalicine (CID:441975) and acetaminophen (CID:1983) both pass through the blood-brain barrier (BBB), but only ajmalicine shows P-gp substrate positivity while acetaminophen shows P-gp substrate negativity in the pharmacokinetic property study. Dexamethasone (CID: 5743), on the other hand, is positive to the P-gp substrate but is unable to cross the BBB. All the three molecules follow Lipinski rule and show high GI absorption (Table 2).

Table2: Pharmacokinetics property of selected compounds in Swiss ADME.

Compounds name	Ajmalicine	Dexamethason	Acetaminophen
Formula	C21H24N2O3	C22H29FO5	C8H9NO2
Molecular weight	352.43 g/mol	392.46 g/mol	151.16 g/mol
Num. heavy atoms	26	28	11
Num. arom. heavy atoms	9	0	6
Fraction Csp3	0.48	0.73	0.12
Num. rotatable bonds	2	2	2
Num. H-bond acceptors	4	6	2
Num. H-bond donors	1	3	2
Molar Refractivity	103.47	101.96	42.78
TPSA	54.56 Å²	94.83 Å²	49.33 Å²
Log Po/w (iLOGP)	3.2	2.26	1.21
Log Po/w (XLOGP3)	2.75	1.94	0.46
Log Po/w (WLOGP)	2.47	2.32	1.16
Log Po/w (MLOGP)	2.13	1.62	0.91
Log Po/w (SILICOS-IT)	2.78	2.58	0.89
Consensus Log Po/w	2.67	2.14	0.93
Log S (ESOL)	-3.88	-3.36	-1.34
Solubility	4.63e-02 mg/ml ; 1.31e-04 mol/l	1.70e-01 mg/ml ; 4.33e-04 mol/l	6.93e+00 mg/ml ; 4.59e-02 mol/l
Class	Soluble	Soluble	Very soluble
Log S (Ali)	-3.55	-3.56	-1.06

Solubility	9.92e-02 mg/ml ; 2.81e-04 mol/l	1.09e-01 mg/ml ; 2.78e-04 mol/l	1.30e+01 mg/ml ; 8.62e-02 mol/l
Class	Soluble	Soluble	Very soluble
Log S (SILICOS-IT)	-4.38	-2.8	-2.19
Solubility	1.47e-02 mg/ml ; 4.16e-05 mol/l	6.25e-01 mg/ml ; 1.59e-03 mol/l	9.72e-01 mg/ml ; 6.43e-03 mol/l
Class	Moderately soluble	Soluble	Soluble
GI absorption	High	High	High
BBB permeant	Yes	No	Yes
P-gp substrate	Yes	Yes	No
CYP1A2 inhibitor	No	No	No
CYP2C19 inhibitor	No	No	No
CYP2C9 inhibitor	No	No	No
CYP2D6 inhibitor	Yes	No	No
CYP3A4 inhibitor	No	No	No
Log Kp (skin permeation)	-6.50 cm/s	-7.32 cm/s	-6.90 cm/s
Lipinski	Yes; 0 violation	Yes; 0 violation	Yes; 0 violation
Ghose	Yes	Yes	No; 1 violation: MW<160
Veber	Yes	Yes	Yes
Egan	Yes	Yes	Yes
Muegge	Yes	Yes	No; 1 violation: MW<200
Bioavailability Score	0.55	0.55	0.55
PAINS	1 alert: indol_3yl_alk	0 alert	0 alert
Brenk	0 alert	0 alert	1 alert: hydroquinone
Leadlikeness	No; 1 violation: MW>350	No; 1 violation: MW>350	No; 1 violation: MW<250
Synthetic accessibility	4.57	5.47	1

Molecular dynamic simulations

The molecular dynamics (MD) simulation, which predicts the stability and intermolecular interactions of docked complexes with respect to time, offers insights into the dynamic behavior of a protein and the conformational changes that occur upon binding with ligands. Here, we analyze a variety of measures to assess the stability of docked protein-ligand complexes. Protein-ligand contact profiling, which assesses the contacts between the protein and ligand in the docked complexes with relation to time during the simulation, is another method of examining the stability of the docked ligand in the protein binding pocket.

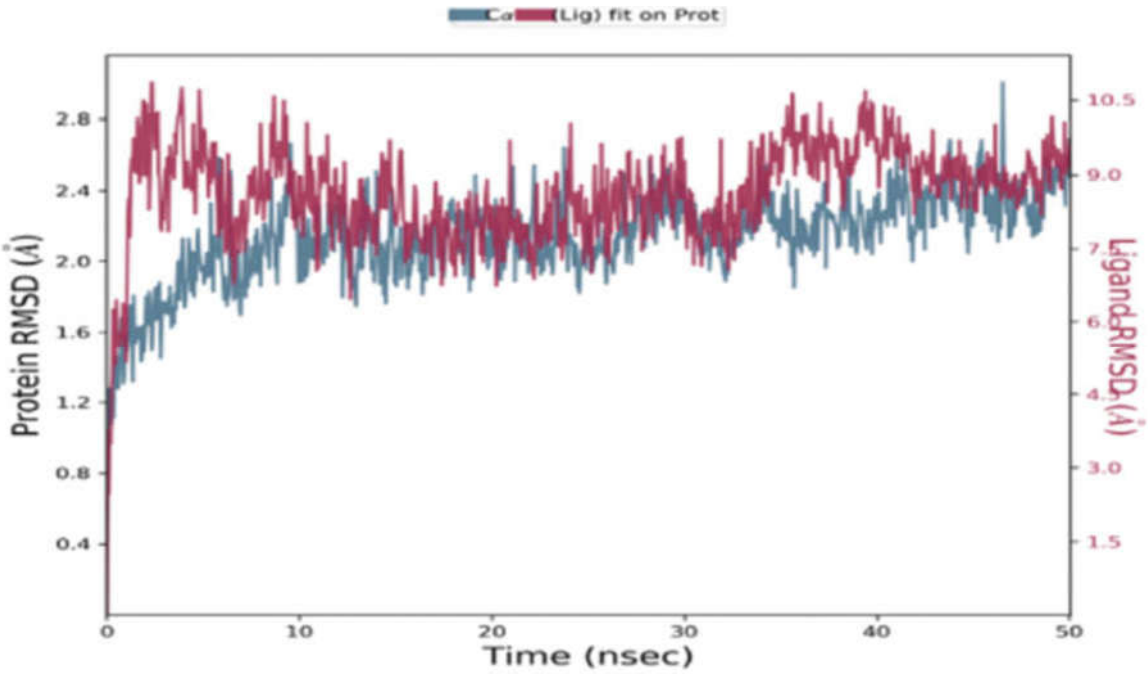


Figure 3: Molecular simulation of Ajmalicine compound on the basis of best docking score

Discussion

Enterococcus faecalis homoserine dehydrogenase *(EfHSD)* is involved in methionine, threonine, and isoleucine biosynthesis pathway and is ubiquitously expressed in all prokaryotic cells. It conducts the first step following the branch point to methionine, which is an important amino acid, and it catalyzes the ATP-dependent phosphorylation of L-homoserine to O-phospho-L-homoserine. Among the numerous and varied oxidoreductases is homoserine dehydrogenase. HSD and other dehydrogenases including malate, lactate, and glyceraldehyde 3-phosphate dehydrogenase have certain similarities. In this work, the 3D structure of HSD was solved using a comparative protein modeling technique, and in silico docking experiments were conducted to examine the beneficial interaction between protein and ligand (Ajmalicine, Dexamethasone and Acetaminophen). Furthermore, all three-ligand (Ajmalicine, Dexamethasone and Acetaminophen) are medicinal compound and has antibacterial activity. These findings encouraged us to conduct in silico docking to analyze the binding of these molecules with *EfHSD*. Docking analyses revealed a strong interaction between the ligand and the *EfHSD*. Hydrogen bonds may be important in substrate binding. It was discovered that L54, L57, G58, M62, Y67, Q72, H73, V75, V93, K94, H96, and Y100 amino acid residues had an H-bond interaction with homoserine and ajmalicine. Dexamethasone and homoserine share amino acid residues with E196, A195, G193, F194, E286, G308, A307, K211, K111, D112, D207, N165, G166, D202, and N201. Acetaminophen and homoserine dehydrogenase share amino acid residues Q126, H129, C130, D131, H105, N326, M335, A330, and G333. The data of binding energy of homoserine and all three-ligand showed that the homoserine involved in bond formation with

ajmalicine with a close margin difference of docking score –2.6 with acetaminophen; -0.8 Kcal/mol close margin difference with dexamethasone, respectively. This result is completely consistent with experimental data (Ejim et al. 2004; Zhan et al. 2014)

The phosphate-binding loop's amino acid residues, such Asp, Asn, Gly, Glu and His are crucial for the creation of H-bonds, with created a groove cavity for ligand docking. Thus, it can be proposed that the ligand (Ajmalicine) binds at the site where this amino acid specially situated (fig 2). As a result, this ligand may be regarded as a potential inhibitor of *EfHSD*, as other 2 ligands occupy the same binding pocket and block the site, which can further use as potent ligand to block the enzyme activity. This shows the similar action in case of Metronidazole to *Enterococcus faecalis* Homoserine Kinase Docking Studies (Yadav et al. 2022; Singh et al. 2020).

A molecular dynamics-based study was carried out to evaluate the docking stability and flexibility of the complex. The Md simulation carried out using the default settings demonstrates that the ligand is constantly present in the pocket and binds with a strong interaction. The artificial environment that surrounds the protein ligand complex and is made up of ions, salts, and water molecules under a constant pressure demonstrates the stability of the compounds that it contains. Additionally, the interaction between the ligand and the protein demonstrates the stability of the ligand and the stable conformation that exists inside the active pocket in the given environment. Molecular dynamic simulations further validated the docking results (Girija et al 2021).

In addition, Lipinski's parameters for drug-likeliness were also analyzed for all three drugs, which states that any orally active drugs should not violate more than one of its parameters (Lipinski, et al. 1997). These compounds follow Lipinski's rule of 5 and possessed moderate to good % human oral absorption. Following the ADME and Lipinski Rule of Five, we were able to arrive at a conclusion on the pharmacokinetic properties of these 3 compounds can be under consideration. These compounds have strong docking scores and binding energies that fall within a wide range of possible values (Table 1). It was found that the ligands should be placed in the appropriate orientation under the active pocket, and both the 2D and 3D figures show that there is an adequate amount of hydrogen bonding, pei-pei interaction, and hydrophobic bonding.

The results of this study can serve as a basis for the development of more powerful chemicals entity that have an even greater affinity for binding and interactions with the protein *Enterococcus faecalis* Homoserine dehydrogenase. It has the potential to contribute to the development of novel compounds that exhibit considerable antibacterial activity, which can lead to rational structure-based inhibitor design.

Conclusion

The research was planned to explore the binding of Ajmalicine, Dexamethasone and Acetaminophen to *E. faecalis* Homoserine dehydrogenase (*EfHSD)*. Homoserine dehydrogenase plays a critical role in aspartate pathway and facilitates the synthesis of methionine, threonine, and isoleucine from aspartate. Due to the unavailability of this enzyme in humans, inhibition of this bacterial enzyme for curing *E. faecalis*-mediated enterococcal infections is a crucial task. A computer-aided approach was used to identify the interaction between *EfHSD* and Ajmalicine, Dexamethasone and Acetaminophen. It is proposed that all molecule can have a significant inhibitory property against *EfHSD* by binding to the catalytic site of the protein. This study confirmed that all the ligands showed significant binding with homoserine dehydrogenase of *E. faecalis* which play an important role in the survival of *E. faecalis*.

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