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Computational modelling, molecular docking, and molecular dynamics simulation studies of Enterococcus faecalis diaminopimelate epimerase

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Abstract

Background: *Enterococcus faecalis* (*E. faecalis*) is an opportunistic bacterial pathogen which is resistant to a several class of antibiotics. *E. faecalis*, infection is increasing in numbers frequently and it is needful to find alternative approaches to fight against the pathogen.

Diaminopimelate epimerase (DapF) enzyme is an essential enzyme in the Lysine biosynthesis pathway which catalyses the formation of meso-2, 6-diaminoheptanedioate from 2,6-diaminoheptanedioate. Lysine is an essential amino acid, therefore, targeting the *E. faecalis* Diaminopimelate epimerase (*EfDapF*) will help us to develop antimicrobial drugs.

Material and Method: We have reported here cloning of *EfDapF* gene its expression, and purification along with the structural prediction of *EfDapF* using computational methods. We have also done molecular docking studies of *EfDapF* with ajmalicine. Docking studies were also validated by MD simulation studies.

Results: These studies indicated the binding of ajmalicine with *EfDapF*. Structural studies indicated a mixed structure of *EfDapF* having both alpha helixes and beta sheets. Docking studies further showed that amino acid residue Ser73 for ajmalicine which is a part of phosphate-binding loop of *EfDapF* plays a significant role in the formation of Hydrogen bond with the ligand. The exact position of ligand binding is deep groove, regarded as the protein's binding cavity.

Conclusion: These studies indicated reasonably good interactions of ajmalicine with *EfDapF* which can eventually diminish the catalytic activity of the protein.

Keywords: *Enterococcus faecalis*; Lysine biosynthesis pathway; Diaminopimelate epimerase; Protein structure predication; molecular docking

Introduction

Diaminopimelate epimerase (DapF) is a PLP-independent amino acid racemase which plays a major role in the biosynthesis of essential amino acid lysine and meso-DAP[1]. The products obtained at the end of this metabolic pathway are biomolecules essential for the formation of

peptidoglycan, housekeeping proteins, virulence factors etc. Hence, they are also considered essential for the survival of the bacteria. Design of *EfDapF* inhibitors will help us to identify a new class of antibacterial drugs[1]. It is important to develop novel class antibiotics against *dapF* because this enzyme is lacking in humans. [2] The three-dimensional structural details of *DapF* has been decoded from various bacterial species including *Escherichia coli*[1], *Haemophilus influenza*[3], *Corynebacterium glutmicum*[4] *Mycobacterium tuberculosis*[5] and *Arabidopsis thaliana*[6] to name a few. The first structure of the enzyme was elucidated in late 90's from *Haemophilus influenza*[7]. *DapF* consists of two structurally similar α/β monomers. Each domain provides an active site cysteine residue which are important for its catalytic activity, and these are always utilized in pair thiolate form. The amino acid Cys acts as a base and it receives a H+ from L-DAP, while thiol form Cys work as acid and releases H+ which led to the formation of meso-DAP.[6] Disulphide linkage are formed by these two cysteine residues under oxidizing condition, which promotes conformational changes at the catalytic site[4]. Recent studies revealed the dual states (solution and crystal) of dimer existence which is also required for optimal activity. It was reported that the dimer interface lies in the middle of the N-terminal domains of the both the monomers and the catalytic site is in a small cleft in the middle of both domains.[1] Superposition of *Bacillus anthracis* and *Escherichia coli DAP* catalytic site structure shows that most of the active site amino acid residues are oriented in a similar manner. To add to this, *DapF* play a key role in bacterial survival but is absent in humans which makes it a reasonably good drug target. This research is a new method to design inhibitors of *DapF* as, which can work as antimicrobial agents. We have reported the expression and purification studies of *EfDapF*. In continuation to which we did structural characterization and molecular docking studies of *EfDapF*. This was followed by MD simulation experiments.

Materials and methods

Amplification expression and purification of *EfDapF* gene

PCR amplification, cloning, expression and purification of EfDapF were done according to the previously reported protocol.[8]

Homology Modelling

The *EfDapF* amino acid sequence (UNIPROT ID: Q838I3) weas retrieved in FASTA format from the Uniprot [https://www.uniprot.org]. The available crystal structures of DapF from

different organisms such as *E.coli* [PDB entry: 4IJZ], *Bacillus anthracis* [PDB entry: 2OTN], *Acinetobacter baumannii* [PDB entry: 5HA4], *Corynebacterium glutamicum* [PDB entry: 5M47], *Haemophilus influenzae* [PDB entry: 1BWZ], *Mycobacterium tuberculosis* [PDB entry: 3FVE], and *Arabidopsis thaliana* [PDB entry: 3EKM] as modelling templates were all derived from the Protein Data Bank[https://www.rcsb.org/]. The I-TASSER server [https://zhanggroup.org/I-TASSER] was used to estimate the 3D structure of *EfDapF* based on the crystal structure of homologous DapF proteins. PyMOL and UCSF Chimera were used to visualise the original model. [10] The model was further subjected to energy minimization using steepest descent for 2000 steps. [11]PDBsum server was used to validate the final 3D model.[12]

Molecular Docking:

The docking of ligands and *EfDapF* was done manually using AutoDock 4.2.6 by docking 'one ligand at a time' to the protein.[13] It is open-source and is regarded as one of the most reliable molecular docking programmes.[14] The 3D structures of putative *EfDapF* ligand Ajmalicine were obtained in SDF format from the PubChem database [http://pubchem.ncbi.nlm.nih.gov] and converted to PDB format using Pymol software. Gasteiger partial charges were assigned to the ligand, and non-polar hydrogen atoms were merged. Through a docking experiment, all torsion angles were set free to rotate and converted into PDBQT format using AutoDockTools v1.5.7. In AutodockTools v1.5.7, the 3D model of *EfDapF* was opened as PDB format, and polar hydrogen atoms, Kollman charges, and macromolecules were assigned and chosen as macromolecules, then saved as PDBQT format. The assignment of grid parameters is the most critical step in molecular docking because it guides the ligand to the receptor's binding site. Around the active and Binding location of *EfDapF*, a grid map of [20 Å × 20 Å × 20 Å] was constructed on coordinates X-1.197, Y-2.552, and Z-2.488. The files for receptors and ligands are saved in the Scripps research folder. For executing the Autodock Vina, a config file with information for running the programmes was created. 20 poses were created with the exhaustiveness set to 32. To validate the docking experiment, a control docking experiment was done using the Co-crystal structure of DapF from *C.glutamicum* [PDB entry:5M47] with its product. Minimum binding energy [kcal/mol], cluster RMS, number of hydrogen bonds, and other interacting residues were used to screen the optimum docked postures for each of the ligands with *EfDapF*. The ideal posture interaction and 2D map for each docked complex were created using the Discovery studio.[15]

Molecular Dynamics Simulation:

The GROMACS 5.1.4 software[16] was used to simulate apo *EfDapF* and the best docked posture of each ligand-enzyme complex on an Intel Xeon system with 32 GB RAM and Ubuntu 14.08 Linux package. PRODRG server was used to construct the ligand topologies. [17] In all simulations, the GROMOS 96al force field was employed. Each protein system was solvated in a pre-equilibrated cubic SPC water box with a distance of 2 from the protein surface to any side of the box. Because the system's overall charge was negative, Na+ ions were introduced to neutralise it. Each system was energy-minimized using 50,000 steps of steepest descent minimization. Particle Mesh Ewald with an interpolation order of 4 was utilised for long-range electrostatic interaction. Using a modified Berendsen thermostat (tau T = 0.1 ps) and a Parrinello–Rahman barostat (tau P = 2 ps), NVT and NPT equilibration was performed for 100 ps at 300 K temperature and 1 bar pressure. Final production of MD was run for 50 nanoseconds. The GROMACS package's tools were used to analyse simulation trajectories. The tools gmx rms and gmx rmsf were used to calculate the root mean square deviation (rmsd) and root mean square fluctuations (rmsf) respectively. The gmx trjconv tool was used to extract the structures from the trajectory. The plots were created with the help of the XMGRACE software suite. [18]

Results and discussion

Overexpression and Purification

The amplified gene of *EfDapF* was cloned in expression vector, overexpressed and the expressed protein was purified using NI-NTA affinity chromatography. The purified protein was further analysed by SDS-PAGE which showed a single band around 36 kDa [Fig.1].

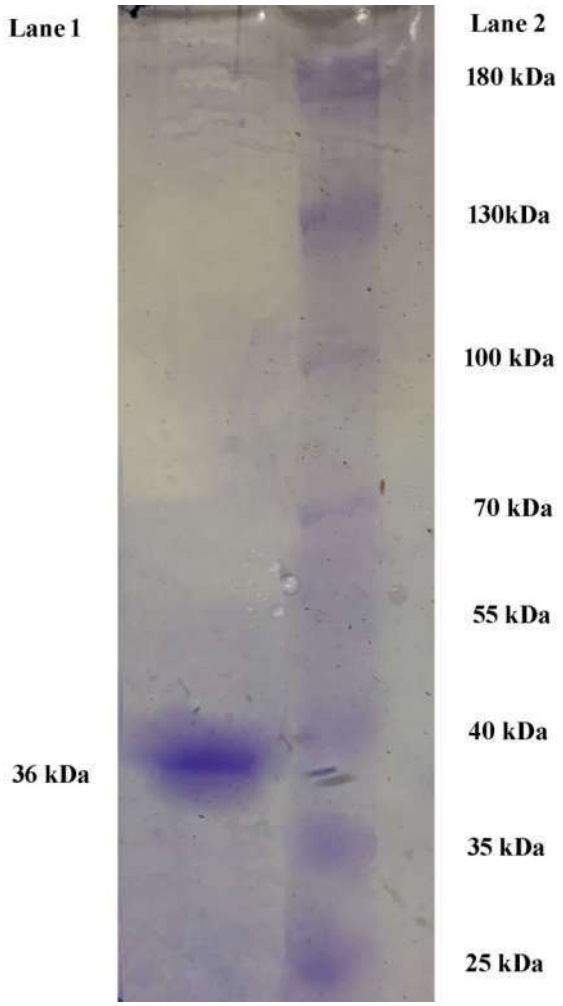

Figure 1. SDS-PAGE showing purified EfDapF protein at around 36 kDa region in Lane 1 against the protein marker in Lane 2. (Ref: 8)

Homology Modelling

Homology modelling was used to predict the three-dimensional structures of *EfDapF*. ModRefiner was utilised to reduce overall energy consumption and improve backbone topology. According to PDBsum server a good quality model would be expected to have over 90% in the most favoured regions and G-factor value less than -0.5. The modelled *EfDapF* generated by PDBsum has 91.4 percent residues in most favoured regions, 8.2 percent residues in additional allowed regions, 0.3 percent residues in generously allowed regions, and no residues in banned regions [Fig.2], indicating a very good quality model.

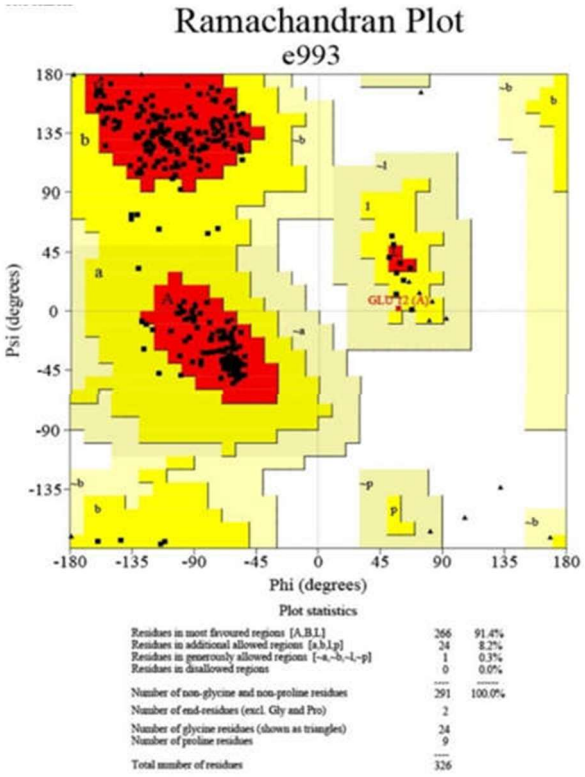

Figure 2. The modelled EfDapF generated by PDBsum has 91.4 percent residues in most favoured regions, 8.2 percent residues in additional allowed regions, 0.3 percent residues in generously allowed regions, and no residues in banned regions,.

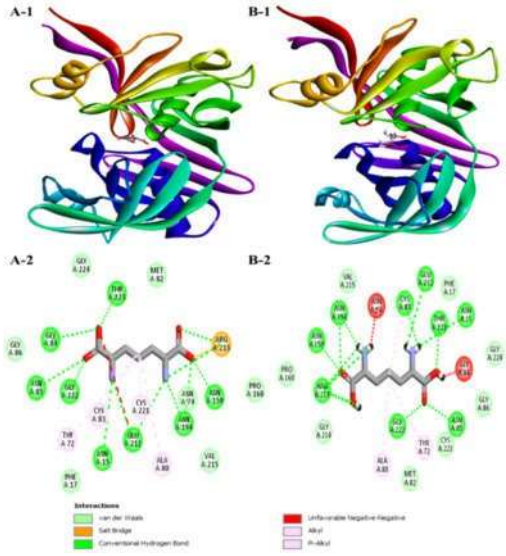

Figure 3. The ligplot of its control docking exhibit identical interacting atoms.

Molecular Docking

Ajmalicine had binding energy of 7.8 kcal/mol and ajmalicine interacted with Asn13 and Asp74 [Fig. 4] near binding pocket of *EfDapF* in its best docked poses **[Table I].**

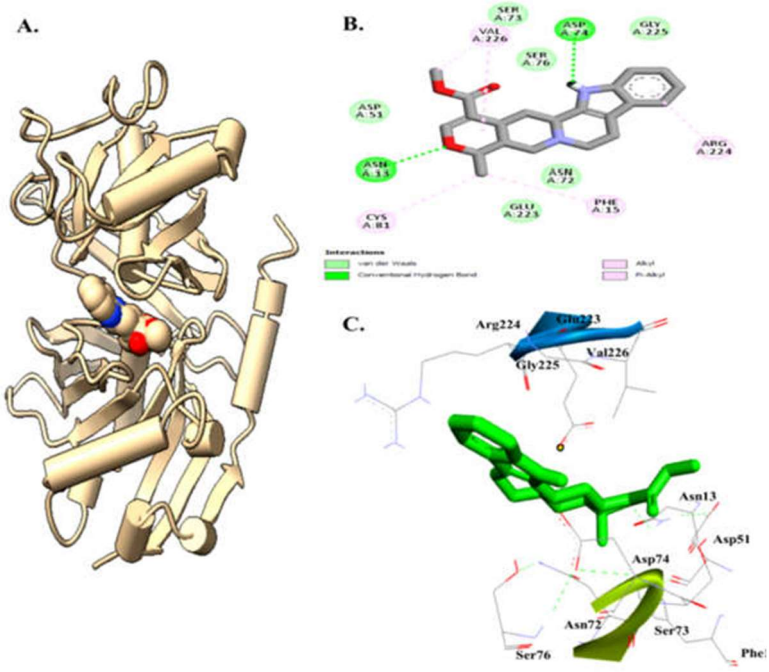

Figure 4. Ajmalicine interacted with Asn13 and Asp74

Protein	Compound	Binding affinity (Kcal/mol)	Van der waals Interactions	Conventional Hydrogen Bond Interaction	Π-Alkyl & Alkyl Interactions
Diaminopimelate epimerase	Ajmalicine	-7.8	Asp51, Ser73, Ser76, Gly225, Asn72, Glu223	Asn13(4.90), Asp74 (4.60)	Val226 (4.60, 6.25), Arg224 (4.25), Phe15 (6.26), Cys81 (5.61)

Table-I: **Binding Affinity and Protein-Ligand interactions profile of the DapF protein with Ajmalicine.**

Molecular Dynamics Simulation

A 50ns molecular dynamics simulation computation was executed, and root mean square displacements (RMSD) and root mean square fluctuations (RMSF) graph were plotted to comprehend the structural dynamics and stability of apo *EfDapF* and the best docked complexes. *EfDapF* Apo as well as its best docked complexes with ajmalicine exhibited of 0.35nm [Fig.5].

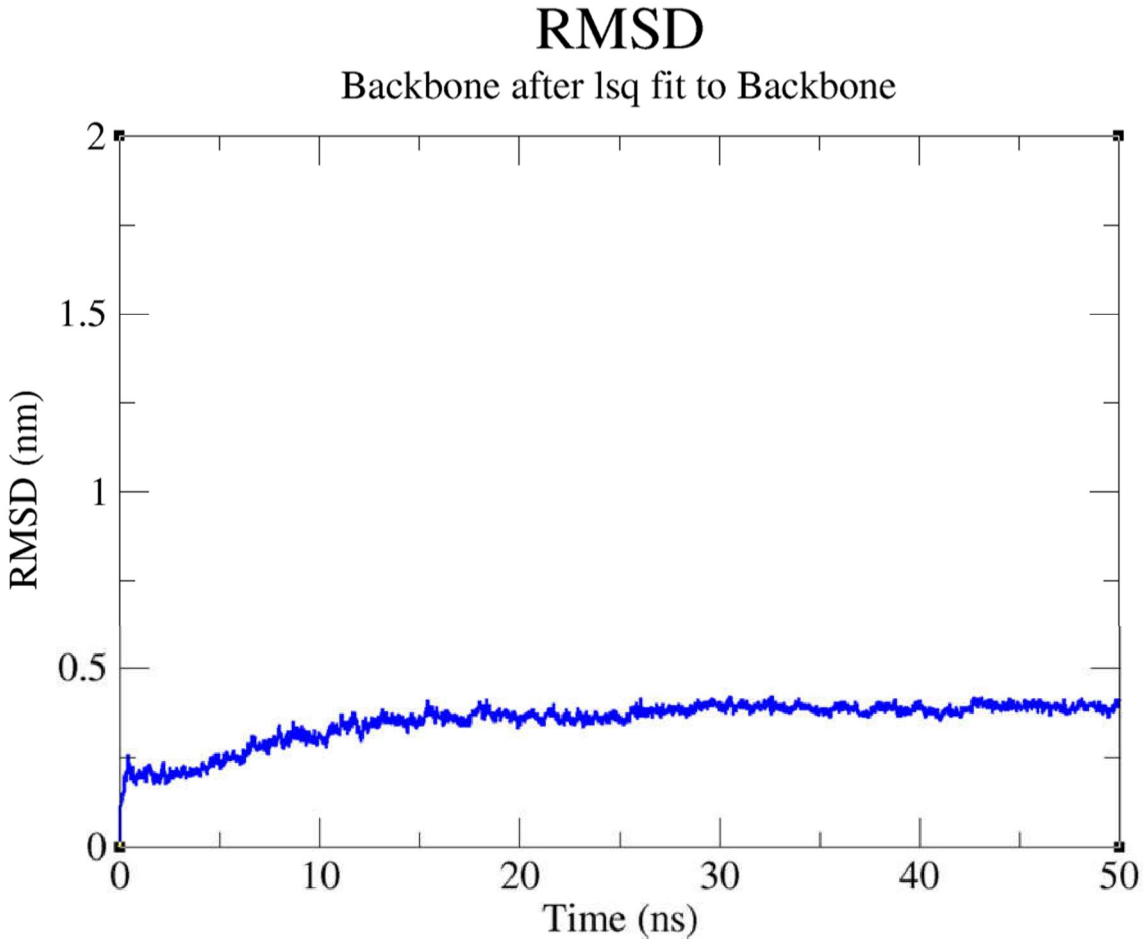

Figure 5. Root mean square deviation of Ajmalicine.

The RMSF plot between these simulations illustrate that the loop region between residues no 140 to 150 and 315 to 325 has by far the most volatility. These dynamic residues are not found in *EfDapF*'s ligand binding site. Interestingly, upon conformational changes, higher stability is seen in regions from residues of binding pocket viz. residues 10-20, 70-90, and 230-240 [Fig6].

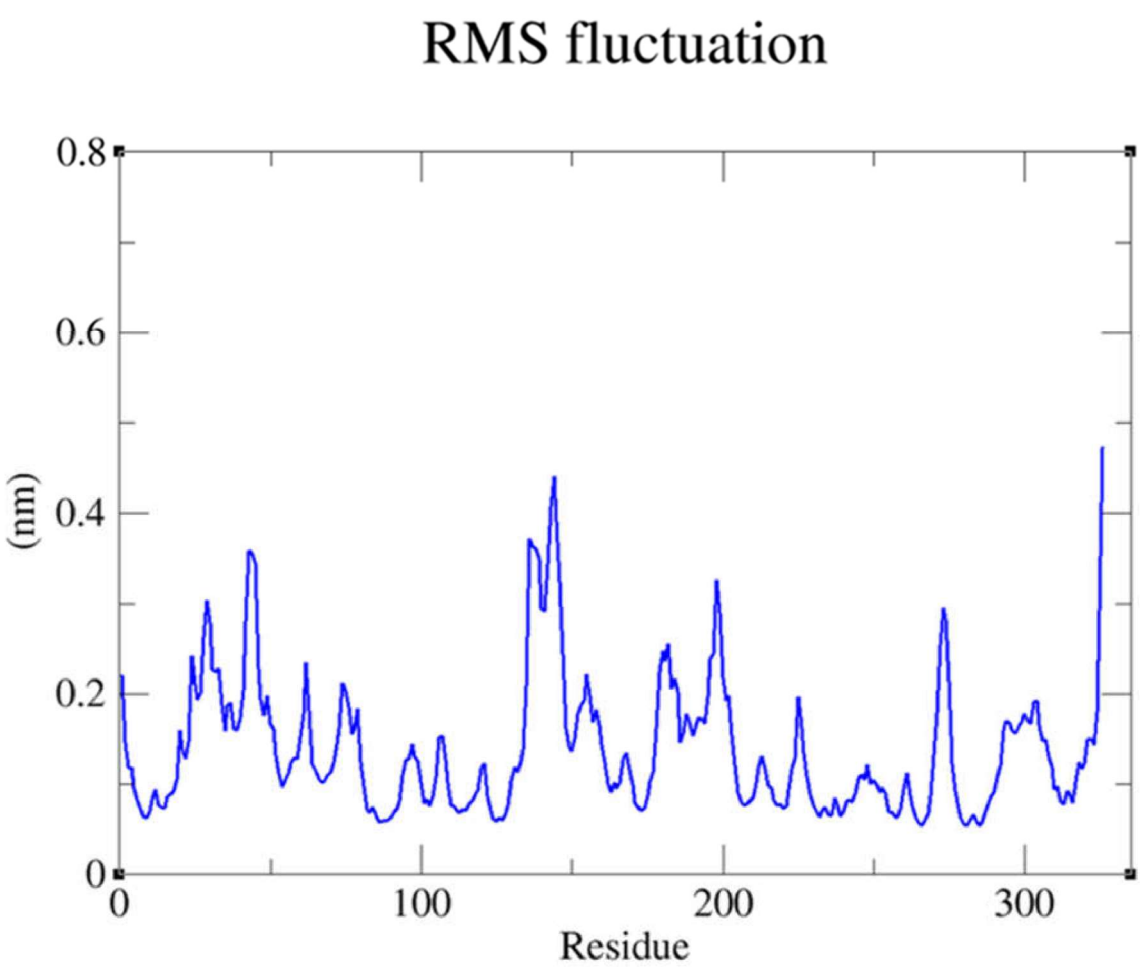

Figure 6 Root mean square fluctuation of Ajmalicine

Conclusion

Diaminopimelate epimerase (DapF) is an essential enzyme which catalyses a major reaction in meso-DAP biosynthesis and lysine biosynthetic pathway. Lysine cannot be synthesised by humans and thus is essential for human beings. Thus, inhibition of bacterial lysine biosynthesis will not have any effect on human beings. In this regard we have analysed the protein and ligands interaction with the help of computer-aided approach. Structural studies of *EfDapF* were performed, which showed the dominant alpha helical structure. Validation and evaluation of structural studies of *EfDapF* protein shows that predicted model is a stable structural model and of good quality because it shows maximum residues (81.9 %) in favoured region. The analysis of protein interaction provides useful information about *EfDapF* enzyme. This study was specifically conducted to study the binding of Ajmalicine to *EfDapF* to estimate the binding of with *EfDapF* and to analyse the types of interactions and the binding pocket of the

ligands on *EfDapF*. Ajmalicine is known to exhibit antibacterial potential, this work confirmed that ajmalicine molecules binds to *EfDapF* with significant interactions. Hence this study provides a new approach to treat *Enterococcus faecalis* infections.

Acknowledgement
None

CONFLICT OF INTEREST:

The authors declare no conflict of interest.

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