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Original Article

In Silico Analyzes for the Inhibition of HIV Protease by Ritonavir and Indinavir

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ABSTRACT

Introduction: This research was conducted to investigate the molecular interaction of HIV protease inhibitor drugs using molecular docking. HIV protease is responsible for processing gag and gag-polyproteins during virion maturation. The activity of this enzyme is essential against viral infections and has beneficial therapeutic effects on HIV treatment.
Materials and Methods: To meet the aim of the study, indinavir and ritonavir were selected as HIV Protease inhibitor drugs. The necessary information on molecular docking was collected through information servers, such as Drug bank and Program database (PDB). Then, molecular docking was performed using Molegro virtual docker software. In order to check the stability of the resulting complex structure and its cellular penetration, a molecular dynamics simulation was run for 50 nanoseconds using GROMACS2019.6 package and Amber99SB force force field. During the molecular dynamics simulation, root mean square deviations (RMSD), root mean square fluctuations (RMSF), the radius of gyration (RG), hydrogen bonds, and distance between ligands and complex were investigated.
Results: The obtained results indicated that the RMSD of the complex of the ligands and HIV protease at the end of 50 nanoseconds had a linear slope. Hydrogen bonds decreased at beginning of simulation but they increase at the end of simulation However RG was decreased at the end of the simulation Also the RMSF was decreased at the end of simulation rather than beginning of simulation, So all the obtained results showing the stability and strength of the structure.
Conclusion: Molecular docking method can indicate the relationship between structure-activity and the effectiveness of ligands on HIV protease based on the level of interaction between the ligands and the receptors.

1. Introduction

Acquired Immune Deficiency Syndrome (AIDS) is a type of disease that occurs when the immunodeficiency virus severely attacks the immune system. The disease caused by the HIV virus has three main stages. In the first stage (acute infection), the person may be experiencing a short influenza-like illness, which is not the same in all people. For this reason, the disease usually follows for an extended period without any symptoms, called the latent period of the disease[1]. The more the disease progresses, the more weakness is found in the body's immune system leading to infections, opportunistic cancers, and tumors although it is usually ineffective in people whose immune system is functioning well[2]. Finally, the disease will enter the third stage of AIDS when CD4 T cell count should be less than 200 cells per microliter. HIV is a virus from a group of retroviruses that attacks the body's immune system cells[3]. This virus was discovered by Frenchman Luc Montagnier and American Robert Galloway[4].

Indinavir is taken orally and its consumption with fatty foods reduces its bioavailability and maximum blood

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concentration, but when consumed with low-fat food, food has no effect on drug absorption. The protein binding of indinavir is 60%. The metabolism of indinavir is carried out by the liver and by cytochrome A4[3] enzymes and turns the drug into an inactive form[5].

Retroviruses are viruses whose genetic material or genome consists of RNA. AIDS viruses need an enzyme called reverse transcriptase to copy their RNA genome into DNA, so that it can be inserted into the genome of the host cell with the help of the integrase enzyme, and finally the virus can be replicated by the protease enzyme[6]. Therefore, the word retro-reverse in the name of this type of virus is quite justified as DNA is usually copied into RNA in cells. It causes AIDS by infecting a group of cells of the immune system called CD4+ T lymphocytes[7]. These cells are a subset of white blood cells that naturally regulate the immune response to infection. When the amount of CD4+ T cells in an HIV-infected person decreases to a certain extent, that person becomes susceptible to a range of diseases the body cannot typically control, and these are opportunistic infections that cause the person's death.

Six different classes of drugs are approved and used to treat HIV infection[8], including nucleoside inhibitors of reverse transcription, non-nucleoside inhibitors of reverse transcription, protease inhibitors, entry inhibitors (co-receptor antagonists), integrase inhibitors, and viral fusion inhibitors[9]. Currently, some protease inhibitors, namely indinavir, saquinavir, ritonavir, nelfinavir, amprenavir, fosamprenavir, lopinavir, atazanavir, tipranavir, and darunavir, have been licensed for clinical use. The increasing prevalence of this disease necessitates the discovery of drugs with greater activity and effectiveness. Since conducting experimental tests is time-consuming and costly; therefore, the development of theoretical methods to evaluate and predict the stability of the inhibition of drug compounds to protease receptors is of significant importance.

Ritonavir is a potent inhibitor of CYP3A4 and affects many transporters and enzymes. Its drug interactions are very common and should always be investigated[10, 11]. Currently, this drug is only used to strengthen other protease inhibitors. Ritonavir was initially considered as a therapeutic agent, but it showed very low tolerability in therapeutic doses. With the advent of computer science and calculations, traditional and random methods of drug design have been replaced by logical and computational methods[12], which can save time and money. Considering the overall process of drug development needs a lot of money and time, it is necessary to use any computing tool which can help to achieve successful results quickly[13]. When researchers implement computational drug design, they have to solve a range of problems, such as efficiency, activity, and toxicity. Molecular docking is probably the most widely used tool for computational drug design, which can solve the mentioned limitations. It is a reliable method that can predict molecules' inhibitory ability towards a specific protein[14,15]. Since docking calculations simulate the interaction between a compound and the active site of a protein, the results are similar to those obtained from biochemical investigations. One of the main reasons for using docking is to design the structure of compounds that bind well to the protein, and to observe the three-dimensional geometric structure of the compound attached to the active site of the protein[16,17]. Gromacs is a multi-purpose database software package used to run molecular dynamics simulations. This software was originally designed to simulate biochemical molecules, such as proteins, lipids, and nucleic acids that have complex bonding interactions. Nowadays, this software is also used to simulate biological systems, such as polymers. It can be installed and run in the Linux environment after downloading it from the Gromacs website. Performing a molecular dynamics simulation in Gromacs requires several steps[1]. With this in mind, the current study aimed to investigate the interaction between ritonavir and indinavir drugs with HIV protease using the molecular docking method.

2. Materials and Methods

2.1. Prediction of Physicochemical Characterization of ligands and Protein

Indinavir $C_{36}H_{47}N_5O_4$ and ritonavir $C_{37}H_{48}N_6O_5S_2$ were selected as inhibitors of the HIV Protease enzyme in the present study. The protein structure of the enzyme was extracted from the Protein Data Bank database (PDB= 1RPI). D and also Drug Bank database was used to check the two mentioned drugs and find the enzymes related to this drug and Mol format of these two ligands was downloaded. Also, to find the structure of these to drugs Zink database was used and. followed by spatial modeling. After examining and selecting the effective drugs for the enzyme, the swiss model online database was used to check the three-dimensional structure and modeling of drugs and enzymes. The FASTA enzyme format was downloaded from the NCBI online database, and the appropriate format was used to model the enzyme in the Swiss Model database[18,19] (Figure 1).

2.2. Investigation of protein-ligand interaction

Molecular docking was performed using Molegro Virtual Docker software to find the interaction of ritonavir, indinavir as ligands and HIV protease and the connection between enzyme and drug was investigated. This software can use biochemical and biophysical information obtained from laboratory methods to predict interaction. This

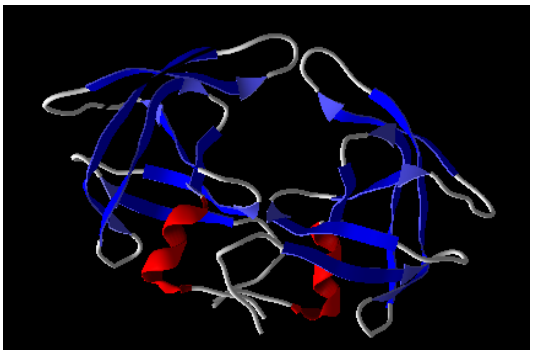


Figure 1. 3D structure of HIV protease enzyme by Swiss Model software

program performs ligand-protein docking in a completely flexible manner. So to run molecular docking, First, the PDB file of the enzyme was entered into the software, and the protein preparation steps were performed, then the desired ligands, consisting of ritonavir and indinavir drugs, were entered into the software, and the docking results were checked. The high negative binding energy (ΔG) leads to stronger ligand-protein binding.

This energy is the sum of electrostatic energies, Van der Waals. In this method, amino acids that more than 50% of their external surfaces are exposed to water are considered active amino acids. the RMSD analysis of protein and ligands provides useful information about their structural changes during docking by this software. RMSD is a quantity commonly used to compare two configurations. This quantity is calculated for all atoms of a molecule at a certain time, relative to a reference state, which is usually the primary structure using the following equation:

$$\mathrm{RMSD(t)} = [\frac{1}{M}\sum_{i=1}^{N} m_i|r_i(t) - r_i^{ref}|^2]^{1/2}$$

2.3. The binding affinity of HIV protease by Indinavir and ritonavir

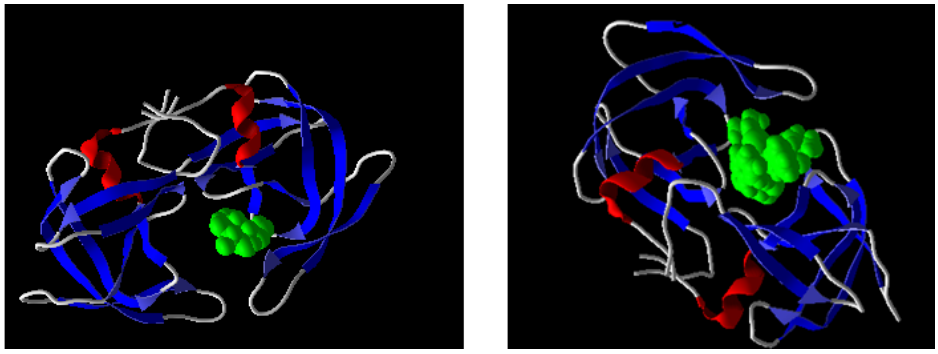
In this study, molecular dynamics simulation calculations were performed for HIV protease protein , ritonavir and indinavir as ligands by using the GROMACS2019.6 software package and the Amber99SB force field .The molecular dynamics simulation of a protein or nucleic acid results in the behavior of the macromolecule or system during a specific and desired time, which is obtained using Newton's equations of motion and potential energy functions. The simulation output is a series of structures created during the simulation time, which presents the process of changes in the structure reflecting dynamic changes in the system or molecule. Most of the molecular dynamic simulations were performed in the canonical ensemble (constant number of atoms, volume, and temperature). Recently, the simulations have been conducted under the conditions of a constant number of atoms, pressure, and temperature because simulation results are more consistent with the laboratory conditions under these conditions, and the real behavior of the system can be observed.

In the molecular dynamic's simulation, the forces that atoms bring to each other are estimated using a force field model. Then the movement of atoms is simulated based on the laws of classical Newtonian physics. This simulation method provides many details of molecular events, but data analysis raises new challenges. Studying the proteins' mechanism of action requires mathematical methods that correctly show the correlations and coordination of the movement of atoms. Molecular dynamics simulation is one of the most useful and effective methods in identifying the structure and function of enzymes; therefore, a researcher can design a more targeted and accurate drug due to dynamics and functional movements in the enzyme. In this study MD simulations were utilized using GROMACS2019.6 and Amber99SB force force fields to assess the conformational changes of protein complex. Pressure and temperature were kept at 1 bar and 300 K, and the system ran for 50 nanoseconds. After conducting a MD simulation, structural stability and molecular penetration per time unit can be evaluated. For this purpose, root mean square deviation (RMSD), root mean square fluctuations (RMSF), and gyration radius (GR) were extracted from the relevant file and the results were observed and analyzed using Pymol and VMD software, and the obtained data curve was drawn using GRACE software.

3. Results

3.1. Results of Molecular docking of these two ligands and HIV protease

Molegro virtual Docker software was used to check the correct binding of the protein-ligand in question and after molecular docking, the best conformation with the lowest binding energy was selected as the result. So, after molecular docking, the best conformation with the lowest binding energy was selected as the result. The aim of this project was to investigate interactions between these two ligands and HIV protease. So, molecular docking of HIV protease-ritonavir complex was determined and the best cluster had a score of -156.126 and also HIV protease- indinavir complex was -160.58, with a size of 29 complexes. In addition, Z-score was equal to -2.1 and -2.8 respectively. The image of the complex of HIV protease- ritonavir and HIV protease-indinavir has been shown in Figure 2 A and B. So Molecular



A B

Figure 2. Final result of molecular docking of complex of ritonavir and indinavir as ligand and HIV protease by Molegro virtual Docker. A) Complex of ritonavir and HIV protease B) Complex of indinavir and HIV protease

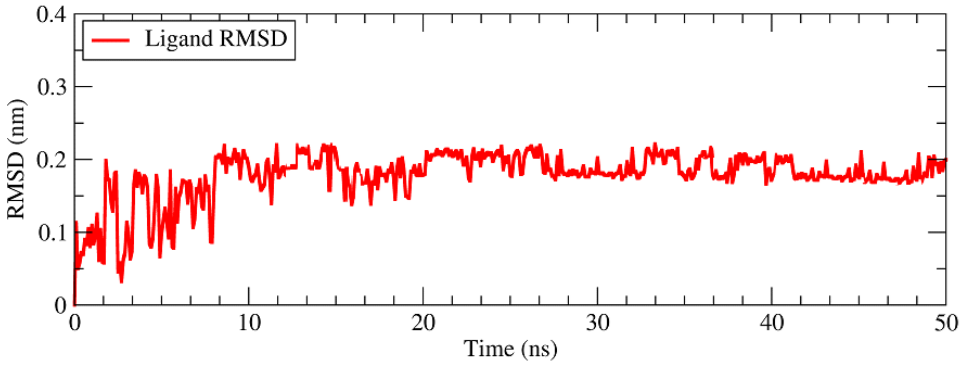


Figure 3. RMSD changes of indinavir ligand per time unit. RMSD has an almost linear slope at the end of 50 nanoseconds

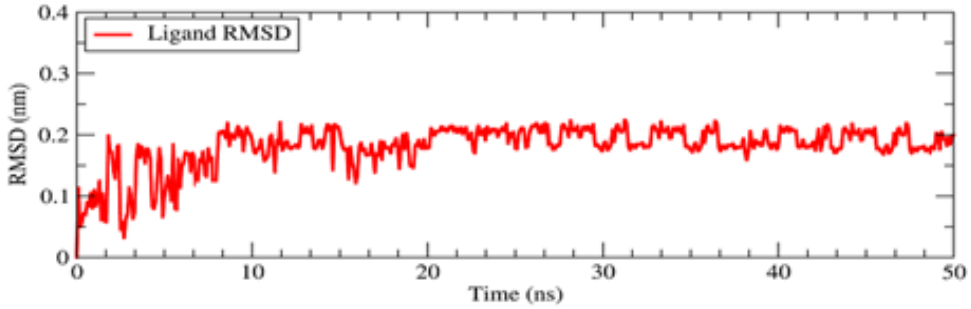


Figure 4. RMSD changes of ritonavir ligand per unit of RMSD time has an almost linear slope at the end of 50 nanoseconds

docking result show that the stability of these two ligands to HIV protease are so similarity and both of them can be used to treat HIV.

3.2. The results of molecular dynamics simulation

During molecular dynamics simulation, structure stability of these two ligand and enzyme was evaluated per unit of time for this purpose, ligands and complex distance, RMSD, RMSF, GR, and hydrogen bonds, were evaluated, and the results were prepared as a graph. The RMSD between the structures created during the molecular dynamic simulation process was a suitable and conventional measure to ensure the stability of the protein structure. So, the slope of the RMSD diagram indicated the stability of the model during the simulation. Figures 3 and 4 show The RMSD of these two ligands in the unit of time and figure 5 shows RMSD of HIV protease. At the end of the simulation, RMSD fluctuations decreased and indicated a linear slope. So, it showed that these two ligands had stability structures and they can be used as drugs to inhibit HIV protease.

As can be seen in figure 6 and 7, The RMSD of complex of indinavir- HIV protease and also indinavir- HIV protease at the beginning of the simulation, was less than 0.25, which increased over time with an upward slope and reached its maximum at 1.25 nm in 43-44 nanoseconds, then decreased slightly in 50 nanoseconds. It reached almost 1 nm and had a linear slope until the end of the simulation.so these results show that these two ligands have stable linkage to HIV protease and they can use as suitable drugs to inhibit the function of HIV protease.

To investigate the dynamic behavior of HIV Protease protein, the RMSF of its amino acids was studied during the simulation time. According to Figure 6, the presence of

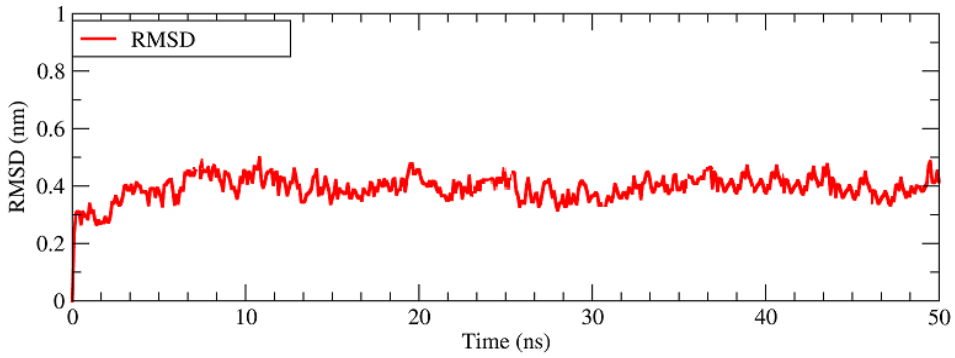


Figure 5. RMSD changes of HIV protease per unit of RMSD time has an almost linear slope at the end of 50 nanoseconds

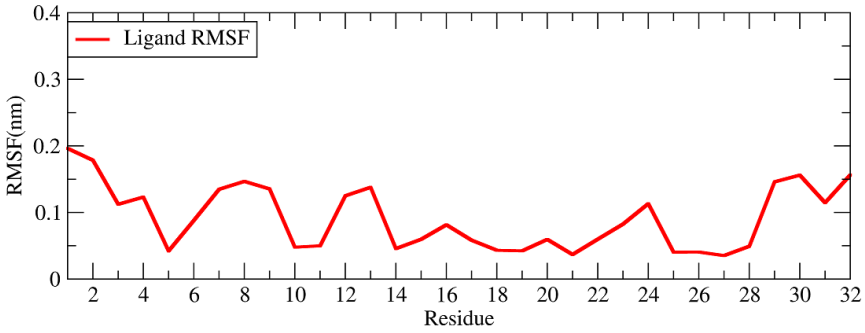


Figure 6. The RMSF of its amino acids was studied during the simulation time. presence of ritonavir compound next to HIV protease protein, has reduced the amount of fluctuations of protein amino acids.

ritonavir compound next to HIV protease protein, has reduced the amount of fluctuations of protein amino acids. As can be seen in figure 7, in the last 50 nanoseconds of the simulation, the average amount of RMSF of HIV protease protein has decreased in the presence of both ligands compared to the state of the protein alone.

Also, to further investigate the stability of the protein, the number of changes in hydrogen bonds per unit of time was measured. As can be seen in Figure 7 at the beginning of the simulation, the number of bonds or bonds was less, but at the end, the simulation value increased. Since it is impossible to determine the relative distance of each atom to the center of the protein mass, the gyration factor was used as a suitable measure for folding or unfolding of proteins. The smaller the radius, the smaller the radius. The system is more spherical and more folded, and the bigger the radius, the more open the system is and it turns from fold to unfold (Figure 8).

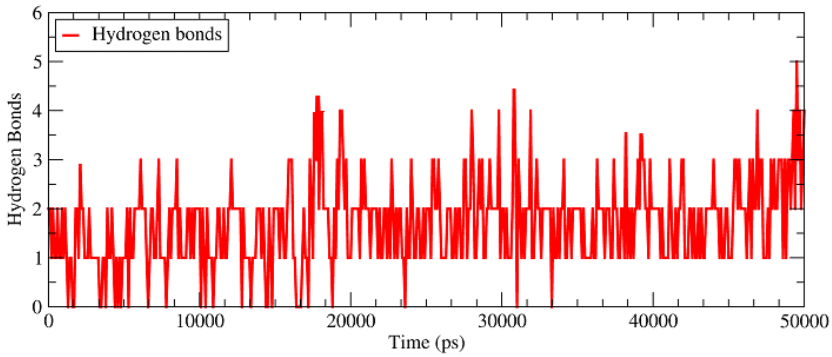


Figure 7. Changes of hydrogen bonds per unit of time. Hydrogen bonds of HIV Protease – ritonavir complex at the beginning of the simulation, the number of links or bands was less, but at the end of the simulation, the value increased, which also indicates the stability of the structure

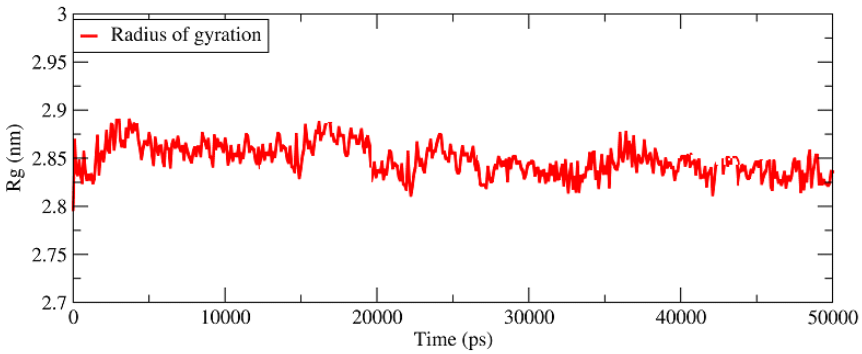


Figure 8. Radius of gyration changes per unit of time. The RG result of HIV protease and ritonavir was larger at the beginning of the simulation but decreased at the end of the simulation, indicating the bond strength.

4. Discussion

In this research, the effect of ritonavir and indinavir, which are important drugs to inhibite effect of HIV protease enzyme to cure HIV disease. So, this study conducted on ritonavir and indinavir as important drugs using molecular docking to investigate the intraction of these two drugs on HIV protease and find the stability of this complex by Molecular dynamics simulation by Gromcs software. The RMSD between the structures during the molecular dynamic's simulation process was suitable and conventional, confirming the stability of the protein

structure. The slope of the RMSD diagram expressed the stability of the model during the simulation. The slope closer to zero indicates the higher stablilty of the model10. The findings of the current study indicated that RMSD of ligand and protein had almost a linear slope, indicating the stability of protein and receptor, and was consistent with previous published results[20, 21].

In order to further investigate the stability of the protein, the number of changes in hydrogen bonds per unit of time was used. In this case, a larger number of bonds indicates a higher level of structure stability[20, 21].

In proteins, it is not easily possible to determine the relative distance of each atom to the center of the protein mass. Therefore, RG factor could be used as a suitable criterion for folding or unfolding proteins. The smaller RG reflects a more spherical and folded system. On the other hand, when the RG is larger, the system is more open and turns folded proteins into unfolded ones. The obtained results showed that the RG decreased at the end of the simulation, confirming the strength of the bond. This finding is consistent with[21-23].

The RMSD is not a suitable parameter to reflect the mobility of structural elements. The root mean square of RMSF fluctuations is used to check the flexibility of the structure. In the RMSF chart, averaging is done over the total time for each particle. The obtained results indicated the flexibility was slightly reduced, compared to the beginning of the simulation[22,23]. The approach of the ligands to the receptors indicated the correct connection and the stable bond. The obtained results showed that the distance between the ligands and the receptors is decreasing.

5. Conclusion

This experiment confirmed that these two ligands can be used as drugs to inhibit HIV protease function to treat HIV disease. Also, in silico analysis is useful methods to investigate the interaction of drugs and their receptors. So, in this project, by using the molecular docking r method, we have achieved the understanding of the structure-activity relationship of the ligands effective on HIV Protease based on the level of interaction between the ligand and the receptor. Also, the dynamic behavior and structural of HIV protease were analyzed by MD simulation in the presence and absence of these two ligands during the simulation time which was considered 50 nanoseconds. For this purpose, quantities such as RMSD, RMSF, RG, hydrogen bonds, second structure and binding free energy were investigated. Examining the results of the measurement and analysis of these quantities shows that the complex of HIV protease and these two ligands are more stable. Therefore, it can be concluded that these two drugs which approved and used to treat HIV infection have protease inhibitors and they can link strongly to HIV protease. So, in silico analysis can be use as preclinical treatment and discover drugs with great activity and effectiveness. Since conducting experimental tests is time-consuming and costly; therefore, the development of theoretical methods to evaluate and predict the stability of the inhibition of drug compounds to protease receptors is of significant importance

Declarations

Competing interests

The authors have no conflicts of interest to declare.

Authors' contribution

All authors discussed the results and contributed to the final manuscript.

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Availability of data and materials

All data generated or analysed during this study are included in this published article.

Ethical considerations

The authors checked for plagiarism and consented to the publishing of the article. The authors have also checked the article for data fabrication, double publication, and redundancy.

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