Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

Elsevier hereby grants permission to make all its COVID-19-related research that is available on the COVID-19 resource centre - including this research content - immediately available in PubMed Central and other publicly funded repositories, such as the WHO COVID database with rights for unrestricted research re-use and analyses in any form or by any means with acknowledgement of the original source. These permissions are granted for free by Elsevier for as long as the COVID-19 resource centre remains active.

Saudi Journal of Biological Sciences 29 (2022) 3140–3150

Contents lists available at ScienceDirect

Saudi Journal of Biological Sciences

journal homepage: www.sciencedirect.com

Original article

# Assessing the therapeutic potential of agomelatine, ramelteon, and melatonin against SARS-CoV-2

Pradeep Kumar Yadalam [a], Thodur Madapusi Balaji [b], Saranya Varadarajan [c], Khalid J. Alzahrani [d], Mohammad S. Al-Ghamdi [d], Hosam Ali Baeshen [e], Mohammed Farhan A. Alfarhan [f], Zohaib Khurshid [g], Shilpa Bhandi [h], Raghunathan Jagannathan [i], Vikrant R. Patil [j], A. Thirumal Raj [k], Jithendra Ratnayake [l], Shankargouda Patil [m,*]

[a] Department of Periodontics, Saveetha Dental College and Hospital, Saveetha Institute of Medical and Technical Sciences, Saveetha University, Chennai, India
[b] Department of Periodontology, Tagore Dental College and Hospital, Chennai, India
[c] Department of Oral Pathology and Microbiology, Sri Venkateswara Dental College and Hospital, Chennai, India
[d] Department of Clinical Laboratories Sciences,College of Applied Medical Sciences, Taif University, Taif, Saudi Arabia
[e] Department of Orthodontics, Faculty of Dentistry, King Abdulaziz University, Jeddah, Saudi Arabia
[f] Department of Surgery, College of Medicine, King Faisal University, Al-Ahsa, Saudi Arabia
[g] Department of Prosthodontics and Dental Implantology, College of Dentistry, King Faisal University, Al-Ahsa, Saudi Arabia
[h] Department of Restorative Dental Science, College of Dentistry, Jazan, University, Saudi Arabia
[i] Department of Periodontology, Tagore Dental College and Hospital, Chennai 600127, India
[j] Biogenre Private Limited, Pune, India
[k] Department of Oral Pathology and Microbiology, Sri Venkateswara Dental College and Hospital, Chennai, India
[l] Department of Oral Science, Faculty of Dentistry, University of Otago, 310 Great King Street, Dunedin 9016, New Zealand
[m] Department of Maxillofacial Surgery and Diagnostic Sciences, Division of Oral Pathology, College of Dentistry, Jazan University, Jazan, Saudi Arabia

ARTICLE INFO

*Article history:*
Received 19 December 2021
Revised 12 January 2022
Accepted 20 January 2022
Available online 25 January 2022

*Keywords:*
ACE 2
In silico
Melatonin
SARS-CoV-2
Spike protein

ABSTRACT

*Background:* The SARS-Cov-2(severe acute respiratory syndrome coronavirus 2) infection affecting human populations worldwide is now a very concerning issue considering the morbidity and mortality rates. Despite several measures followed by the medical fraternity and general public, there is no resolution. Therapeutic measures to tackle the infection have been based on researching new designer drug molecules that could prevent viral entry into the human host. Melatonin has been tried as an adjuvant in the management of COVID 19(coronavirus disease) illness but its specific antiviral role has not been investigated. Objectives: The objectives of the present study were to conduct an in-silico analysis to investigate if melatonin and related drugs namely ramelteon and agomelatine could be used as antiviral agents in SARS-CoV-2 infection based on their binding to the SARS-CoV-2 receptor binding site (RBD) and Angiotensin-converting enzyme 2 (ACE 2).

*Methods:* For docking studies (Pdb Id 1M0J), the SARS-CoV-2 spike protein receptor-binding domain (RBD) crystal structure which was ACE2 cell receptor bounded was employed. From the PubChem database, the three-dimensional configuration of the ligands melatonin, ramelteon, and agomelatine was retrieved, and conceptual density functional theory (CDFT) was performed to determine molecular descriptors. Charges were added and optimized with the universal force field to prepare the ligands for the process of docking. For facilitation of readability by the AutoDock software conversion to PDBQT(Protein Data Bank, Partial Charge (Q), & Atom Type (T)) format was performed. AutoDock version 4.2.6 docking program and AutoDock Tools (ADT) version 1.5.6 were used for molecular docking. Desmond, a Package of Schrödinger LLC was used to simulate molecular dynamics for hundred nanoseconds using.

* Corresponding author.
*E-mail address:* dr.ravipatil@gmail.com (S. Patil).

Peer review under responsibility of King Saud University.

Production and hosting by Elsevier

https://doi.org/10.1016/j.sjbs.2022.01.049
1319-562X/© 2022 The Author(s). Published by Elsevier B.V. on behalf of King Saud University.
This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

*Results:* Data from the present study reveal that melatonin, ramelteon, and agomelatine demonstrate significant binding with SARS-CoV-2 RBD and ACE 2 demonstrating the fact that they can strongly prevent viral entry into the host cells through dual binding effects. However, Ramelteon was found to be the most superior amongst the 3 drugs analyzed in its antiviral properties against SARS-CoV-2.
*Conclusion:* Results advocate further research in exploring the potential therapeutic applications of melatonin, ramelteon, and agomelatine for the management of SARS-CoV-2 infection.
© 2022 The Author(s). Published by Elsevier B.V. on behalf of King Saud University. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

## 1. Introduction

SARS-Cov-2 virus detrimental effects are well documented all over the globe (Khurshid et al., 2020; Warsi et al., 2021), which in turn is validated by WHO(World Health Organization) designating COVID-19 as a pandemic (Mohan and Vinod, 2020). Currently, there is a second wave of viral infection in many continents and mutant strains of the virus have been implicated in causing this condition and are blamed for disease recurrence in already afflicted individuals (Pedro et al., 2020). The main factor that leads to fatality following infection is acute dyspnoea and pneumonia (Li and Ma, 2020). Another important phenomenon that results in significant morbidity and mortality is the cytokine storm which implies excessive production of proinflammatory cytokines following infection (Tang et al., 2020). Thus, the scientific community is in an uphill battle investigating novel therapeutic modalities.

The advent of vaccines against SARS-Cov-2 is well known and it is to be reiterated that a vaccination drive and campaign is operating globally to vaccinate humans to prevent severe disease (Mathieu et al., 2021). Inhibiting host viral entry is another major strategy being explored. A major viral entry portal is the oral cavity (Sakaguchi et al., 2020). Host entry is accomplished when the viral spike glycoprotein attaches to the host cell angiotensin-converting enzyme 2 receptors (ACE 2) (Li et al., 2003) (Lan et al., 2020). The expression of ACE 2 has been documented in the oral cavity and hence the oral tissues are vulnerable to viral attack and entry (Xu et al., 2020). At this point, it is to be emphasized that a plethora of designer drugs are being developed by bioinformatic methods to bind with ACE 2 and viruses receptor-binding domain (RBD) which could deny viral entry. The drawbacks of inventing new drug molecules for this purpose are mainly based on the fact that clinical tangibility remains a question. Moreover, given the time restraint, investigating natural host-derived molecules which have been approved for interventions not specific to COVID-19, could be explored. Investigation focusing on the binding of these natural molecules to ACE 2 and the viral RBD could potentially prevent viral entry without inducing any significant adverse effects.

It is in this situation that we propose melatonin as a molecule that could prevent viral entry by binding to ACE 2 as well as the viral RBD. Melatonin (N-Acetyl-5-methoxytryptamine) is an indoleamine produced mainly by the stationary cells of the pineal gland during the night (Reiter, 1991). Tissues other than the pineal gland are also endowed with the capacity for melatonin biosynthesis, one such being the oral cavity. The biosynthesis of melatonin and the presence of melatonin receptors have been documented in the gingiva (Madapusi and Rao, 2018) and salivary glands (Shimozuma et al., 2011). It is hence understandable that melatonin and ACE 2 are simultaneously expressed in the oral tissues. It is already known that melatonin has an intimate connection with the Renin-Angiotensin system of which ACE 2 is a member (Burrell et al., 2004). Melatonin has already been documented to possess antiviral properties against viruses other than SARS-Cov-2 such as porcine epidemic diarrhea virus (PEDV), Transmissible gastroenteritis virus (TGEV) and porcine delta coronavirus (PDCoV) (Zhai et al., 2021). Melatonin has already been suggested and implemented in SARS-Cov-2 as an adjunctive measure due to its antioxidant and anti-inflammatory properties (Sehirli et al., 2020; Zhang et al., 2020). There is also preliminary bioinformatic data available concerning melatonin as a SARS-Cov-2 viral inhibitor (Feitosa et al., 2020). But the binding of melatonin to ACE 2 and the viral RBD has not been researched. Hence, we propose a preliminary in silico model which has identified the binding of melatonin to ACE 2 and the viral RBD which could prevent SARS-Cov-2 binding to the ACE 2 and could consequently inhibit viral entry. In addition to melatonin, we have also applied the in silico analysis to ramelteon (Zammit, 2007) and Agomelatine (Kennedy and Eisfeld, 2007) which are drugs related to melatonin. Our primary pursuit was to investigate if melatonin and related drugs could also be used in the management of the SARS-Cov-2 pandemic.

## 2. Materials and methods

### 2.1. Preparation of the protein and ligands:

For docking studies (Pdb Id 1M0J), the SARS-CoV-2 spike protein RBD crystal structure which was ACE2 cell receptor bounded was employed. Processing of proteins was carried out by removing specific ligands and was prepared for docking using the PyRx virtual screening software. Processing of the protein to the PDBQT file format was done so that AutoDock software could detect and read the processed protein. The ligands used in this study were melatonin, ramelteon, and agomelatine. Extraction of 3-D configuration of the ligands from the PubChem database was performed. Charges were added and optimized with the universal force field to prepare the ligands for the process of docking. For facilitation of readability by the AutoDock software conversion to PDBQT format was performed Fig. 1 represents the two-dimensional structure of the ligands.

### 2.2. Quantum chemical Descriptor's calculations using conceptual DFT

Conceptual DFT (CDFT) (conceptual density functional theory) protocol was used (Geerlings and De Proft, 2008). It derives its working principles from the Hohenberg–Kohn theorem. Calculation of around ten types of molecular descriptors that represent the activity of the components at a molecular level was carried out for the three ligands being studied namely melatonin, ramelteon, and agomelatine. They are the highest occupied molecular orbital (HOMO), total energy, absolute hardness ($\eta$), the energy gap between LUMO(lowest unoccupied molecular orbital)and HOMO ($\Delta E$) of the ligands, chemical potential ($\mu$), softness ($\sigma$), molecular dipole moment, electronegativity ($\chi$), lowest unoccupied molecular orbital (LUMO), and electrophilicity index ($\omega$). Molecular structure–activity associations can be understood through these descriptors. The chemical structure of the drug molecules used in this study is shown in Fig. 1. Table 1 depicts the above-defined Quantum chemical descriptors for the assessed ligands (melatonin, ramelteon, and agomelatine).

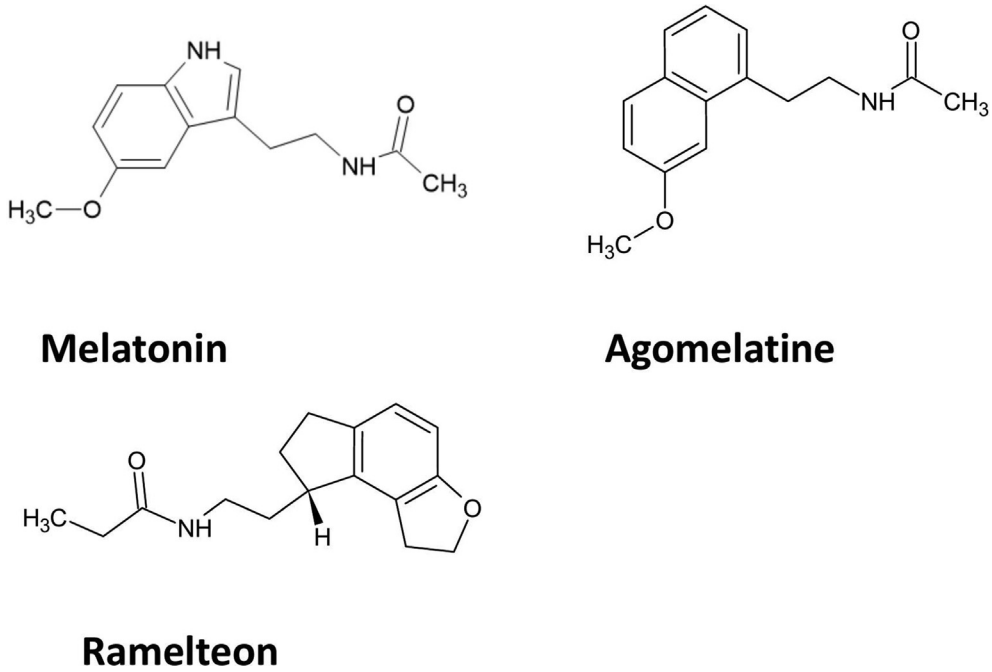


**Fig. 1.** Structure of drug molecules used in this study.

### 2.3. Molecular docking

AutoDock version 4.2.6 docking program and AutoDock Tools (ADT) version 1.5.6 were used for molecular docking (Humphrey et al., 1996). The calculation for docking was carried out using the Gasteiger charges. Molecular docking of ACE2 and RBD domain of SARS-CoV-2 with the drug molecules Melatonin, Ramelteon, and Agomelatine was executed. UCSF (University of California, San Francisco) Chimera program (version 1.15) was used for the minimization of the obtained best pose. For the drug molecules and the protein minimalization, the GAFF force field and Amber14SB force field were employed. VMD (visual molecular dynamics) program was employed for both the visualization of the binding mode and its analysis. In addition, the VMD program was also used for analyzing the interactions of the obtained poses binding pocket. Analysis and further interpretation were carried out.

### 2.4. Molecular dynamics simulation

Desmond, a Package of Schrödinger LLC was used to simulate the molecular dynamics for a duration of 100 ns (23). Docking studies provided data on the primitive stage of the complex between the ligand and protein. The status of binding of ligands during stationary conditions can be predicted with molecular docking. Determination of the status of ligand binding at physiologic conditions was assessed by carrying out simulations. Protein Preparation Wizard or Maestro was used for optimizing, preprocessing, and minimizing complexes. of complexes. Preparation of the entire system was done by the System Builder tool. For TIP3P (Transferable Intermolecular Interaction Potential 3 Points), the orthorhombic box Solvent Model was selected. The simulation was done with the help of the OPLS (Optimized Potentials for Liquid Simulations)_2005 force field. The addition of counter ions was done wherever needed for the neutralization of the models. The addition of 0.15 M sodium chloride (NaCl) was done to replicate the physiological conditions. For simulation selection of the NPT ensemble with 300 K temperature and 1 atm pressure was done. Before the simulation, relaxation of the models was carried out. Following 100 ps for analysis, trajectories were saved. Periodic calculation of root means square deviation (RMSD) of the protein and ligand was executed to evaluate simulation stability.

Analysis of the desmond simulation trajectories was performed. Analysis of MD trajectory provided data for calculation of root mean square fluctuation (RMSF), protein–ligand contacts, and root mean square deviation (RMSD).

## 3. Results

### 3.1. Conceptual DFT data interpretation

Gaussian 09 (Frisch et al., 2009) with B3LYP (Becke, 3-parameter, Lee–Yang–Parr) function (Becke, 1993) and SVP (Split valence polarization) basis set was used to optimize the ligands and calculate component descriptors. Fukui's theory was used as a basis to compute molecular orbital energy (Fukui, 1982). Table 1 provides the statistical details of the DFT-based molecular descriptors. The present study revealed larger $\Delta E$ values attributed

**Table 1**
Quantum chemical descriptors for the studied Ligands.

| Compound | Total Energy (Hartree) | Dipole Moment (debye) | $E_{HOMO}$ (eV) | $E_{LUMO}$ (eV) | H-L Gap ($\Delta$E) | Hardness ($\eta$) | Softness ($\sigma$) | Electro-negativity (X) | Chemical Potential ($\mu$) | Electro-philicity ($\omega$) |
|---|---|---|---|---|---|---|---|---|---|---|
| **Melatonin** | −764.432 | 3.16 | −5.31 | −0.45 | 4.86 | 2.43 | 1.21 | 2.88 | −2.88 | 10.09 |
| **Agomelatine** | −786.485 | 5.20 | −5.71 | −1.30 | 4.41 | 2.20 | 1.10 | 3.50 | −3.50 | 13.52 |
| **Ramelteon** | −826.979 | 4.69 | −5.40 | −0.10 | 5.30 | 2.65 | 1.33 | 2.75 | −2.75 | 10.05 |

to the Ramelteon ligand and the lowest $\Delta E$ to the Agomelatine ligand. It has been documented that the lesser the energy difference, the more the molecular activity, which could be corroborated with the molecular shift from HOMO to the LUMO state. The ligand that had the highest HOMO energy is the ligand Agomelatine ($E_{HOMO}$ = −5.71 eV). This higher energy allows it to be the best electron donor among the three studied ligands. Similarly, the ligand that had the lowest LUMO energy is the ligand Ramelteon ($E_{LUMO}$ = -0.10 eV) which signifies that it could be the best electron acceptor. The electron density charts of the three molecules representing the density of electrons in the HOMO and LUMO state are presented in Fig. 2.

Concerning dipole moment, Agomelatine recorded the highest of 5.198 debyes, followed by Ramelteon and melatonin with 4.695 debyes and 3.160 debyes respectively (Data from Table 1). Ramelteon had the highest value of chemical hardness, thus it is more reactive among the three studied ligands. Ramelteon and melatonin also had the lowest electronegativity index (2.75 and 2.88 respectively).

### 3.2. Inferences from molecular docking

Molecular docking could help to determine the best intermolecular interaction between macromolecules and drug molecules. Docking was carried out in the present study using the RBD domain of SARS-CoV-2 and ACE2. Table 2 shows binding energies obtained after molecular docking studies for best-docked conformations.

It can be seen that among the docked drug molecules, melatonin, ramelteon, and agomelatine demonstrated comparable and significant binding with the RBD domain of SARS-CoV-2 and ACE2. Ramelteon had the lowest binding energy, which meant higher interaction energy and stability for both SARS-CoV-2 as well with ACE2 protein Fig. 3 demonstrates the docking of all the 3 chosen ligands in ACE2. It can be observed that all the ligands are bound to ACE 2 at the same location (Fig. 3A and B). Fig. 3C and D demonstrate the interaction between Ramelteon with ACE2, respectively. Fig. 4 shows the interaction between the studied ligands with SARS-CoV-2 RBD Fig. 4A and B shows the interaction of Ramelteon with SARS-CoV-2.

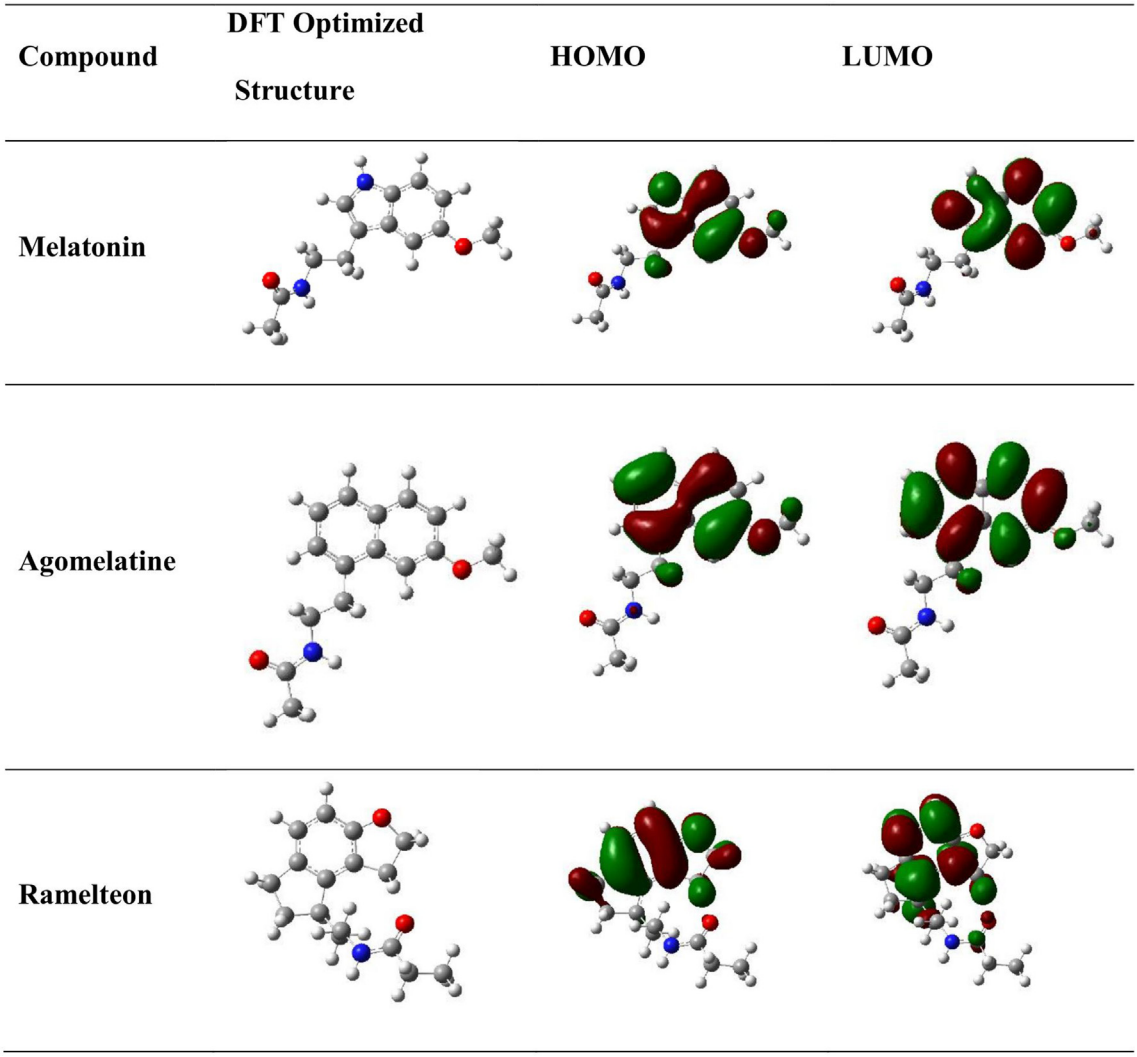


**Fig. 2.** Electron density maps of the HOMO and LUMO of three ligands under study.

**Table 2**
Binding energy (kcal/mol) for docked complexes.

| Drug/Protein | SARS-CoV-2 RBD | ACE2 |
|---|---|---|
| Melatonin | −5.37 | −5.69 |
| Agomelatine | −5.81 | −5.92 |
| Ramelteon | −5.83 | −6.50 |

### 3.3. Inferences from molecular dynamics simulation

Figs. 5 and 6 show the emergence of RMSD scores with time for the C-alpha atoms. The RMSD plot of the Melatonin_cov2 complex shows stability at twenty nanoseconds and the RMSD graph of the Ramelton-cov2 complex shows stability attained at five nanoseconds. A mean RMSD value of 1.5 Å for (Melatonin_cov2 protein) and 0.5 Å for (Ramelton-cov2 protein) lasts for hundred nanoseconds. During simulation, an acceptable level of variation of RMSD values of 0.5 Å remained that indicates ideal ligand receptor-ligand binding during simulation. At forty nanoseconds a sudden rise of a sudden increase of Ramelton-cov2 ligand to twelve was observed indicating an alteration of the mode of ligand binding. Figs. 7 & 8 depicts the RMSF value of the ligand-bound to protein. The residues depicting greater peaks represent the loop regions, recognized from MD(Molecular dynamics) trajectories (Figs. 9 and 10), or N and C-terminal zones. Low RMSF values of residues of the site of binding site residues show ligand-receptor binding stability.

Results of MD shed light on the fact that hydrogen bonds and hydrophobic interactions are the predominant ligand-receptor interaction as shown in Figs. 11 and 12. GLU_471, ILE_472 and GLN_474 are significant considering H-bonds for (Melatonin_-cov2). TYR_453 and GLY_502 are significant considering of H-bonds for (Ramelton-cov2). Some residues made significant strong hydrophobic interactions (TYR_473 for Ramelton-cov2 and ARG_103, PHE_456 and TYR_505 for Ramelton-cov2). Normalization of bar graphs during the trajectory course was done.

## 4. Discussion

The present study aimed to investigate if infection from SARS-CoV-2 could be treated by melatonin and related drugs ramelteon and agomelatine. The reason behind this objective was the fact that several naturally derived molecules are being investigated as potential modalities against the virus. Moreover, there is enough evidence that melatonin is a ubiquitous and evolutionarily conserved molecule found in almost all living beings (Cipolla-Neto and Amaral, 2018). Melatonin has been endowed with a spectrum of properties such as antioxidant (Reiter et al., 2016), anti-inflammatory (Nabavi et al., 2019), immunomodulatory (Giannoulia-Karantana et al., 2007), anti-cancer (Di Bella et al., 2013), and bone sparing (Maria and Witt-Enderby, 2014). Melatonin has already been proposed and implemented in clinical trials

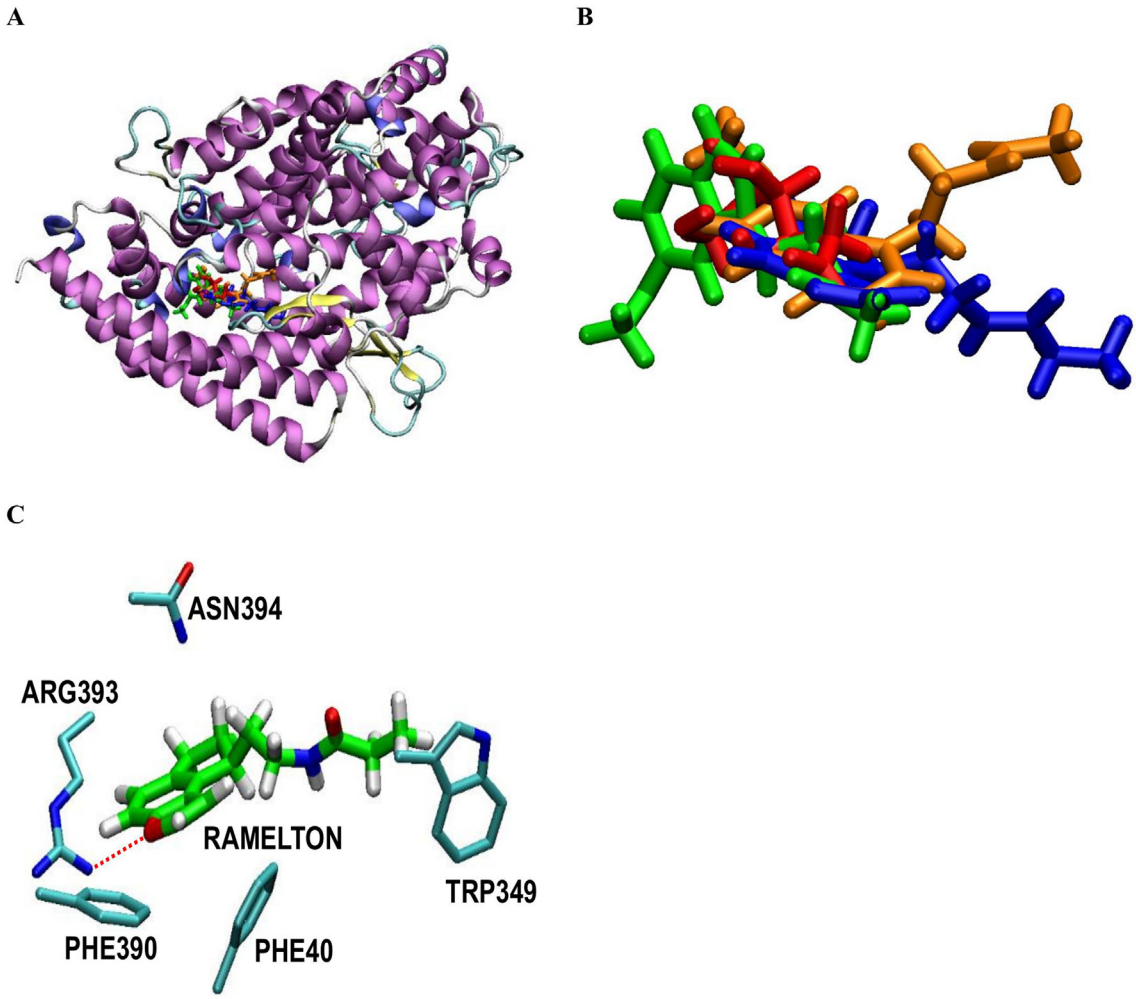


**Fig. 3.** A) Docked conformation of Melatonin (green), Agomelatine (orange), Ramelteon (blue) in ACE2. B) Only ligands are shown. C) Docked conformation of Ramelton in ACE2. Hydrogen bonds are shown with red dotted lines. For clarity, only sidechains are shown (except if the backbone forms a hydrogen bond with ligand).

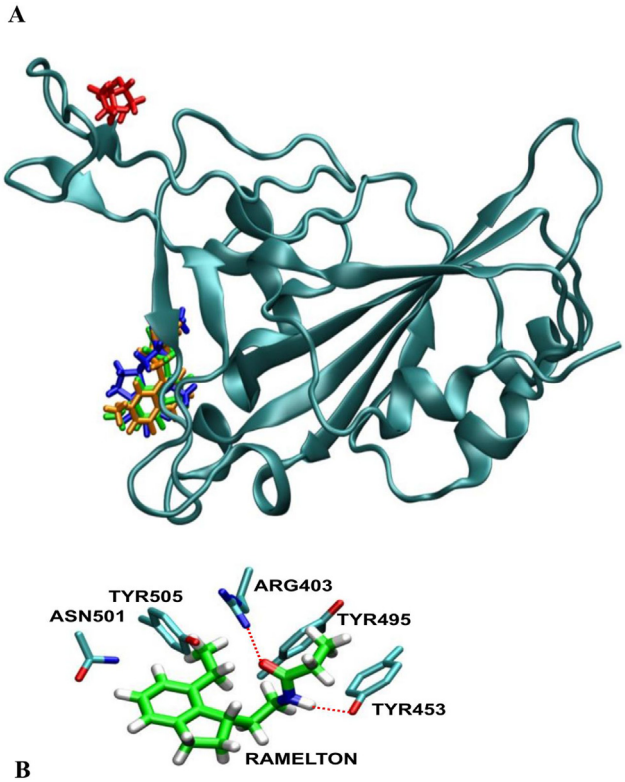


**Fig. 4.** A) Docked conformation of Melatonin (green), Agomelatine (orange), Ramelteon (blue), and 2-DG (red) in SARS-CoV-2 RBD. B) Docked conformation of Ramelton in SARS-CoV-2 RBD. Hydrogen bonds are shown with red dotted lines. For clarity, only sidechains are shown (except if the backbone forms a hydrogen bond with ligand).

as an adjuvant in the management of COVI 19 illness based on its antioxidant and anti-inflammatory properties (Sehirli et al., 2020; Zhang et al., 2020). However, its direct inhibitory effects on SARS-CoV-2 viral entry into the human host have not been well explored.

It is with this background that we went on to perform conceptual DFT, molecular docking, and Molecular dynamics simulation. The simulation aimed to assess the potential role of melatonin and its receptor agonists namely ramelteon and agomelatine in inhibiting the entry of the virus into the host cells. SARS-CoV-2 utilizes its spike glycoprotein which has a homotrimeric structure to enter into the host cells. This spike glycoprotein RBD interacts with the ACE 2 on the host cells. The interaction causes a series of molecular changes which causes the S1 subunit of the glycoprotein to bind the ACE 2 while the S2 subunit transforms a metastable prefusion state to a stable postfusion configuration to facilitate membrane fusion of the virus and further post-entry events (Song et al., 2018). There is enough evidence to support that ACE 2 is pivotal in mediating the cellular entry of the SARS-CoV-2 virus. Data from HeLa cell lines demonstrate that cells without ACE 2 do not internalize the virus as compared to cells with the ACE 2 receptor (Zhou et al., 2020). Hence a pursuit for drugs that could exert strong antiviral activity would test them for binding to both the SARS-CoV-2 receptor binding domain as well as to ACE 2. The present study also performed molecular docking to assess the ligands for binding to both the SARS-Cov-2 virus RBD and the ACE 2.

Before the molecular docking, a DFT analysis was performed to determine and quantify the chemical descriptors of the 3 ligands namely melatonin, ramelteon, and agomelatine. It is known that the DFT process quantifies various parameters of chemical significance in the ligands. In this connection, the present study revealed some important data. The energy difference between the LUMO and HOMO was represented as $\Delta E$ values which were highest for ramelteon and lowest for agomelatine. Ramelteon was found to be the best electron acceptor and Agomelatine was found to be the best electron donor. With regards to chemical hardness values, ramelteon scored over the other 2 ligands namely melatonin and ramelteon. Electronegativity scores revealed the lowest scores obtained by melatonin and ramelteon which meant that these 2 ligands could have a significant inhibitory effect after binding to the receptor.

Concerning docking experiments, it was found that all 3 ligands namely, melatonin, ramelteon, and agomelatine bound significantly to the ACE 2 enzyme. However, the highest interaction of

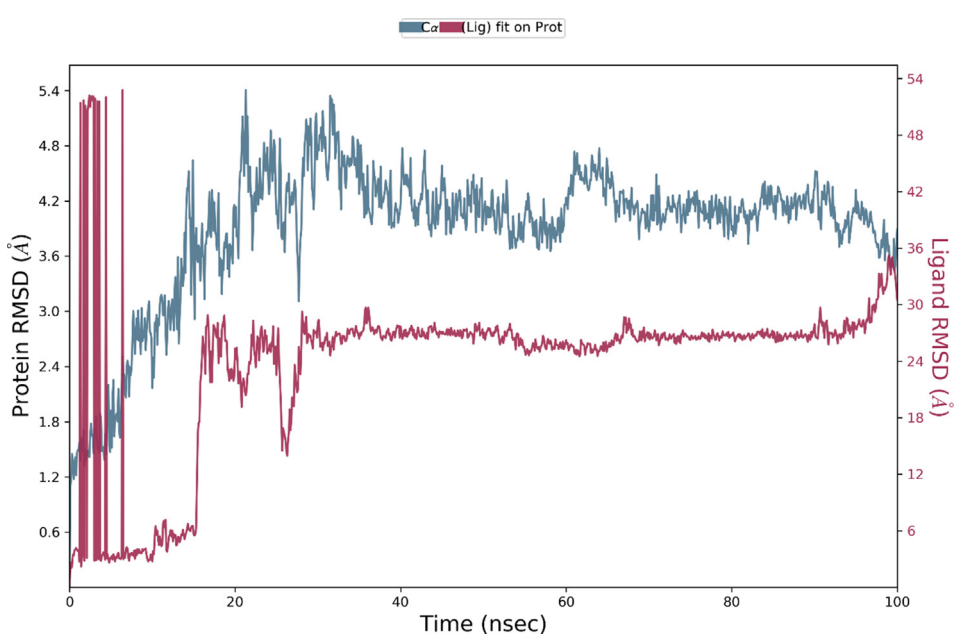


**Fig. 5.** Root means square deviation (RMSD) of the C-alpha atoms of protein and the ligand with time (Melatonin_cov2 complex). The left Y-axis shows the variation of protein RMSD through time. The right Y-axis shows the variation of ligand RMSD through time.

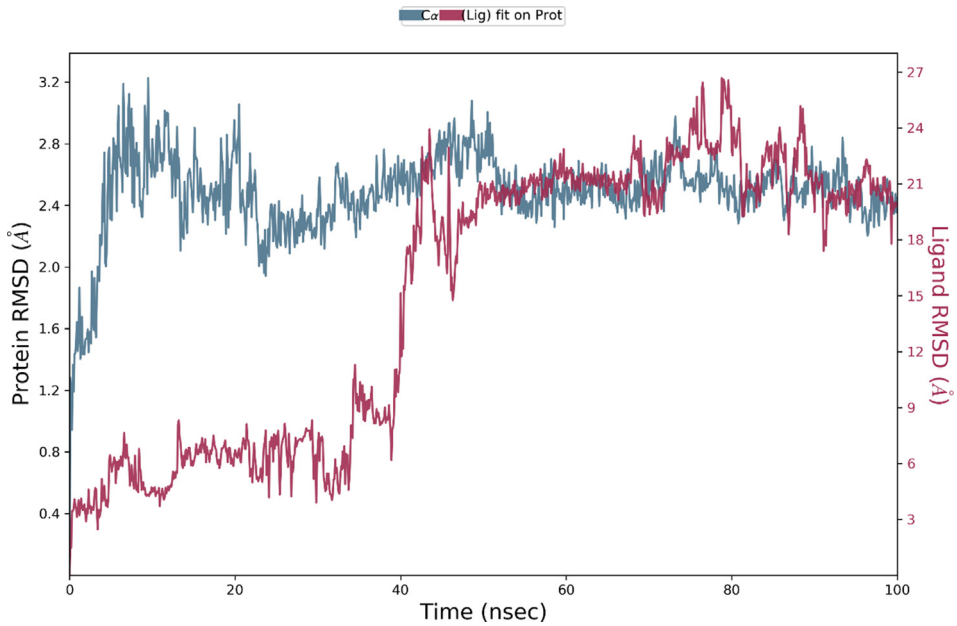


**Fig. 6.** Root means square deviation (RMSD) of the C-alpha atoms of protein and the ligand with time (Ramelton-cov2). The left Y-axis shows the variation of protein RMSD through time. The right Y-axis shows the variation of ligand RMSD through time.

binding with ACE 2 was recorded by ramelteon, followed by agomelatine and melatonin. However, it was observed that ramelteon followed by agomelatine and melatonin had the highest binding interaction with the SARS-CoV-2 RBD and was bound to the same site.

The findings of molecular dynamics stimulation demonstrate the plot of the complex's RMSD. The RMSD plot of the complex Ramelton-cov2 reveals that the complex attains stability at twenty nanoseconds, while Melatonin cov2 depicts that stability of the complex is attained at five nanoseconds. After being steady for (Melatonin cov2), ligand fit to protein RMSD values fluctuates within 5.0 Angstrom. These results show that the ligand remains stably bound to the receptor's binding site all through simulated time. However, after 40 ns, there is a dramatic spike of 12 Angstrom in (Ramelton-cov2 ligand) and then it returns to normal. This explains why the ligand binding mode has changed. In terms of H-bonds, TYR 453 and GLY 502 are the most important (Ramelton-cov2). Some residues (TYR 473 for Ramelton-cov2 and ARG 103, PHE 456, and TYR 505 for Ramelteon-cov2) made significant strong hydrophobic contacts. Low RMSD values indicate that ramelteon is more stable than other compounds.

Data from the present study reveal that melatonin, ramelteon, and agomelatine demonstrate significant binding with SARS-CoV-2 RBD and ACE 2 demonstrating the fact that they can strongly prevent viral entry into the host cells through dual binding effects. As above mentioned ramelteon was found to have the most superior effects as an anti-SARS-Cov-2 drug as revealed by the present in silico analysis. Many chemical compounds and naturally derived essential oils have shown significant binding to the SARS-CoV-2 RBD (Yadalam et al., 2021). However, data from the present study are novel as we have opened up a new vista in exploring the antiviral properties of melatonin and its related receptor agonists ramelteon and agomelatine.

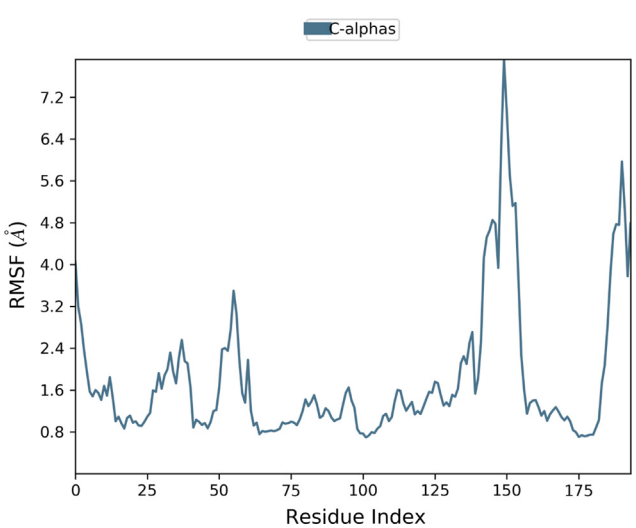


**Fig. 7.** Residue-wise Root Mean Square Fluctuation (RMSF) of protein (Melatonin_cov2).

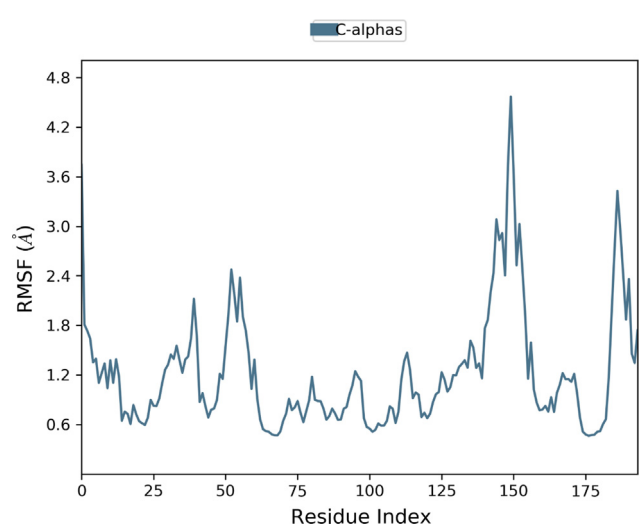


**Fig. 8.** Residue-wise Root Mean Square Fluctuation (RMSF) of protein (Ramelton-cov2).

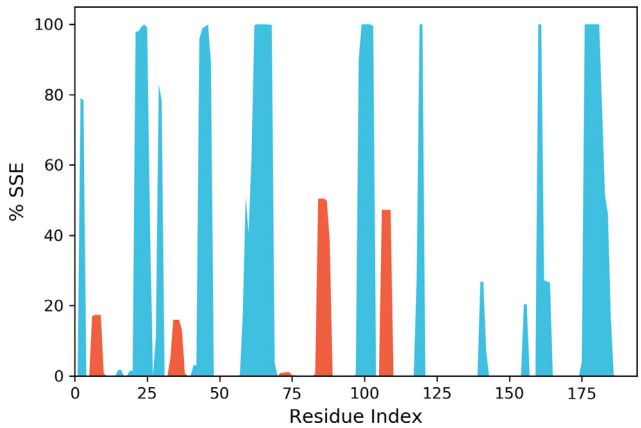


**Fig. 9.** Protein Secondary Structure element distribution by residue index throughout the protein structure (Melatonin_cov2). Red columns indicate alpha helices, and blue columns indicate beta-strands.

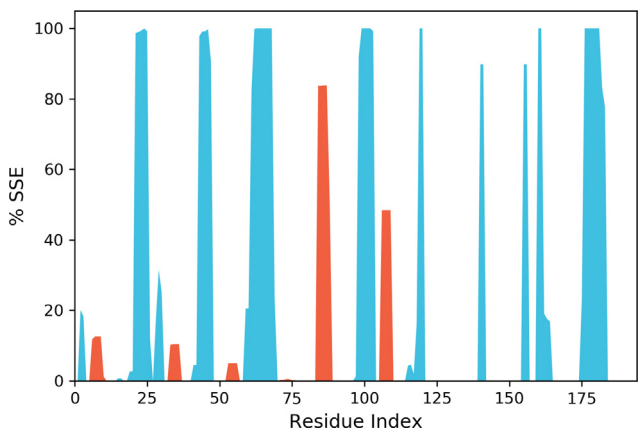


**Fig. 10.** Protein Secondary Structure element distribution by residue index throughout the protein structure (Ramelton-cov2). Red columns indicate alpha helices, and blue columns indicate beta-strands.

Melatonin has been already projected as an antiviral agent in the global COVID 19 pandemic. It has been hypothesized that melatonin significantly inhibits inflammasome stimulation which could indirectly reduce the intensity of the cytokine storm and lung destruction (Shneider et al., 2020). Moreover, it can cause a restoration of circadian rhythm and mitochondrial metabolism (Anderson and Reiter, 2020). By restoring antioxidant status and restoring sleep patterns in critically ill COVID 19 patients, melatonin could serve as an adjuvant in COVID 19 management (Brusco et al., 2021). One preliminary in silico study has also demonstrated the effects of melatonin as a SARS-CoV-2 main protease (Mpro) inhibitor (Feitosa et al., 2020). We have elucidated a novel finding that melatonin can bind to both SARS-CoV-2 RBD and ACE 2. It is also known that melatonin is a significant calmodulin inhibitor (Huerto-Delgadillo et al., 1994). Calmodulin is required for the stability and activation of ACE 2 (Lai et al., 2009). So from our findings, it appears that melatonin has a 2 pronged effect on ACE 2, one by binding it and the other by inhibiting calmodulin. With all the above evidence and the numerical docking data, melatonin can be used as a candidate drug for SARS-Cov-2 infection.

Ramelteon which demonstrated the most superior effects in the study is a melatonin receptor agonist that acts predominantly by binding to the MT 1 and MT 2 receptors (McGechan and Wellington, 2005). This drug was predominantly discovered and used for the management of insomnia and delirium in chronically ill patients (Zammit et al., 2007). Ramelteon has good efficacy and a safety profile and can be used in SARS -CoV-2 according to our data as an antiviral that prevents viral entry into the host cells. There is one preliminary in silico study on ramelteon demonstrating its anti-SARS-CoV-2 effects as a SARS-CoV-2 papain-like protein (PLpro) inhibitor (Hosseini et al., 2021). But the present study data give a novel insight into the antiviral actions of ramelteon.

Agomelatine is also an agonist of the melatonin receptors and an inhibitor of the serotonin 5-$HT_{2C}$ receptor (Zupancic and Guilleminault, 2006). It is an antidepressant (de Bodinat et al., 2010) and has been used for the management of glioblastomas (Kast, 2015). The present study has highlighted its antiviral role. It is to be reiterated that agomelatine could also be used as an

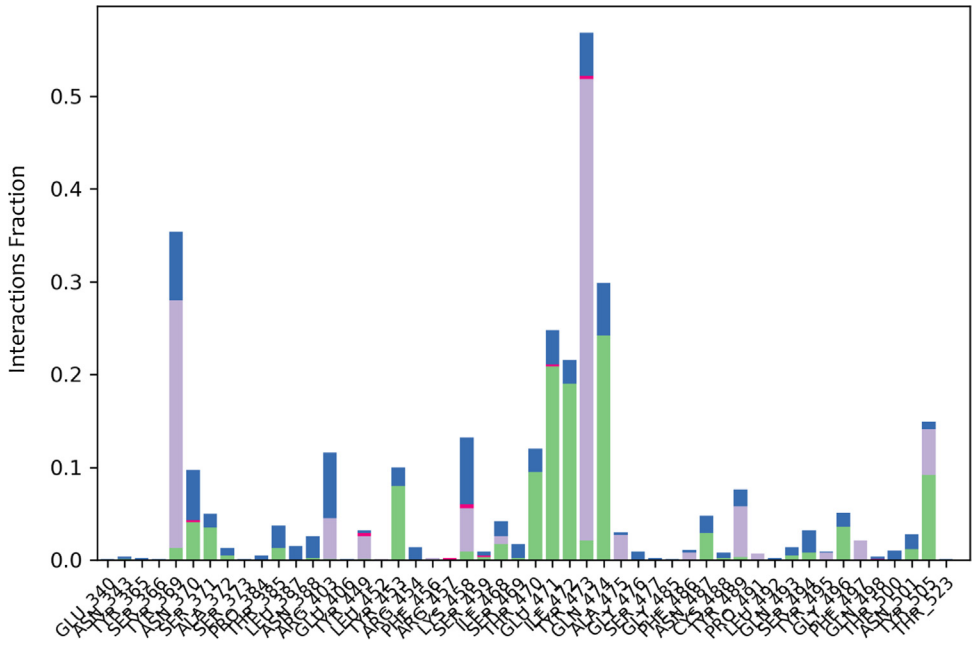


**Fig. 11.** Protein-ligand contact histogram (Melatonin_CoV2).

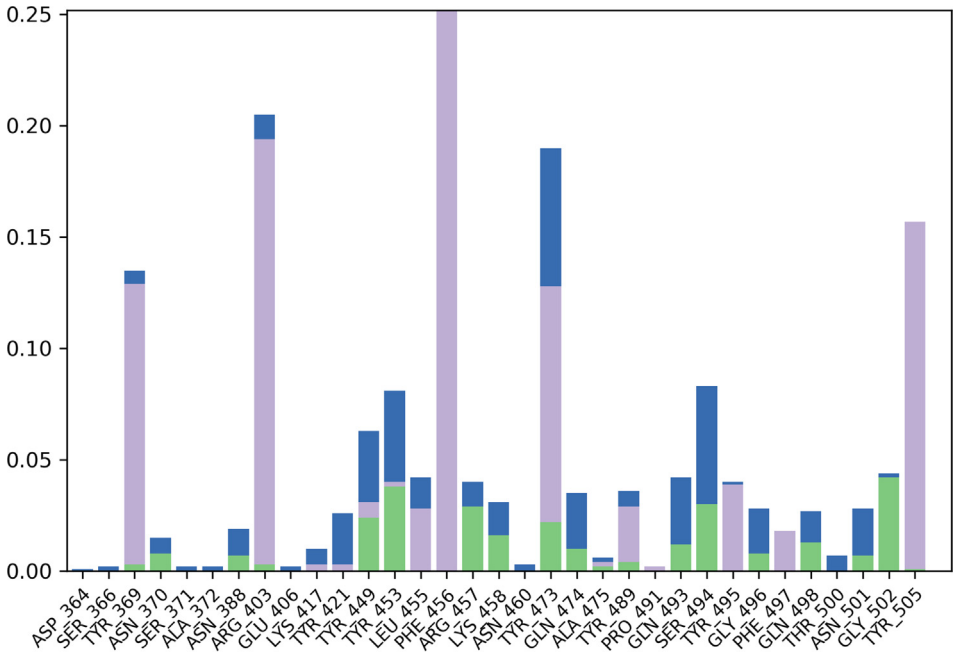


**Fig. 12.** Protein-ligand contact histogram (Ramelton-CoV2).

antidepressant to manage the psychiatric complications of COVID 19 infection. It was found that melatonin, ramelteon, and agomelatine were superior in their binding properties to SARS-CoV-2 RBD and ACE 2 and demonstrated better antiviral effects in this regard.

Another intriguing question that needs to be justified and answered before further testing the drugs for COVID 19 management is the adverse effect of ACE 2 blockade. The data from the present study reveal that melatonin, ramelteon, and agomelatine are ACE 2 inhibitors. If this is the case, it is expected that ACE 2 blockade can cause hypertension as an adverse effect as there could be an elevation of Angiotensin 2 consequent to ACE 2 inhibition (Hamming et al., 2007). This could worsen COVID 19 symptoms and cause respiratory distress. However, this side effect could be circumvented by melatonin and ramelteon as both these drugs can reduce and counteract increased blood pressure by multiple mechanisms (Li et al., 2009; Stroethoff et al., 2018). Hence with all the available data, it appears that melatonin and related drugs could be used in the management of the SARS-CoV-2 illness. Since the oral cavity is a documented portal of entry of the virus, it would be worthwhile using local drug formulations containing melatonin which could be applied onto the oral tissues and can thereby combat entry of the SARS-CoV-2 virus. Such topical formulations containing melatonin for oral application have already been devised and used in periodontitis management and have proven effective as antioxidant and anti-inflammatory agents for periodontitis management (Balaji et al., 2021). In the same lines, we suggest that melatonin and related drugs could be formulated as local drug delivery formulations for use to prevent the SARS-C0V-2 illness thereby making our idea clinically translatable. However, there are a few limitations in this regard as melatonin is a molecule with a very short half life (Tordjman et al., 2017). Moreover, it is very quickly sequestered in the presence of free radicals and reactive oxygen species into a plethora of metabolites (Reiter et al., 2016) which may not have the expected antiviral activity. Hence it would be worthwhile to titrate and arrive at an ideal dose of melatonin that would be required for our intended use. Moreover, we also need to employ the latest techniques of drug delivery to enhance quick absorption of melatonin into the oral tissues without loss, which would further enhance the anti SARS-CoV-2 effects of our preparation.

## 5. Conclusions

In conclusion, vital data from the present study highlight the antiviral role of melatonin-related drugs. As earlier described, the oral cavity is a known portal of entry of the virus due to the presence of ACE 2 expressing cells. Hence if local drug delivery formulations could be developed using melatonin, ramelteon, and agomelatine with novel drug formulation methods, these drugs could easily be used by patients. After thorough and diligent pharmacokinetic and safety profile studies, these drugs could also be used in the systemic formulation for the management of SARS-CoV-2 infection.

## Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

## Acknowledgment

The publication of the article was supported by Taif University Researchers Supporting Program (Grant number: TURSP-2020/128), Taif University, Saudi Arabia.

## Institutional Review Board Statement

Not applicable.

## Informed Consent Statement

Not applicable.

## References


Anderson, G., Reiter, R.J., 2020. Melatonin: Roles in influenza, Covid-19, and other viral infections. Rev. Med. Virol. 30 (3). https://doi.org/10.1002/rmv.v30.310.1002/rmv.2109.

Balaji, T.M., Varadarajan, S., Jagannathan, R., Mahendra, J., Fageeh, H.I., Fageeh, H.N., Mushtaq, S., Baeshen, H.A., Bhandi, S., Gupta, A.A., Raj, A.T., Reda, R., Patil, S., Testarelli, L., 2021. Melatonin as a Topical/Systemic Formulation for the Management of Periodontitis: A Systematic Review. Materials (Basel) 14, 2417. https://doi.org/10.3390/ma14092417.

Becke, A.D., 1993. Density-functional thermochemistry. III. The role of exact exchange. J. Chem. Phys. 98 (7), 5648–5652. https://doi.org/10.1063/1.464913.

Brusco, L.I., Cruz, P., Cangas, A.V., Rojas, C.G., Vigo, D.E., Cardinali, D.P., 2021. Efficacy of melatonin in non-intensive care unit patients with COVID-19 pneumonia and sleep dysregulation. Melatonin Res. 4, 173–188. https://doi.org/10.32794/mr11250089.

Burrell, L.M., Johnston, C.I., Tikellis, C., Cooper, M.E., 2004. ACE2, a new regulator of the renin-angiotensin system. Trends Endocrinol. Metab. 15 (4), 166–169. https://doi.org/10.1016/j.tem.2004.03.001.

Cipolla-Neto, J., Amaral, F.G., 2018. Melatonin as a Hormone: New Physiological and Clinical Insights. Endocr. Rev. 39, 990–1028. https://doi.org/10.1210/er.2018-00084.

de Bodinat, C., Guardiola-Lemaitre, B., Mocaër, E., Renard, P., Muñoz, C., Millan, M.J., 2010. Agomelatine, the first melatonergic antidepressant: discovery, characterization and development. Nat. Rev. Drug Discov. 9 (8), 628–642. https://doi.org/10.1038/nrd3140.

Di Bella, G., Mascia, F., Gualano, L., Di Bella, L., 2013. Melatonin anticancer effects: review. Int. J. Mol. Sci. 14, 2410–2430. https://doi.org/10.3390/ijms14022410.

Feitosa, E.L., Júnior, F.T.D.S.S., Nery Neto, J.A.D.O., Matos, L.F.L., Moura, M.H.D.S., Rosales, T.O., De Freitas, G.B.L., 2020. COVID-19: Rational discovery of the therapeutic potential of Melatonin as a SARS-CoV-2 main Protease Inhibitor. Int. J. Med. Sci. 17 (14), 2133–2146. https://doi.org/10.7150/ijms.48053.

Frisch, M.J., Trucks, G.W., Schlegel, H.B., Scuseria, G.E., Robb, M.A., Cheeseman, J.R., Scalmani, G., Barone, V., Mennucci, B., Petersson, G.A., Nakatsuji, H., Caricato, M., Li, X., Hratchian, H.P., Izmaylov, A.F., Bloino, J., Zheng, G., Sonnenberg, J.L., Hada, M., Ehara, M., Toyota, K., Fukuda, R., Hasegawa, J., Ishida, M., Nakajima, T., Honda, Y., Kitao, O., Nakai, H., Vreven, T., Montgomery, J.A., Peralta, J.E., Ogliaro, F., Bearpark, M., Heyd, J.J., Brothers, E., Kudin, K.N., Staroverov, V.N., Kobayashi, R., Normand, J., Raghavachari, K., Rendell, A., Burant, J.C., Iyengar, S.S., Tomasi, J., Cossi, M., Rega, N., Millam, J.M., Klene, M., Knox, J.E., Cross, J.B., Bakken, V., Adamo, C., Jaramillo, J., Gomperts, R., Stratmann, R.E., Yazyev, O., Austin, A.J., Cammi, R., Pomelli, C., Ochterski, J.W., Martin, R.L., Morokuma, K., Zakrzewski, V.G., Voth, G.A., Salvador, P., Dannenberg, J.J., Dapprich, S., Daniels, A.D., Farkas, Foresman, J.B., Ortiz, J. V, Cioslowski, J., Fox, D.J., 2009. Gaussian 09, Revision B.01. Gaussian 09, Revis. B.01, Gaussian, Inc., Wallingford CT 1–20.

Fukui, K., 1982. Role of frontier orbitals in chemical reactions. Science 218 (4574), 747–754.

Geerlings, P., De Proft, F., 2008. Conceptual DFT: the chemical relevance of higher response functions. Phys. Chem. Chem. Phys. 10, 3028–3042. https://doi.org/10.1039/b717671f.

Giannoulia-Karantana, A., Vlachou, A., Polychronopoulou, S., Papassotiriou, I., Chrousos, G.P., 2007. Melatonin and immunomodulation: Connections and potential clinical applications. Neuroimmunomodulation 13 (3), 133–144. https://doi.org/10.1159/000097258.

Hamming, I., Cooper, M.E., Haagmans, B.L., Hooper, N.M., Korstanje, R., Osterhaus, A.D.M.E., Timens, W., Turner, A.J., Navis, G., van Goor, H., 2007. The emerging role of ACE2 in physiology and disease. J. Pathol. 212 (1), 1–11. https://doi.org/10.1002/path.2162.

Hosseini, M., Chen, W., Xiao, D., Wang, C., 2021. Computational molecular docking and virtual screening revealed promising SARS-CoV-2 drugs. Precis. Clin. Med. 4, 1–16. https://doi.org/10.1093/pcmedi/pbab001.

Huerto-Delgadillo, L., Antón-Tay, F., Benitez-King, G., 1994. Effects of melatonin on microtubule assembly depend on hormone concentration: role of melatonin as a calmodulin antagonist. J. Pineal Res. 17 (2), 55–62.

Humphrey, W., Dalke, A., Schulten, K., 1996. VMD: visual molecular dynamics. J. Mol. Graph. 14 (33–8), 27–28. https://doi.org/10.1016/0263-7855(96)00018-5.

Kast, R.E., 2015. Agomelatine or ramelteon as treatment adjuncts in glioblastoma and other M1- or M2-expressing cancers. Contemp. Oncol. (Poznan, Poland) 19, 157–162. https://doi.org/10.5114/wo.2015.51421.

Kennedy, S.H., Eisfeld, B.S., 2007. Agomelatine and its therapeutic potential in the depressed patient. Neuropsychiatr. Dis. Treat. 3, 423–428.

Khurshid, Z., Asiri, F.Y.I., Al Wadaani, H., 2020. Human Saliva: Non-Invasive Fluid for Detecting Novel Coronavirus (2019-nCoV). Int. J. Environ. Res. Public Health 17, 2225. https://doi.org/10.3390/ijerph17072225.

Lai, Z.W., Lew, R.A., Yarski, M.A., Mu, F.-T., Andrews, R.K., Smith, A.I., 2009. The identification of a calmodulin-binding domain within the cytoplasmic tail of angiotensin-converting enzyme-2. Endocrinology 150, 2376–2381. https://doi.org/10.1210/en.2008-1274.

Lan, J., Ge, J., Yu, J., Shan, S., Zhou, H., Fan, S., Zhang, Q., Shi, X., Wang, Q., Zhang, L., Wang, X., 2020. Structure of the SARS-CoV-2 spike receptor-binding domain bound to the ACE2 receptor. Nature 581, 215–220. https://doi.org/10.1038/s41586-020-2180-5.

Li, H.-L., Kang, Y.-M., Yu, L., Xu, H.-Y., Zhao, H., 2009. Melatonin reduces blood pressure in rats with stress-induced hypertension via GABAA receptors. Clin. Exp. Pharmacol. Physiol. 36, 436–440. https://doi.org/10.1111/j.1440-1681.2008.05080.x.

Li, W., Moore, M.J., Vasilieva, N., Sui, J., Wong, S.K., Berne, M.A., Somasundaran, M., Sullivan, J.L., Luzuriaga, K., Greenough, T.C., Choe, H., Farzan, M., 2003. Angiotensin-converting enzyme 2 is a functional receptor for the SARS coronavirus. Nature 426, 450–454. https://doi.org/10.1038/nature02145.

Li, X., Ma, X., 2020. Acute respiratory failure in COVID-19: is it "typical" ARDS? Crit. Care 24, 198. https://doi.org/10.1186/s13054-020-02911-9.

Madapusi, B.T., Rao, S.R., 2018. Preliminary Evaluation of Human Gingiva as an Extrapineal Site of Melatonin Biosynthesis in States of Periodontal Health and Disease. J. Clin. Diagnostic Res. https://doi.org/10.7860/JCDR/2018/32451.11078.

Maria, S., Witt-Enderby, P.A., 2014. Melatonin effects on bone: potential use for the prevention and treatment for osteopenia, osteoporosis, and periodontal disease and for use in bone-grafting procedures. J. Pineal Res. 56, 115–125. https://doi.org/10.1111/jpi.12116.

Mathieu, E., Ritchie, H., Ortiz-Ospina, E., Roser, M., Hasell, J., Appel, C., Giattino, C., Rodés-Guirao, L., 2021. A global database of COVID-19 vaccinations. Nat. Hum. Behav. https://doi.org/10.1038/s41562-021-01122-8.

McGechan, A., Wellington, K., 2005. Ramelteon. CNS Drugs 19, 1057–1065. https://doi.org/10.2165/00023210-200519120-00007. discussion 1066–7.

Mohan, B., Vinod, N., 2020. COVID-19: An Insight into SARS-CoV2 Pandemic Originated at Wuhan City in Hubei Province of China. J. Infect. Dis. Epidemiol. 6. https://doi.org/10.23937/2474-3658/1510146.

Nabavi, S.M., Nabavi, S.F., Sureda, A., Xiao, J., Dehpour, A.R., Shirooie, S., Silva, A.S., Baldi, A., Khan, H., Daglia, M., 2019. Anti-inflammatory effects of Melatonin: A mechanistic review. Crit. Rev. Food Sci. Nutr. https://doi.org/10.1080/10408398.2018.1487927.

Pedro, S.A., Ndjomatchoua, F.T., Jentsch, P., Tchuenche, J.M., Anand, M., Bauch, C.T., 2020. Conditions for a Second Wave of COVID-19 Due to Interactions Between Disease Dynamics and Social Processes. Front. Phys. 8, 428. https://doi.org/10.3389/fphy.2020.574514.

Reiter, R.J., 1991. Melatonin: the chemical expression of darkness. Mol. Cell. Endocrinol. 79, C153–C158. https://doi.org/10.1016/0303-7207(91)90087-9.

Reiter, R.J., Mayo, J.C., Tan, D.X., Sainz, R.M., Alatorre-Jimenez, M., Qin, L., 2016. Melatonin as an antioxidant: under promises but over delivers. J. Pineal Res. https://doi.org/10.1111/jpi.12360.

Sakaguchi, W., Kubota, N., Shimizu, T., Saruta, J., Fuchida, S., Kawata, A., Yamamoto, Y., Sugimoto, M., Yakeishi, M., Tsukinoki, K., 2020. Existence of SARS-CoV-2 Entry Molecules in the Oral Cavity. Int. J. Mol. Sci. 21. https://doi.org/10.3390/ijms21176000.

Sehirli, A.O., Sayiner, S., Serakinci, N., 2020. Role of melatonin in the treatment of COVID-19; as an adjuvant through cluster differentiation 147 (CD147). Mol. Biol. Rep. 47, 8229–8233. https://doi.org/10.1007/s11033-020-05830-8.

Shimozuma, M., Tokuyama, R., Tatehara, S., Umeki, H., Ide, S., Mishima, K., Saito, I., Satomura, K., 2011. Expression and cellular localizaion of melatonin-synthesizing enzymes in rat and human salivary glands. Histochem. Cell Biol. 135, 389–396. https://doi.org/10.1007/s00418-011-0800-8.

Shneider, A., Kudriavtsev, A., Vakhrusheva, A., 2020. Can melatonin reduce the severity of COVID-19 pandemic? Int. Rev. Immunol. 39, 153–162. https://doi.org/10.1080/08830185.2020.1756284.

Song, W., Gui, M., Wang, X., Xiang, Y., 2018. Cryo-EM structure of the SARS coronavirus spike glycoprotein in complex with its host cell receptor ACE2. PLoS Pathog. 14,. https://doi.org/10.1371/journal.ppat.1007236 e1007236.

Stroethoff, M., Behmenburg, F., Spittler, K., Raupach, A., Heinen, A., Hollmann, M.W., Huhn, R., Mathes, A., 2018. Activation of Melatonin Receptors by Ramelteon Induces Cardioprotection by Postconditioning in the Rat Heart. Anesth. Analg. 126, 2112–2115. https://doi.org/10.1213/ANE.0000000000002625.

Tang, Y., Liu, J., Zhang, D., Xu, Z., Ji, J., Wen, C., 2020. Cytokine Storm in COVID-19: The Current Evidence and Treatment Strategies. Front. Immunol. 11. https://doi.org/10.3389/fimmu.2020.01708.

Tordjman, S., Chokron, S., Delorme, R., Charrier, A., Bellissant, E., Jaafari, N., Fougerou, C., 2017. Melatonin: Pharmacology, Functions and Therapeutic Benefits. Curr. Neuropharmacol. 15, 434–443. https://doi.org/10.2174/1570159X14666161228122115.

Warsi, I., Khurshid, Z., Shazam, H., Umer, M.F., Imran, E., Khan, M.O., Slowey, P.D., Goodson, J.M., 2021. Saliva Exhibits High Sensitivity and Specificity for the Detection of SARS-COV-2. Diseases 9, 38. https://doi.org/10.3390/diseases9020038.

Xu, H., Zhong, L., Deng, J., Peng, J., Dan, H., Zeng, X., Li, T., Chen, Q., 2020. High expression of ACE2 receptor of 2019-nCoV on the epithelial cells of oral mucosa. Int. J. Oral Sci. 12, 8. https://doi.org/10.1038/s41368-020-0074-x.

Yadalam, P.K., Varatharajan, K., Rajapandian, K., Chopra, P., Arumuganainar, D., Nagarathnam, T., Sohn, H., Madhavan, T., 2021. Antiviral Essential Oil Components Against SARS-CoV-2 in Pre-procedural Mouth Rinses for Dental Settings During COVID-19: A Computational Study. Front. Chem. 9,. https://doi.org/10.3389/fchem.2021.642026 642026.

Zammit, G., Erman, M., Wang-Weigand, S., Sainati, S., Zhang, J., Roth, T., 2007. Evaluation of the efficacy and safety of ramelteon in subjects with chronic insomnia. J. Clin. Sleep Med. 3, 495–504.

Zammit, G.K., 2007. Ramelteon: a novel hypnotic indicated for the treatment of insomnia. Psychiatry (Edgmont). 4, 36–42.

Zhai, X., Wang, N., Jiao, H., Zhang, J., Li, C., Ren, W., Reiter, R.J., Su, S., 2021. Melatonin and other indoles show antiviral activities against swine coronaviruses in vitro at pharmacological concentrations. J. Pineal Res. 71. https://doi.org/10.1111/jpi.12754.

Zhang, R., Wang, X., Ni, L., Di, X., Ma, B., Niu, S., Liu, C., Reiter, R.J., 2020. COVID-19: Melatonin as a potential adjuvant treatment. Life Sci. 250,. https://doi.org/10.1016/j.lfs.2020.117583 117583.

Zhou, P., Yang, X.-L., Wang, X.-G., Hu, B., Zhang, L., Zhang, W., Si, H.-R., Zhu, Y., Li, B., Huang, C.-L., Chen, H.-D., Chen, J., Luo, Y., Guo, H., Jiang, R.-D., Liu, M.-Q., Chen, Y., Shen, X.-R., Wang, X., Zheng, X.-S., Zhao, K., Chen, Q.-J., Deng, F., Liu, L.-L., Yan, B., Zhan, F.-X., Wang, Y.-Y., Xiao, G.-F., Shi, Z.-L., 2020. A pneumonia outbreak associated with a new coronavirus of probable bat origin. Nature 579, 270–273. https://doi.org/10.1038/s41586-020-2012-7.

Zupancic, M., Guilleminault, C., 2006. Agomelatine: a preliminary review of a new antidepressant. CNS Drugs 20, 981–992. https://doi.org/10.2165/00023210-200620120-00003.