Research Article

Potential therapeutic intervention of melatonin against COVID-19: A comparative pharmacokinetic study

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ABSTRACT

Melatonin synthesis is primarily regulated by environmental light-dark cycle and is well known for its biological rhythm regulation and its potent antioxidant and anti-inflammatory properties across species. The present investigation focuses on the potential actions of melatonin as a therapeutic agent against COVID-19 and these actions are compared with other commonly used pharmacological agents of this kind including methylprednisolone, doxycycline, oseltamivir, and remdesivir. The comprehensive comparisons of pharmacokinetic profiles include their absorption, distribution, metabolism, and excretion (ADME) properties. The further in-depth analyses on their target identification, functional enrichment are performed by Gene Ontology (GO), Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways, construction of protein-protein interaction (PPI) networks, and molecular docking. These analyses elucidate the potential correlation of melatonin with critical hub targets implicated in COVID-19 pathogenesis. The results from pharmacokinetics indicates that melatonin has the higher bioavailability than the other tested drugs due to its low molecular weight, lipophilicity, lack of P-glycoprotein (P-gp) along with inhibitory action on cytochrome P450 1A2 (CYP1A2). GO and enriched KEGG analyses suggests that melatonin-mediated modulation of COVID-19 pathogenesis likely targets the AGE-RAGE pathway, HIF-1α signaling, and apoptosis. Furthermore, PPI network analysis also shows that melatonin has the highest nodes and edges, as well as the greatest average node degree score and highest common potential targets with the genes associated with the development of COVID-19. Notably, molecular docking study demonstrates the substantial interactions of melatonin with principal hub targets TP53, AKT1, IL6, TNF, IL1B, BCL2, EGFR, STAT3, CASP3, and NFKB1. Hence, melatonin has several significant pharmacokinetic advantages compared to selected therapeutic agents which may appear to modulate multiple facets of COVID-19 pathology. Based on the significant pharmacokinetic advantages of melatonin over the commonly used other drugs, the substantial clinical studies are necessary to establish its methods of application as a potential therapeutic against SARS-CoV-2 in the near future.

Key words: Melatonin, SARS-CoV-2, covid-19, cytokine storm, molecular docking, pharmacokinetics

1. INTRODUCTION

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), the causative virus of the COVID-19 pandemic, is characterized by its exceptional transmissibility and its profound damage to the global public health and socio-economic systems. Since its emergence in late 2019, the virus has caused widespread morbidity and mortality, posing unprecedented challenges to healthcare infrastructures and international response efforts. According to Worldometer (https://www.worldometers.info/coronavirus/) report, as of April 13, 2024, the confirmed infected cases have surpassed 700 million, with an estimated 7 million deaths attributed to the disease. These astounding statistics addresses the magnitude of the pandemic and highlight the urgent need for continued research, public health interventions, and policy measures to mitigate its far-reaching consequences. This virus initiates a host-pathogen interaction through binding of its spike protein with the human membrane receptor, angiotensin-converting enzyme 2 (ACE2), facilitated by transmembrane serine protease 2 (TMPRSS2) (1). ACE2 is expressed across a diverse range of human tissues, including enterocytes, ciliated cells in both upper and lower respiratory tracts, type II alveolar pneumocytes (2). The perturbation of ACE2 activity due to viral invasion initiates a cascade of biological and pathological responses, delineating COVID-19 pathophysiology (3). Based on the symptoms, SARS-CoV-2 infection can be classified into asymptomatic, mild, moderate, and severe cases as outlined by the National Institute of Health (NIH). In primate animal model of SARS-CoV-2 infection, virus replicates in the lungs until Day 10 post-infection. Surprisingly, lung inflammation intensified after virus clearance, peaking at Day 14 and remaining higher until Day 28 (4). Immune responses also vary over the course of the infection. The levels of interleukin (IL)-10, IL-6, and tumor necrosis factor (TNF)-α increase with the progression of infection and are higher in ‘severe’ patients along with a reduction in CD8+ and CD4+ T cells (5). This ‘cytokine storm’ produced by macrophages leads to destructive inflammation and host cell damage (6). Cyclic GMP/AMP synthase (cGAS) triggers a further large-scale proinflammatory cytokine release known as the ‘secondary cytokine storm’, creating a vicious cycle. If not interrupted, this cycle results in widespread apoptosis, pyroptosis, and necrosis of even in the non-infected cells (6). Therefore, preventing the ‘cytokine storm’ is crucial for the treatment of COVID-19 patients.

The ramifications of the COVID-19 pandemic have spurred extensive efforts to devise preventative and therapeutic measures. These initiatives have thus far culminated in the remarkably swift development of multiple efficacious vaccines (7, 8), alongside the assessment of a diverse array of prospective treatments in clinical trials, some of which are easily available in the market. Drawing from six decades of antiviral drug discovery, two principal categories of anti-SARS-CoV-2 agents emerge (9). The first category of these medicines is to target viral proteins exemplified by remdesivir (GS-5734), a potent inhibitor of viral RNA-dependent RNA polymerase. Early identification of remdesivir as a promising therapeutic candidate for COVID-19 stemmed from its demonstrated ability to inhibit SARS-CoV-2 *in vitro* (10). These agents potentially offer high selectivity due to the absence of human homologues, yet they carry the risk of drug resistance due to emerging viral variants (11). The second category of medicines is to target the host proteins implicated in the viral life cycle, such as receptors facilitating viral entry. Oseltamivir, being illustrative of this group, a neuraminidase inhibitor sanctioned by the Food and Drug Administration (FDA) for treating influenza A and B (12), has been proposed in the treatment of critically ill COVID-19 patients (13, 14). This agent might exhibit broad spectrum antiviral activity but often has low selectivity and potentially adverse safety profiles (15). Additionally, compounds targeting human proteins, like systemic glucocorticoid dexamethasone, play a crucial role in mitigating cytokine storms (11). Methylprednisolone, a glucocorticoid, is widely used for treating

COVID-19 due to its anti-inflammatory and immune-regulatory actions (16). In acute respiratory distress syndrome (ARDS) induced by SARS-CoV-2, methylprednisolone administration significantly enhances oxygen diffusion from the pulmonary alveoli into the bloodstream within two days (17). However, an antibiotic, doxycycline, with strain-dependent antiviral activity was also used widely in COVID-19 treatment as it inhibits matrix metalloproteinases (MMPs), especially MMP-9, which is likely essential for the initial block of viral entry into host cells (18). These selective compounds have significant impact on COVID-19 treatment but their major adverse effects are the concerning.

Melatonin, an endogenously chronobiotic molecule, is also a promising adjunctive factor for viral infections due to its anti-inflammatory, anti-apoptotic, immune-modulatory, and powerful antioxidant properties (19). The comprehensive beneficial properties of melatonin have been proved in attenuating and preventing the 'cytokine storm', resulting in reduced morbidity and mortality from the disease (20). Being an effective antioxidant and anti-inflammatory agent, melatonin mitigates inflammation by suppressing pro-inflammatory cytokines, enhancing anti-inflammatory responses, regulating macrophage polarization via SIRT1, and modulating VEGF, ROS, and HIF secretion to reduce ischemia, anemia, and COVID-19 severity (21). With its ability to lower oxidative stress, enhance immunity, and improve outcomes in elderly and comorbid patients, melatonin shows promise as a safe and effective therapy for severe cases, long haul COVID-19, and emerging variants (21). Interestingly, administration of this molecule over a period of 14 days significantly reduces plasma levels of IL-4, IL-2, IL-1β, IFN-γ, and IL-6 in COVID-19 patients (age group was >18 years, diagnosed with moderate to severe level) (22). It notably suppresses the oxidative agents like nitric oxide and malondialdehyde, while enhances antioxidative factors like superoxide dismutase (SOD) (22, 23). In twenty COVID-19 patients (12 males, 8 females), expression level of regulatory genes related to 'cytokine storm' (e.g., T-bet, GATA3, STAT4, STAT6, CAS, CASP1) is decreased after melatonin treatment (24). In a study by Hasan and co-workers (2022), application of oral melatonin decreases sepsis and thrombosis development, resulting in lower mortality rates in severe COVID-19 patients (25). Melatonin, by interacting with α7nAChR, mitigates COVID-19 severity through its dual antiviral and host-modulating effects, reducing vulnerability, symptoms, and recovery time while enhancing humoral and cellular immunity, therefore, melatonin could serve as a potential adjuvant in the management of COVID-19 (26).

Based on the evidence mentioned above, this study undertakes an *in silico* comparative pharmacokinetic evaluation to elucidate the potential therapeutic efficacy of melatonin, an endogenous molecule, as an intervention against SARS-CoV-2 infection. The investigation will juxtapose pharmacodynamic and pharmacokinetic properties of melatonin with commonly deployed therapeutics in COVID-19 management, including methylprednisolone, doxycycline, oseltamivir, and remdesivir. Leveraging advanced bioinformatics platforms, integrative network pharmacology, and high-precision molecular docking analyses, the research aims to delineate mechanistic and pharmacological plausibility of melatonin in addressing the intricate and multifactorial challenges posed by SARS-CoV-2 pathogenesis.

2. METHODS

2.1. Identification of COVID-19 associated genes from RNA-seq dataset.

Gene Expression Omnibus (GEO) (*https://www.ncbi.nlm.nih.gov/geo/*) was searched for gene expression datasets regarding COVID-19 progressions like asymptomatic, mild, moderate and severe (accession number: GSE196822). The selected dataset comprised 34 (8 asymptomatic, 9 mild, 10 moderate and 7 severe) cases.

2.2. Pre-processing (filtering and transformation) of RNA-seq dataset.

Data pre-processing was done in iDEP (v2.0; an integrated web application for differential expression and pathway analysis of RNA-Seq data) (27). The genes not expressed in any samples or expressed at extremely low levels were discarded for further analysis. A gene must have more than 0.5 counts per million (CPM) in at least one sample. In CPMs calculation, read counts were normalized by the total counts per sample in EdgeR. Only genes with levels above min CPM in at least nLibraries (n) = 1 were retained. The CPM was then transformed for clustering by log2 (CPM+c) method using EdgeR where the pseudo count (c) was taken 4.

2.3. Identification of differentially expressed genes (DEGs) from RNA-seq dataset.

DEGs among the groups (asymptomatic, moderate, mild and severe) were analyzed using DESeq2 package, based on R programme (v4.2.1), in iDEP (v2.0) with the criteria of FDR < 0.1 and minimum fold change, |log2FC| > 2.

2.4. Identification of COVID-19 associated genes from different databases.

Furthermore, the targets associated with COVID-19 were obtained by searching the following databases: DisGeNET (*http://www.disgenet.org/*), GeneCards (*https://www.genecards.org/*), and NCBI Gene (*https://www.ncbi.nlm.nih.gov/*). Targets gathered from public databases and GEO datasets were then combined followed by removal of the common targets.

2.5. Absorption, distribution, metabolism, excretion (ADME) profiling of melatonin and other selected drugs.

The ADME analysis pertains to the pharmacokinetic profiling of a compound, encompassing its absorption, distribution, metabolism, and excretion characteristics. The *in silico* ADME screening and drug-likeness assessment for the chosen compounds were conducted utilizing the SwissADME web tool (*https://swissadme.ch*) (28).

2.6. Findings the genetic targets of melatonin and other selected drugs.

The Simplified Molecular-Input Line-Entry System (SMILES) and 3D structures of melatonin, methylprednisolone, doxycycline, oseltamivir and remdesivir were obtained from the PubChem database (*https://pubchem.ncbi.nlm.nih.gov/*). The possible targets were determined by the SwissTargetPrediction database (*https://swisstargetprediction.ch/*), using the SMILES information, and also by Drug Bank (*https://go.drugbank.com/*), Comparative Toxicogenomics Database (CTD, *http://ctdbase.org/*), Chemical Association Networks (STITCH, *http://stitch.embl.de/*), Drug Gene Interaction Database (DGIdb, *https://www.dgidb.org/*).

2.7. Analysis of Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment of common targets.

ShinyGO online server (*https://bioinformatics.sdstate.edu/go/*, v0.80) was used for the analysis of GO and KEGG pathway enrichment of the common targets.

2.8. Protein-protein interaction (PPI) network analysis of the common targets.

The Search Tool for the Retrieval of Interacting Genes/Proteins (STRING; v11.5) database was employed to PPI network for common drug targets. The analysis included active interaction sources such as experimental repositories, computational prediction methods, and public text collections, all confined to the species "*Homo sapiens*" with a stringent interaction confidence score of 0.700, ensuring high reliability of the information. The resultant PPI network was subsequently analyzed using Cytoscape (v3.9.0). The top 10 core proteins, identified as hub targets, were determined using the Cytoscape plugin CytoHubba (*https://apps.cytoscape.org/apps/cytohubba*; v0.1), based on their respective degree value (29).

2.9. Study of molecular docking.

2.9.1. Preparation of proteins.

The three-dimensional crystal structures of the shared hub targets were accessed in PDB format from the RCSB Protein Data Bank. For docking simulations, the Autodock tools (v1.5.7) were used to incorporate charges and eliminate water molecules from the protein structures, converting them into the PDBQT format (29).

2.9.2. Preparation of ligand (melatonin).

Using Open Babel software (v3.1.1), the SDF 3D structures (retrieved from the PubChem database) of melatonin was converted into PDB format (29). Subsequently, Autodock tools (v1.5.7) were employed to generate the PDBQT format for the ligand.

2.9.3. Docking and visualization.

Utilizing Autodock software (v4.2), docking was conducted to assess the interactions and binding affinities between melatonin and its hub target proteins. The Kollman charge was assigned to the proteins, and grid maps were generated using Autogrid, with the docking grid box centered on the initial ligand position and set to dimensions of 60 Å × 60 Å × 60 Å. The grid resolution was configured at 0.375 Å. We employed the Lamarckian Genetic Algorithm (LGA) for the docking process, with 100 participants in the genetic algorithm population out of a total of 300. The maximum iterations were set to 27,000 for energy generations and 25,000,000 for energy evaluations. Post-docking, the protein-ligand complex structures were analyzed based on affinity parameters, and the optimal binding conformations were also visualized using DS visualizer software.

2.10. Statistical analysis.

The docking parameters were subjected to a detailed statistical evaluation using principal component analysis (PCA) to elucidate the relationships among the data based on their multidimensional distribution. The analysis was performed using the statistical software R (v4.1.0) with RStudio, employing the "FactoMineR" and "factoextra" packages (30). Coordinate scores, correlation coefficients, contributions, and squared cosine values for each variable (i.e., docking parameters) and individual (i.e., studied proteins) were computed. Additionally, the average contribution for each principal component (PC) was determined as 100 divided by the total number of variables or individuals. Higher squared cosine values, indicating superior quality of representation, were also calculated to assess the significance of each variable or individual in the principal components.

3. RESULTS

3.1. Identification of DEGs.

The DEGs were then identified using DESeq2 method where FDR cutoff was 0.1 and minimum fold change taken was 2. Initially we compared the DEGs in different severity levels of COVID-19 and after comparison, 791 DEGs (252 upregulated and 539 downregulated) were identified in total (Figure 1). It was observed that comparison between mild and severe cases showed 98 and 23, asymptomatic and moderate cases showed 61 and 223, whereas asymptomatic and mild cases showed 93 and 293 genes to be upregulated and downregulated, respectively and represented in volcano plot. The comparison between asymptomatic and severe, mild and moderate, moderate and severe cases did not reveal any significant upregulation and downregulation of DEGs (Figure 1 C and 2).

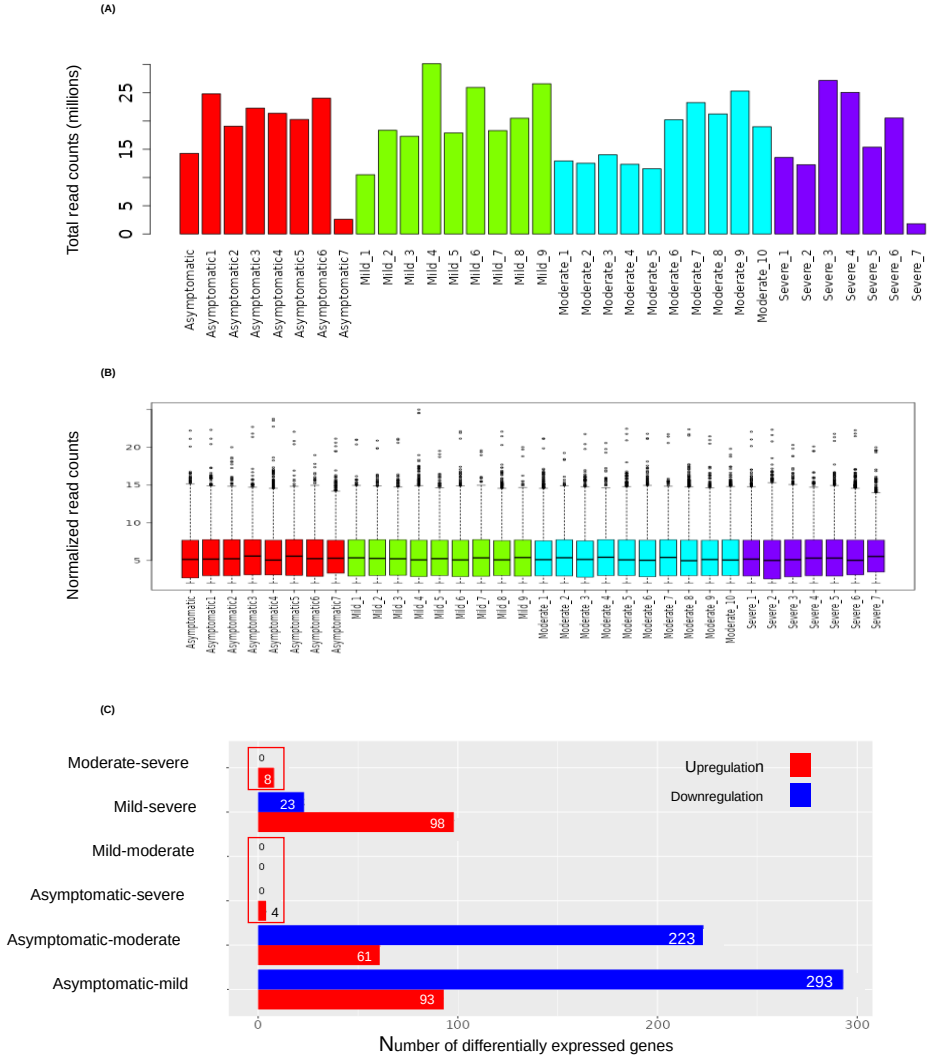

Fig. 1. Bar diagram representing data pre-processing (filtering and transformation) and identification of DEGs of RNA-seq dataset regarding COVID-19 progressions (accession number: GSE196822) obtained from GEO.

(A) total read counts (in million), (B) normalized read counts and (C) number of differentially expressed genes, where red and blue bar are denoted for the numbers of up and downregulated genes, respectively in different studied cases and non-significant variations in DEGs are highlighted with red rectangle box.

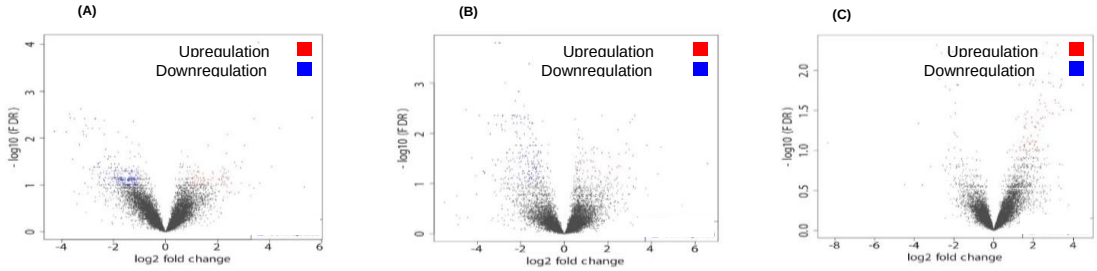

Fig. 2. Volcano plots representing the up and downregulated genes which are significant in DEG dataset (red and blue dots, respectively) by comparing.

(A) Asymptomatic with mild, (B) Asymptomatic with moderate, and (C) Mild with severe cases in COVID-19 progressions. log2 fold change and –log10 (FDR) are plotted on X and Y-axis, respectively.

3.2. Prediction of ADME properties of melatonin and the selected drugs.

Computation of ADME properties like physicochemical, solubility and pharmacokinetics of melatonin and the other selected drugs were performed (Figure 3, Table 1). In comparisons with the selected drugs, melatonin has lowest molecular weight and least number of heavy atoms; and octanol-water (o/w) partition coefficient also reflected its significant lipophilicity. It was found that melatonin was readily soluble to water (~1.05 mg/ml or 4.53×10^{-3} mol/l), permeable to gastrointestinal (GI) tract and blood-brain barrier. It showed no P-glycoprotein (P-gp) substrate properties but exhibited inhibitory action on CYP1A2 enzyme.

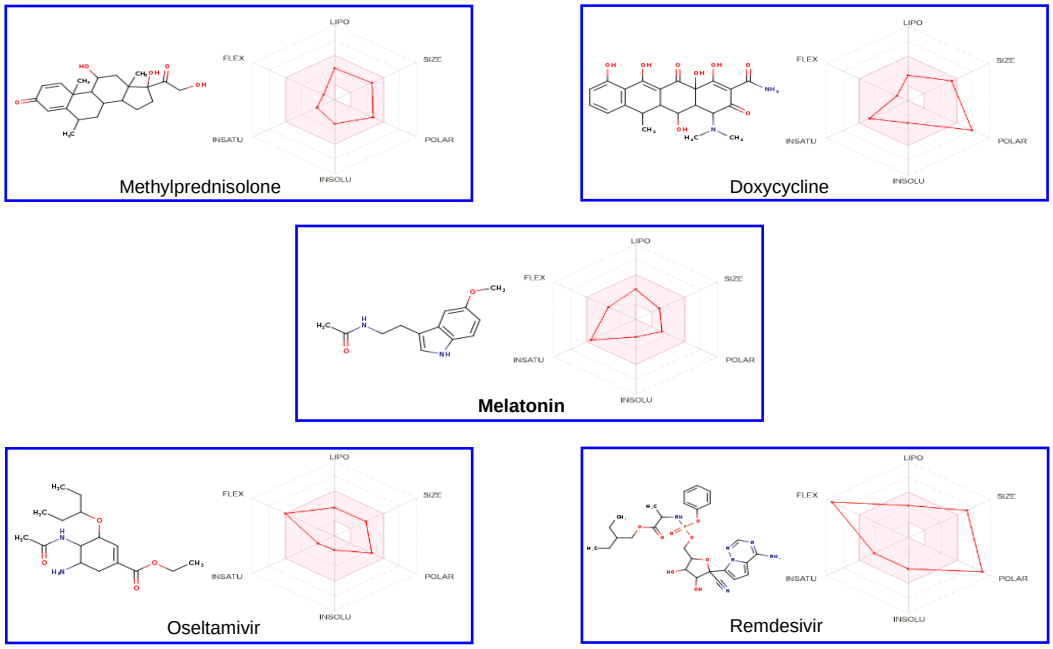

Fig. 3. Demonstration of ADME profiling of melatonin and the selected drugs (methylprednisolone, doxycycline, oseltamivir, remdesivir) utilising the bioavailability radar including molecular structure of each component.

The optimal range for each property is represented by pink area; lipophilicity (LIPO): −0.7 ≤ XLOGP3 ≤ +5.0, molecular weight (SIZE): between 150 and 500 g/mol, polarity (POLAR): 20 ≤ TPSA ≤ 130 Å, solubility (INSOLU): log S ≤ 6, saturation (INSATU): fraction of carbons in the sp3 hybridization ≥ 0.25 and flexibility (FLEX): less than nine rotatable bonds.

Table 1: ADME profiling of melatonin and the other selected drugs against COVID-19 utilizing the SwissADME web tool.

Physicochemical Properties					
Characteristics	**Melatonin**	**Methylprednisolone**	**Doxycycline**	**Oseltamivir**	**Remdesivir**
Formula	$C_{13}H_{16}N_2O_2$	$C_{22}H_{30}O_5$	$C_{22}H_{24}N_2O_8$	$C_{16}H_{28}N_2O_4$	$C_{27}H_{35}N_6O_8P$
Molecular Weight	232.28	374.47	444.43	312.40	602.58
No. of Heavy atoms	17	27	32	22	42
No. of aromatic heavy atoms	9	0	6	0	15
No. of rotatable bonds	5	2	2	9	14
No. of H-bond acceptors	2	5	9	5	12
No. of H-bond donors	2	3	6	2	4
Lipophilicity					
Log Po/w (iLOGP)	1.98	2.26	1.64	2.82	3.24
Log Po/w (XLOGP3)	1.59	1.95	0.54	1.10	1.91
Log Po/w (WLOGP)	1.86	1.8	–0.5	1.29	2.21
Log Po/w (MLOGP)	0.97	1.52	–2.08	0.63	0.18
Log Po/w (SILICOS-IT)	2.78	2.18	–0.98	1.33	–0.05
Consensus Log Po/w	1.83	1.94	–0.28	1.43	1.50
Water Solubility					
Log S (ESOL)	–2.34	–3.26	–2.94	–1.88	–4.12
Solubility (mg/ml)	1.05E+00	2.07E–01	5.07E–01	4.16E+00	4.58E–02
Solubility (mol/l)	4.53E–03	5.52E–04	1.14E–03	1.33E–02	7.59E–05
Class	Soluble	Soluble	Soluble	Very soluble	Moderately soluble
Pharmacokinetics					
GI absorption	High	High	Low	High	Low
BBB permeant	Yes	No	No	No	No
P-gp substrate	No	Yes	Yes	Yes	Yes
CYP1A2 inhibitor	Yes	No	No	No	No
CYP2C19 inhibitor	No	No	No	No	No
CYP2C9 inhibitor	No	No	No	No	No
CYP2D6 inhibitor	No	No	No	No	No
CYP3A4 inhibitor	No	No	No	No	Yes
Bioavailability Score	0.55	0.55	0.11	0.55	0.17

3.3. Identification of genetic targets associated with COVID-19.

Total 15,721 COVID-19 related genes were retrieved from DisGeNET [580], GeneCards [14,606] and NCBI Gene [535]. Finally, we obtained 10,154 COVID-19 targets by merging its associated genes and DEGs from the GEO after removing duplications.

3.4. Common potential genetic targets of melatonin, other selected drugs on COVID-19.

Common potential targets for melatonin and the selected drugs on COVID-19 related genes, viz. melatonin [285], methylprednisolone [113], doxycycline [114], oseltamivir [47] and remdesivir [72] were isolated by Venn Diagram tool (Figure 4A-E, Table 2) and notably, it was highest for melatonin in compare to other studied drugs.

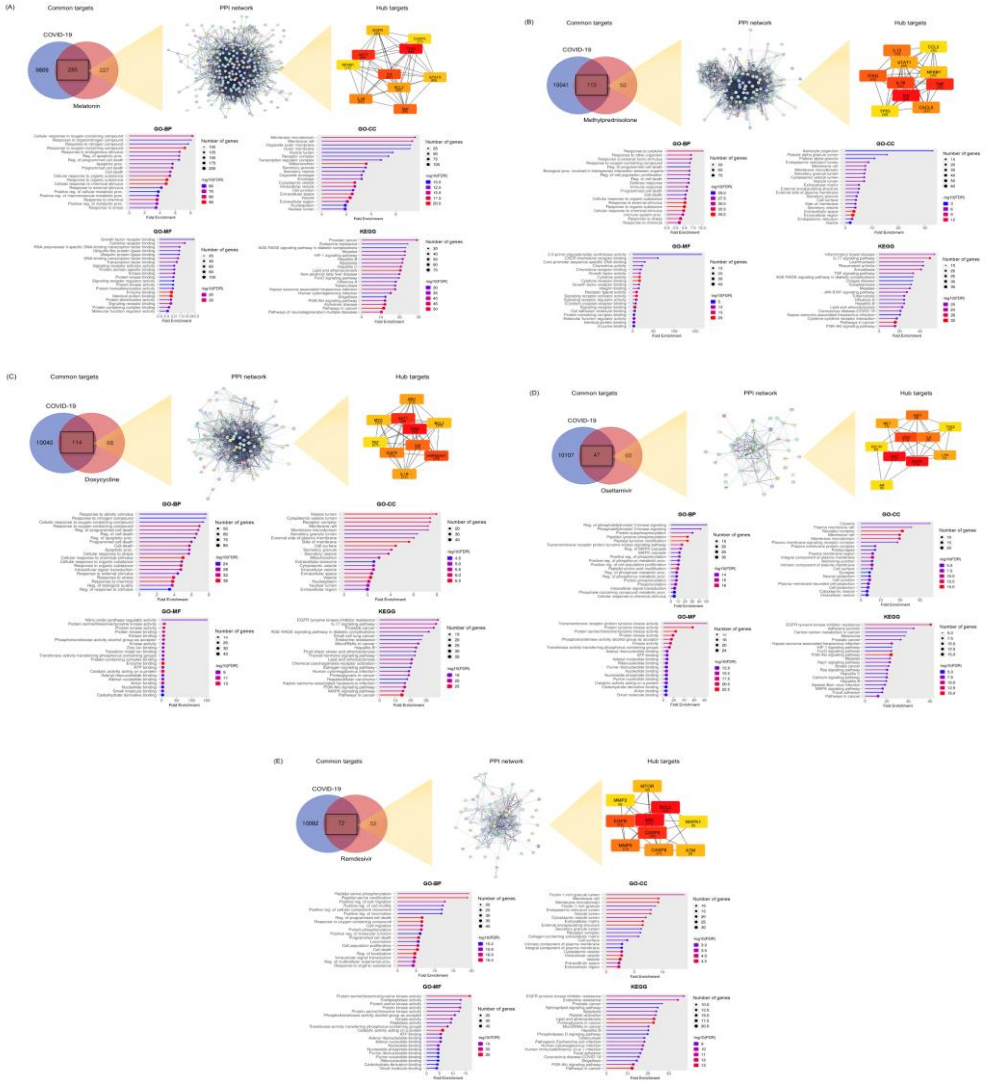

Fig. 4. The common potential targets in COVID-19 related genes.

PPI network of common targets (values within the rectangular box in Venn diagram), PPI network of hub targets (according to degree values mentioned within bracket for each protein, GO enrichment analysis of significantly enriched terms in biological processes (GO-BP), cellular components (GO-CC) and molecular functions (GO-MF) and significantly enriched KEGG pathways are demonstrated for (A) melatonin, (B) methylprednisolone, (C) doxycycline, (D) oseltamivir, (E) remdesivir.

3.5. GO enrichment analysis of common targets.

GO enrichment analyses on common targets were performed to investigate the biological processes, cellular components and molecular functions of melatonin and the selected drugs against COVID-19 (Figure 4A-E, Table 2). As a consequence, for melatonin: 2050 [BP: 1000, CC: 377, and MF: 673], GO terms were highlighted. Important representative GO-BP terms included: cellular response to oxygen-containing and organonitrogen compound, endogenous stimulus, regulation of apoptotic process.

3.6. KEGG enrichment analysis of common targets.

The KEGG enrichment analysis highlighted 245 pathways for melatonin. Important representatives were prostate cancer [hsa05215], endocrine resistance [hsa01522], AGE-RAGE signaling in diabetic complications [hsa04933], HIF-1 [hsa04066] and FoxO [hsa04068] signaling, apoptosis [hsa04210] (Figure 4A-E, Table 2).

3.7. Analysis of PPI network of common potential targets of melatonin and the selected drugs.

The PPI network revealed 282 nodes and 8088 edges with 57.4 average node degree for melatonin (Table 2). The network was constructed and subsequently the top ten hub targets for melatonin and the selected drugs were obtained on the basis of degree score analysis (Figure 4A-E). These top ten targets might play an essential role in the modulation of COVID-19 progression.

Table 2: Comparison of melatonin with other selected drugs against COVID-19 related genes on the basis of the values (in number) of common genetic targets.

Molecules	Number of CGT	GO			KEGG enrichment	PPI network analysis			Hub targets
		BP	CC	MF		Node	Edge	ADS	
Melatonin	285	1000	377	673	245	282	8088	57.4	TP53, AKT1, IL6, TNF, IL1B, BCL2, EGFR, STAT3, CASP3, NFKB1
Methylpre-dnisolone	113	1000	135	327	188	112	1816	32.4	IL6, TNF, IL1B, IFNG, IL10, CXCL8, STAT1, NFKB1, CCL2, TP53
Doxycycline	114	1000	524	274	229	114	1322	23.2	TP53, AKT1, HSP90AA, IL6, EGFR, IL1B, SRC, MYC, BCL2, TNF
Oseltamivir	47	1000	198	275	157	47	169	7.19	SRC, EGFR, JAK2, JAK1, IL6, LYN, MET, IGF1R, AR, TYK2
Remdesivir	72	1000	201	276	182	72	378	10.5	SRC, BCL2, CASP3, EGFR, MMP9, CASP8, ATM, MTOR, MMP2, MAPK1

BP: biological process. CC: cellular components, MF: molecular function, ADS: Average degree score, CGT: common genetic targets.

3.8. Molecular docking and PCA analyses.

Investigation of enrichment analyses (GO and KEGG), and the PPI network revealed that melatonin exhibited the highest values for enriched pathways, nodes and edges, as well as the greatest average node degree in compare to selected drugs against COVID-19 related genes. Consequently, ten hub targets were selected for molecular docking studies with melatonin (Figure 5A, B). Docking parameters (Table 3**)** such as binding energy (kcal/mol), ligand efficiency, reference Root Mean Square Deviation (RMSD), inhibition constant (Ki in μM), and the number of hydrogen bonds were obtained and analysed through PCA. It showed that the first two principal components (PC1 and PC2) have eigenvalues exceeding 1 (3.04 for PC1 and 1.00 for PC2) and collectively accounted for over 85% of the variance (68.15% for PC1 and 19.85% for PC2) (Figure 6). For each target protein, factor scores, contributions, and squared cosine values were calculated (Table 4). PC1 distinguished BCL2, TP53, and CASP3 from NFKB1, AKT1, and TNF, while PC2 differentiated IL6 from all other studied proteins. To identify the variables which influence the distinctions, the coefficient of correlation, contribution, and squared cosine values of the variables with respect to each principal component were examined (Table 5). It was found that binding energy, ligand efficiency, inhibition constant, and number of hydrogen bonds were most critical for interpreting PC1, while reference RMSD was pivotal for PC2. Ultimately, PCA identified seven key target proteins—BCL2, TP53, CASP3, NFKB1, AKT1, TNF, and IL6—based on their contribution and representation quality (as indicated by squared cosine values) in relation to the first two principal components and the studied variables.

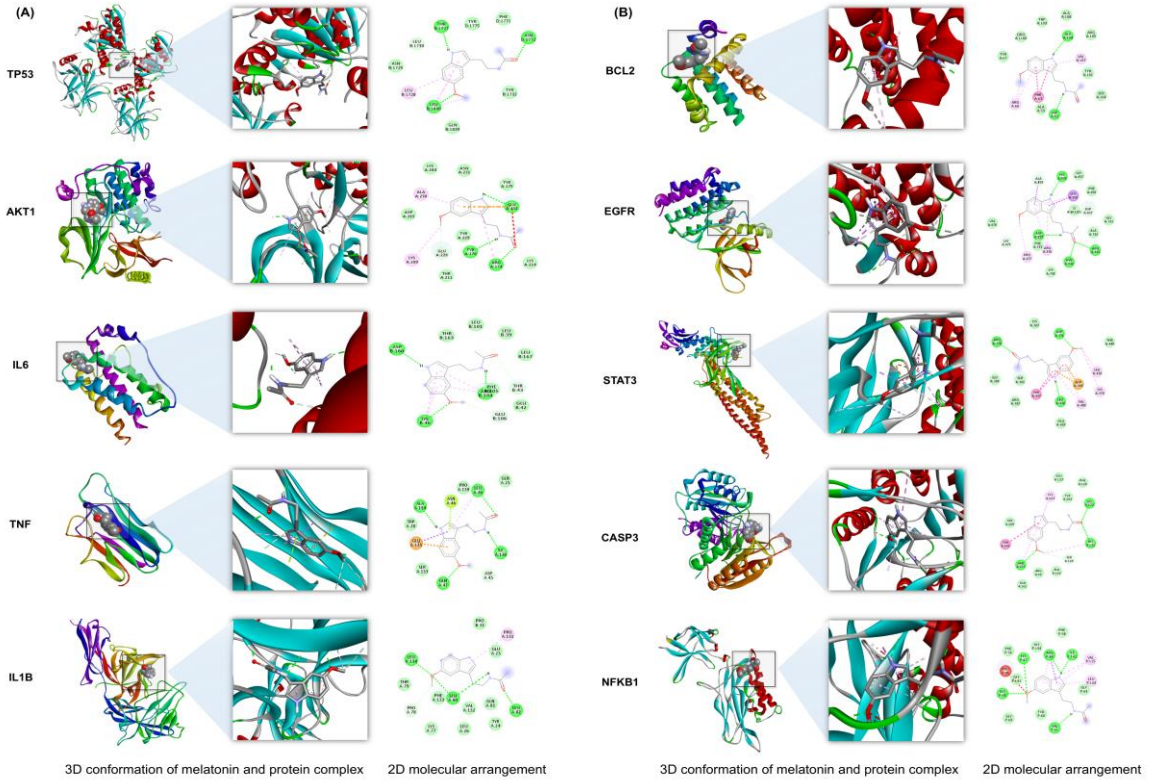

Fig. 5. Molecular conformations and the interaction of melatonin with regulatory proteins.

The 3D in left panel and corresponding 2D arrangements in right panel along with specific site of bond formation (inset) obtained by molecular docking analysis (A) TP53, AKT1, IL6, TNF, IL1B and (B) BCL2, EGFR, STAT3, CASP3, NFKB1.

Table 3: Results of the molecular docking analysis between hub targets and melatonin enlisting binding energy (kcal/mol), ligand efficiency, inhibition constant, Ki (µM), reference RMSD and number of hydrogen bonds.

Protein	Binding energy (kcal/mol)	Ligand efficiency	Inhibition constant (Ki) (µM)	Reference RMSD	Number of hydrogen bonds
TP53	–5.63	–0.33	74.81	61.08	3
AKT1	–6.95	–0.41	8.06	11.73	3
IL6	–5.96	–0.35	42.65	189.75	3
TNF	–6.39	–0.38	20.88	36.08	4
IL1B	–6.58	–0.39	15.03	33.62	3
BCL2	–5.28	–0.31	133.85	54.75	2
EGFR	–5.82	–0.34	54.15	49.98	4
STAT3	–6.23	–0.37	26.92	28.79	3
CASP3	–5.66	–0.33	70.60	45.36	3
NFKB1	–7.37	–0.43	3.97	66.40	5

Table 4: Values of factor score, contribution and squared cosine values for each individual (hub targets) obtained from PC analysis.

PC / SV	Factor scores		Contribution		SCV	
	PC1	PC2	PC1	PC2	PC1	PC2
TP53	**1.53**	–0.05	**6.89**	0.03	**0.96**	0
AKT1	**–1.78**	–1.03	**9.29**	10.59	**0.61**	0.20
IL6	0.88	**2.65**	2.27	**70.76**	0.09	**0.83**
TNF	**–1.16**	–0.19	**3.93**	0.36	**0.77**	0.02
IL1B	–1.02	–0.56	3.06	3.20	0.53	0.16
BCL2	**3.42**	–0.61	**34.34**	3.69	**0.93**	0.03
EGFR	0.39	0.08	0.45	0.06	0.09	0
STAT3	–0.29	–0.66	0.24	4.45	0.10	0.56
CASP3	**1.41**	–0.37	**5.82**	1.36	**0.87**	0.06
NFKB1	**–3.39**	0.74	**33.71**	5.51	**0.89**	0.04

*PC: principal components, SV: studied variable, SCV: squared cosine values. Values in bold are crucial for PC analysis***.**

Table 5: The correlation, contribution and squared cosine values for each variable.

PC / SV	PC1			PC2		
	Correlation	Contribution	SCV	Correlation	Contribution	SCV
Binding energy (kcal/mol)	**0.975**	**27.883**	**0.950**	0.009	0.008	0
Ligand efficiency	**0.973**	**27.803**	**0.947**	0.033	0.110	0.001
Inhibition constant (Ki, µM)	**0.937**	**25.740**	**0.877**	–0.077	0.602	0.006

Reference RMSD	0.234	1.602	0.055	**0.960**	**92.813**	**0.922**
Number of hydrogen bonds	**–0.760**	**16.972**	**0.578**	0.253	6.467	0.064

The values were generated by PCA analysis. The values in bold are important for PC analysis. PC: principal components, SV: studied variable, SCV: squared cosine values.

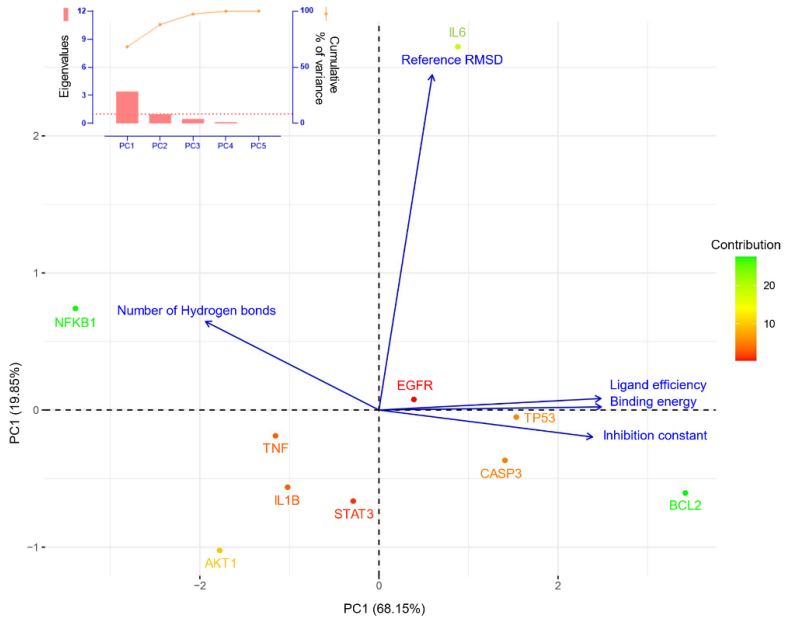

Fig. 6. Ordination diagram along with scree plot (inset) depicts the hub targets along with five major parameters, represented as a bi-plot across two principal components (PCs).

In this analysis, the data is condensed into two dimensions, PC1 and PC2, which account for 68.15% and 19.85% of the variance, respectively. The loading of each variable is visualized as pointed arrows corresponding to these principal components. The analysis focuses on five key parameters: binding energy, reference RMSD, inhibition constant, ligand efficiency, and the number of hydrogen bonds. Arrows lying on the same axis signify a positive correlation, while those on different axes indicate a negative correlation. The proteins are displayed as coloured dots, with the colour gradient ranging from green to red, representing the descending order of their contribution values.

4. DISCUSSION

Network pharmacology serves as a valuable approach for elucidating the role of a molecule within intricate physiological systems. In our current investigation, we tried to explore the potential efficacy of an endogenous molecule, melatonin as a therapeutic agent in compare to four commonly used drugs against COVID-19 including methylprednisolone, doxycycline, oseltamivir and remdesivir. In this context, ADME profiling, genetic target identification, PPI and enrichment analyses, hub target identification and molecular docking were combined to elucidate the effectiveness of melatonin against SARS-CoV-2 infection. In addition to intersecting with the greatest number of genetic targets associated with COVID-19, melatonin was demonstrated as the most significant representation among the other mostly used anti-COVID-19 drugs.

In this study, we first checked the physicochemical, pharmacokinetics, absorption and permeability of melatonin compared to the other selected drugs using the SwissADME *in silico* suite (31) (Table 1). The results showed that the relatively low molecular weight of melatonin (232.28) may enhance its GI tract permeability which is necessary for effectiveness of an orally administered drug. Melatonin is also present significant lipophilicity, thereby, impacting its cell membrane permeability (32). In addition, melatonin is unique in compare to the tested other medicines as it is not a substrate for P-gp, which generally functions as an efflux transporter to pump drugs back into the GI lumen, thereby, this feature renders its high availability in plasma and tissues (33). ADME data further revealed that melatonin exhibited inhibitory action on CYP1A2 enzyme (responsible for hepatic metabolism and excretion), it also argues in favour of its sustained bioavailability resulting prolonged physiological functions (34). Therefore, based on ADME profiles, melatonin emerges as a potent molecule which may serve as an effective therapeutic agent.

PPI network analysis and hub target identification enlisted 10 most crucial genetic targets of melatonin in regulating COVID-19 associated pathogenesis (Figure 4, Table 2). TP53 is one of these genetic targets with the highest degree score of 98. In terms of immune response modulation across various organs, TP53 has evolved to be recognized as the custodian of overall immune integrity, a role that becomes particularly pertinent in context of SARS-CoV-2 infections (35). In the realm of viral infections, it is thus unsurprising that TP53 deficiency renders the host more vulnerable and susceptible to infections (36). SARS-CoV-2 adopts a survival strategy to downregulate the basal TP53 levels by stabilizing its principal inhibitor, MDM2 (37). In this study, we observed that melatonin proficiently interacts TP53 with binding energy (kcal/mol) of –5.63, along with –0.33 ligand efficiency, having 3 participating hydrogen bonds (Table 3) and it is supposed to upregulate TP53 in COVID-19 (36). In accordance with the various approaches employed by different drugs to inhibit viral replication, potent and selective activator of TP53 could prove effective against SARS-CoV-2 infection (36, 38). Emerging researches suggested that pharmacological activation of TP53 by various natural compounds may offer an effective strategy to combat SARS-CoV-2 infection (38, 39). Furthermore, in our analysis, AKT1 emerged as a significant hub target with the degree score of 96 (second highest) (Table 2), underscoring its efficacy in inflammatory processes. AKT1, a member of the serine/threonine protein kinase, plays a pivotal role in both systemic and localized inflammatory responses by differentiation, proliferation, and migration of immune cells (40). Consequently, AKT1 emerges as a promising target with broad-spectrum antiviral activity (41). The present study highlighted the potential interaction of melatonin with AKT1 with –6.95 kcal/mol binding energy along with three hydrogen bonds and –0.41 ligand efficiency and melatonin may inhibit the activity of AKT1 (42) (Table 3). The excessive activation of AKT during viral infections may precipitate T-cell exhaustion and markedly heighten the mortality risk in SARS-CoV-2 patients (5). Thus, inhibiting the excessive activation of AKT during COVID-19 may serve to modulate the immune response and improve patient outcomes.

Proinflammatory cytokines, such as TNF-α, IL-1B, IL-6, IL-2, IL-7, and IL-10, implicated in the pathogenesis of ‘cytokine storm’ associated COVID-19 (43). Interleukin-6 (IL-6) is an essential cytokine exhibiting pleiotropic effects and functional redundancy, crucial for host defense mechanisms (5). In our analysis, IL-6 was ranked third based on the value of degree score (Table 2). As a pivotal component of the cytokine network, elevated levels of IL-6 have been recurrently reported in COVID-19 studies, indicating its potential utility as a prognostic marker for disease severity (39, 44). Furthermore, patients with severe COVID-19 exhibited a markedly higher IL-6/IFN ratio compared to those with mild symptoms, potentially attributable to a more intense ‘cytokine storm’ (45). Consequently, targeting IL-6 in COVID-19 appears to be a promising therapeutic strategy. We also observed that melatonin interacts

with IL-6 with a binding energy of –5.96 kcal/mol and a ligand efficiency of –0.35, forming three hydrogen bonds with the target (Table 3). This interaction suggests that melatonin may effectively engage with IL-6, exhibiting an inhibition constant (Ki) of 42.65 μM, indicative of its potential role against IL-6 induced 'cytokine storm'. Upon viral invasion, another key proinflammatory cytokine TNF-α, secreted by macrophages and monocytes, acts as an early effector to activate the immune defence mechanisms (46). It was ranked fourth among hub targets based on its degree score of 90 in our *in silico* analysis (Table 2). TNF-α signaling dysregulation can result in severe pathological outcomes in COVID-19 including the 'cytokine storm' induced inflammation and increased mortality of patients. The molecular docking analysis revealed that melatonin potentially interacts with TNF-α with a binding energy of –6.39 kcal/mol, with four hydrogen bonds and a ligand efficiency of –0.38, indictive of the inhibitory action of melatonin on this molecule. These findings suggest that melatonin may offer significant potential in treating COVID-19 by targeting TNF-α. Interleukin-IL1B, or IL-1β, is a key pro-inflammatory cytokine and a member of the IL-1 cytokine family. IL1B was ranked fifth as the degree score was 85 (Table 2). Elevated levels of IL-1β have been observed during direct infection of peripheral blood mononuclear cells (PBMCs) with SARS-CoV-2 (47). Consequently, several hypotheses and studies suggested that blocking IL-1β may mitigate the severity of 'cytokine storm' and alleviate symptoms in COVID-19 patients. So, the significant interaction of melatonin with IL1B, characterized by a docking score of –6.58 kcal/mol, a ligand efficiency of –0.39, an inhibition constant (Ki) of 15.03 μM, and three hydrogen bonds, suggesting that melatonin may modulate IL1B in the treatment of COVID-19 infection (Table 3). A few numbers of clinical evidences clearly confirmed that melatonin supplement significantly reduced levels of cytokines (IL6, TNF-α, IL1B) in COVID-19 patients (20, 22). The strong interactions of melatonin with these cytokine molecules may result in the reduction of 'cytokine storm' during viral infection. The immunoregulatory components of signaling pathways involved in the regulation of 'cytokine storm', contain a key transcription factor, nuclear factor kappa B (NF-κB) (20). In our current investigation, NFKB1 was identified as the 10th ranked hub target, with a degree score of 71 (Table 2). Recent studies underscore the critical role of NF-κB in mediating the pathophysiological features of SARS-CoV-2 infection, highlighting the impact of NF-κB activation within the infected cells (20, 48). The inhibition of the NF-κB pathway has been proposed as a promising useful strategy for mitigating severe SARS-CoV-2 infections (49). Our molecular docking studies also argued that melatonin potentially interacts with NF-κB with a substantial binding energy of –7.37 kcal/mol, a ligand efficiency of –0.43, and an inhibition constant (Ki) of 3.97 μM, with five interactive hydrogen bonds (Table 3). These highlights the involvement of melatonin in targeting NF-κB during neutralization of viral infection. Thus, melatonin may suppress the elevated 'cytokine storm' by modulating several key pro-inflammatory cytokines and their regulator, NF-κB. It was evidenced that, the antiviral and anti-inflammatory properties of melatonin exhibit significant potential in defence against COVID-19, primarily through the augmentation of humoral immunity by suppressing the release of IL-6, IL-1β, and TNFα (50).

Cellular apoptosis and the aberrant activation of caspases have been implicated in the hematological and immunological abnormalities observed in COVID-19 patients (51). The B cell lymphoma 2 (BCL-2) family of proteins is categorized into three distinct groups: anti-apoptotic proteins (BCL-2 and BCL-XL), pro-apoptotic pore-formers, and pro-apoptotic BH3-only proteins. Among these, BCL2, was ranked sixth in our analysis with a degree score of 84, is a prominent anti-apoptotic member of the BCL-2 family. The apoptosis induced by SARS-CoV-2 infection can be inhibited by the overexpression of BCL-2 but till date, no effective treatments targeting BCL-2 family proteins have been developed (52). In our analysis, an interaction between melatonin and BCL2, with a docking score of –5.28 kcal/mol

and a ligand efficiency of –0.31 was found. Additionally, caspases, which mediate apoptosis, are classified into initiator caspases (e.g., caspases 8, 9, and 10) and effector caspases (e.g., caspases 3, 6, and 7) depending on their role in the apoptotic signaling (53). Interestingly, Caspase-3 (CASP3) emerged as one of the top ten hub targets in present study, with a degree score of 71. Increased levels of active caspase-3 have been observed in SARS-CoV-2 infected human cortical organoids and glial cells, underscoring the role of virus in inducing apoptosis (54). Therapeutic strategies targeting caspase associated inflammation and cell death could involve direct modulation of its activity (55). Present molecular docking studies demonstrated a significant binding affinity of melatonin with caspase-3 by binding energy of –5.66 kcal/mol, a ligand efficiency of –0.33, and an inhibition constant (Ki) of 70.6 µM, along with three interacting hydrogen bonds (Table 3). The results hypothesize that melatonin may be used as a therapeutic agent by the suppression of apoptotic activity in COVID-19 pathogenesis (56). Pulmonary fibrosis, represents a severe sequela of respiratory viral infections including COVID-19, results from overactive epidermal growth factor receptor (EGFR) signaling (57). It is a 170-kDa transmembrane glycoprotein classified within the receptor tyrosine kinase (RTK) family. A clinical feature of COVID-19 is marked by EGFR mediated mucus production and hypoxia leads to increased morbidity and mortality (58), thereby EGFR may be a potential therapeutic target. In our study, EGFR emerged as one of the hub targets, with a degree score of 80 and melatonin was demonstrated having a potential interaction with EGFR, with a binding energy of –5.82 kcal/mol, a ligand efficiency of –0.34, and an inhibition constant (Ki) of 54.15 µM, alongside four interacting hydrogen bonds (Table 3). Very recently, advanced docking analyses also revealed that the binding affinity between EGFR and the SARS-CoV-2 spike (S) protein is comparable to that observed between ACE2 and the S protein (59). Another important marker of COVID-19 is lung fibrosis occurs by the activation of signal transducer and activator of transcription (STAT3) protein. In the current study, STAT3 emerged as one of the top ten hub targets, with a degree score of 80. The IL-6/STAT3 axis plays a significant role in the pathogenesis of COVID-19 by the development of 'cytokine storm' (60). Our molecular docking analysis revealed that melatonin exhibits a significant potential to interact with STAT3 with three interacting hydrogen bonds. But till to now, no comparable reports are available to illustrate the potential of melatonin in the regulation of lung fibrosis and hypoxia during COVID-19 pathogenesis in any animal model.

In conclusion, melatonin emerges as a potential modulator of COVID-19 pathology through its interactions with a network of key genetic targets, including TP53, AKT1, IL6, TNF, IL1B, BCL2, EGFR, STAT3, CASP3, and NFKB1. Specifically, the top GO terms enriched by targets of melatonin reflect its involvement in critical pathways related to stress, immune responses, and apoptosis, which are pivotal in the context of SARS-CoV-2 infection. Moreover, effect of melatonin on several significantly enriched KEGG pathways suggests its multifaceted approaches to modulating COVID-19 pathogenesis from inflammation and immune response to cell death and pulmonary fibrosis. Through the integration of information gathered from various pathways, pharmacokinetics, effectiveness, safety profiles, and molecular docking, the study provides a robust framework for understanding how melatonin modulates immune responses and inflammation. These findings highlight the broader evaluation of melatonin as potential prophylactic and therapeutic agent in comprehensive clinical investigations against COVID-19 progression. Despite these promising findings, the proposed application of melatonin against COVID-19 remains speculative without empirical validation regarding its methods of application. For instance, while melatonin did not significantly reduce overall mortality caused by COVID-19, subgroup analyses advocated potential aids for patients under the age of 55 and those who received treatment for more than 10 days (61). In a very recent placebo-controlled trial

(double-blind and randomized), it was indicated that no statistically significant difference was found between the melatonin and placebo groups regarding clinical symptom improvement, mortality rate, adverse effects, or various blood markers suggests that melatonin may not have a measurable impact on these specific outcomes (62). These inconsistences with other reports might be due to the different doses of melatonin used. The general concept is that this devastating SARS-CoV-2 infection, especially the server cases, requires extremely high doses of melatonin intervention and the recommended dose is large than 40 mg/dose (63, 64). Therefore, these inconsistences and its theoretical benefits, highlights the need for further investigation to better understand the role of melatonin in treating COVID-19. However, the current evidence is supported by a few small-scale studies or meta-analyses, necessitating larger, well controlled randomized trials to justify the therapeutic potential of melatonin comprehensively.

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AUTHORSHIP

AA conceptualized the experiment, set up methodologies, investigated and analysed data and written original draft. KNH supervised, acquired funding, conceptualized the experiment, set up methodologies, analyzed data, and performed writing- review & editing.

CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

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