



Research article

A network-biology led computational drug repurposing strategy to prioritize therapeutic options for COVID-19

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ARTICLE INFO

Keywords:

Network-biology
Quercetin
2-DG
N-acetyl-Cysteine

ABSTRACT

The alarming pandemic situation of novel Severe Acute Respiratory Syndrome Coronavirus 2 (nSARS-CoV-2) infection, high drug development cost and slow process of drug discovery have made repositioning of existing drugs for therapeutics a popular alternative. It involves the repurposing of existing safe compounds which results in low overall development costs and shorter development timeline. In the present study, a computational network-biology approach has been used for comparing three candidate drugs i.e. quercetin, N-acetyl cysteine (NAC), and 2-deoxy-glucose (2-DG) to be effectively repurposed against COVID-19. For this, the associations between these drugs and genes of Severe Acute Respiratory Syndrome (SARS) and the Middle East Respiratory Syndrome (MERS) diseases were retrieved and a directed drug-gene-gene-disease interaction network was constructed. Further, to quantify the associations between a target gene and a disease gene, the shortest paths from the target gene to the disease genes were identified. A vector DV was calculated to represent the extent to which a disease gene was influenced by these drugs. Quercetin was quantified as the best among the three drugs, suited for repurposing with DV of -70.19, followed by NAC with DV of -39.99 and 2-DG with DV of -13.71. The drugs were also assessed for their safety and efficacy balance (in terms of therapeutic index) using network properties. It was found that quercetin was a forerunner than other two drugs.

1. Introduction

The 2019 novel coronavirus (2019-nCoV/SARS-CoV-2), as the cause of coronavirus disease 2019 (abbreviated as COVID-19) has become a health concern to the entire world. As of September 2020 there has been 27 million confirmed cases of COVID-19, including 904,000 deaths for the 2019-nCoV/SARS-CoV-2 outbreak worldwide, as reported to WHO. The causative agent is novel Severe acute respiratory syndrome coronavirus 2 (nSARS-CoV-2). Other similar agents were previously known are Middle East Respiratory Syndrome coronavirus (MERS-CoV) and Severe Acute Respiratory Syndrome coronavirus (SARS-CoV). SARS-CoV are enveloped single-stranded, positive-strand ribonucleic acid (RNA) viruses and belong to Coronaviridae family [1]. The structure of SARS-CoV-2 is in close resemblance to that of SARS-CoV. The SARS family contains 14 host-binding amino acids. Out of these, 8 amino acids are specifically conserved for SARS-CoV-2 [1]. The binding residues of the family interact with the ACE-2 (Angiotensin converting enzyme-2) of the host directly. The most common symptoms of COVID-19 include fever, malaise, nasal congestion, dry cough, sore throat, dyspnoea,

diarrhoea, muscle aches, shortness of breath and multiple organ complications. However, some people serve as asymptomatic carriers of the disease. Currently, no drug or vaccine is available for coping this disease. Precautionary measures are presently the sole approach to prevent person to person transmission.

In the meantime, efforts are underway to develop an effective method of treatment or vaccine. The development of a new molecule is labour-intensive and time-consuming process. It has been assessed that an approved new chemical entity costs more than 1.5 billion and takes 13.5 years from discovery to the market [2]. There are several innovative technologies and approaches to reduce the cost, time and labour and increase the efficiency of drug discovery and development. Drug-repurposing, represented as identification of therapeutically potent molecules against the disease from a library of pre-existing molecule; could considerably shorten the time and reduce the cost compared to de-novo drug discovery and randomized clinical trials. Experimental methodologies for drug repurposing are expensive, laborious and slow [3]. In-silico approaches offer approaches for directed systematic drug repositioning. It offers novel testable hypotheses regarding usage,

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effectiveness, harm and hence supplement evidence generated in randomized controlled trials [3, 4, 5, 6, 7].

The repurpose strategy of existing drugs has led to identification of a number of candidate molecules for COVID-19 treatment [8]. As on date, there are nearly 80 clinical trials underway in China on potential treatments [9]. WHO has also recently announced restricted use permission for repurposed anti-HIV, anti-malarial, anti-flu and anti-Ebola drugs [10, 11]. Three promising molecules that have been proposed as effective therapeutic molecules against COVID-19 are Quercetin (<https://clinicaltrials.gov/ct2/show/NCT04377789>) N-acetyl Cysteine (<https://clinicaltrials.gov/ct2/show/NCT04419025>) and 2-Deoxy-D-Glucose (2-DG).

1.1. Quercetin and nSARS-CoV-2

Quercetin is a naturally occurring plant pigment (flavonoid) present in colorful fruits and vegetables like berries, onions, red wine, green tea, and apples. It has innumerable health benefits and distinctive biological properties that may improve mental/physical performance and immunity [12]. These properties include anti-carcinogenic, anti-inflammatory, antiviral, antioxidant, psychostimulant, lipid peroxidation inhibition, platelet aggregation and capillary permeability, mitochondrial biogenesis stimulation [13]. It has antioxidant properties and strong ability to fight free-radical molecules. It also has senolytic or anti-cellular-senescence properties [14]. Thus it is a common ingredient in medicine, supplements, and skincare products. Quercetin and its major metabolites, such as quercetin 3-beta-O-d-glucoside (Q3G, also called isoquercetin), have been found in laboratory studies to inhibit a wide variety of viruses, including SARS-CoV, which is related to COVID-19 [15,16,17,18,19,20]. Some studies have proposed the role of senolytic compounds for the treatment/prevention of corona virus infection [21]. Quercetin is a well-known anti-senescence molecule and thus proposed as an effective molecule against COVID-19 [21]. Additionally, docking analysis have ascertained that 'darunavir-quercetin-3- rhamnoside' is a potent and less toxic drug chemical-cocktail/formulation against SARS-CoV-2-Mpro. Additionally, predicted toxicity and pharmacokinetics profiles also support the hypothesized formulation [22]. In-silico molecular dynamics docking using energy-minimized states of the flavonoids was performed to assess their use against SARS-Cov-2. Quercetin and isoquercetin were among the top 10 flavonoids identified [23]. The S glycoprotein of the virus is cleaved by endosomal proteases cathepsin B/L into S1 and S2 subunits and thereafter the S2 subunit-mediates membrane fusion [24]. Quercetin which is a common and readily available supplement is a strong cathepsin inhibitor with an IC50 in the low micromolar range [25]. Therefore, it is considered as an adjuvant to prevent or to weaken the course of viral infections. The CoV genome encodes two proteases, papain-like protease (PLpro) and 3-chymotrypsin-like protease (3CLpro), also known as the main protease (Mpro), that are important for virus replication [26]. These proteases are among the most promising drug targets. Interestingly, in 3CLpro all residues involved in the dimerization, substrate binding, and catalysis are 100% conserved between SARS-CoV and SARS-CoV-2 [27]. Remarkably, quercetin has a strong binding affinity for 3CLpro [28]. Quercetin thus may block viral replication via inhibition of the viral cysteine protease 3CLpro [29]. Some studies have also proposed quercetin could possibly replace HCQ and be used as prophylactic applications, specifically for the more vulnerable individuals such as the elderly, diabetics, immuno-compromised and those with coronary ailments [29]. A recent study utilises the gene expression profiles of Vitamin D and Quercetin activities to assess them as viable candidates for their potential utility as COVID-19 pandemic mitigation agents [30]. Based on this study, a randomized interventional clinical trial entitled "Phase II Clinical Trial of Estradiol to Reduce Severity of COVID19 Infection in COVID19+ and Presumptive COVID19 + Patients" (<https://clinicaltrials.gov/ct2/show/NCT04359329>) and two interventional randomized clinical trials evaluating effects of Vitamin D on prevention and treatment of COVID-19 NCT04334005 and NCT04344041 have been listed on [ClinicalT](https://clinicaltrials.gov)

[rials.gov](https://clinicaltrials.gov) website. In a recent study, using supercomputer-based in silico drug-docking to the COVID-19 viral spike protein, quercetin was identified among top 5 scoring ligands for viral S-protein-human ACE2 receptor interface [31]. Studies also show that administration of quercetin has resulted in significantly decreased expression of the ACE2 gene during differentiation of human intestinal cells [30]. Quercetin may thus inhibit the binding and entry of the virus in the cell [18, 32]. Molecular docking evaluation of a marine drug metabolite database show that algae polyphenols and quercetin derivatives can be successfully used as treatment against SARS-CoV-2 [33,34]. One of the world's most cited scientists, Michel Chrétien, has received 1 million dollars donation to begin a clinical trial of quercetin against COVID-19. Quercetin has already been approved as safe for human consumption by U.S.-based Food and Drug Administration, which means that if the results are encouraging, the treatment will be readily available.

1.2. 2-DG and nSARS-CoV-2

2-Deoxy-D-Glucose (2-DG) is a dual decoy of glucose and mannose. It has one of the hydroxyl groups found in the chemical structure of natural glucose replaced by hydrogen. This renders 2-DG ineffective to be converted into energy or support proper glycosylation. It is thus an inhibitor of hexokinase during glycolysis. After the entry of the virus, there is an increased demand of nutrients and energy for the replication of the virus. Thus, the host cells have been observed to undergo metabolic reprogramming. 2-DG acts as a dual inhibitor of glycolysis as well as glycosylation [35]. This way it hinders in virus replication; its increased energy needs and viral assembly; thus might serve as a probable drug candidate. 2-DG has already been granted permission for clinical trials for other diseases [36, 37, 38]. Some in silico studies have tried to investigate glucose anti-metabolite, 2-DG as a repurposed drug for the treatment of novel SARS-CoV-2 virus. These studies have revealed that 2-DG can dock efficiently with viral main protease 3CLpro as well as NSP15 endoribonuclease, and efficiently inactivate these viral receptors leading to incapacitation of the virus [39]. A recent study demonstrated that viruses induce an anabolic state in their host cells. This causes these infected cells to upregulate their energy production using glycolysis as compared with their healthy neighbours [35]. Studies show that nontoxic levels 2-DG prevented SARS-CoV-2 replication in Caco-2 cells by blocking carbon metabolism (glycolysis). Importantly, multiple independent studies have shown the disruption of both glycolysis and glycosylation, can have a potent effect against viruses like coronavirus [35, 40, 41, 42, 43, 44]. One prodrug of 2-DG, namely, 3,6-di-O-acetyl-2-deoxy-d-glucose (WP1122) has been developed in Dr. Waldemar Priebe's laboratory and is owned by the US company Moleculin Biotech. This compound is currently being tested as an antiviral drug for targeting the novel Coronavirus [45] and the company is initiating clinical trials on this drug to fight COVID-19 [46]. Proteomics of SARS-CoV-2 infected host cells revealed that the virus reforms central cellular pathways such as translation, splicing, carbon metabolism, protein homeostasis (proteostasis) and nucleic acid metabolism. Inhibitors that target these pathways prevented viral replication in cells. The authors reported that blocking glycolysis with non-toxic concentrations of 2-DG completely prevented SARS-CoV-2 replication in human cells [47].

1.3. N-acetyl cysteine and nSARS-CoV-2

N-acetylcysteine (NAC) is reported as a potential candidate for treatment, preventive and/or adjuvant against nSARS-CoV-2 due to a variety of potential properties that include: increases glutathione, improves T cell response and anti-inflammation thus reducing the effect of viral induced oxidative stress and cell death [48]. The free sulfhydryl group of NAC helps reduce the disulphide bonds in the mucus glycoproteins matrix and hence helps in reducing mucus viscosity [49, 50]. Under oxidative stress, NAC also improves redox status by acting as a precursor to cysteine (required for glutathione synthesis), and the ability

to replenish thiol pools that help to regulate the redox state [48]. Severe SARS-CoV-2 infection is associated with hallmark responses like high viral load, T cell exhaustion, and high levels of TNF- α , IL1 β , IL6, IL10. Thus NAC helps reduce the oxidative stress created by cytokine storm syndrome and production of Reactive Oxygen Species (ROS) may be attenuated by NAC's antioxidant effect [49]. NAC is a precursor of glutathione and has antioxidant properties as it acts as a direct scavenger of Reactive Oxygen Species to regulate redox status and modulate inflammatory response [51]. NAC is also proven to decrease airway inflammation and responsiveness in asthma, by modulating the tight junctional protein claudin 18 expression present in airway epithelial cells [52]. The E protein of SARS-CoV interacts with the S-protein through disulphide bonds which can potentially be broken by NAC, thus reducing the viral infectivity [53]. NAC may also decrease pulmonary disease severity by blocking excessive production of angiotensin II [54]. In vitro and clinical studies have shown that NAC can block ACE. This study also demonstrated the decrease of Angiotensin II (ACE2) plasma concentrations just after 2hrs of NAC administration, suggesting that by blocking ACE, it can reduce angiotensin II expression and can inhibit nSARS-CoV-2 viral endocytosis [49]. NAC is known to prevent protein glycosylation. This way it can attenuate the glycosylation events in nSARS-CoV-2 thus decreasing its infectivity. Severe COVID cases exhibit lower GSH and higher ROS levels [55]. NAC is a known inhibitor of NF- κ B activation and promotes restoration of thiol pools thus allowing ROS scavenging. This way it may reduce the associated pathologies. NAC has been used in many clinical trials investigating the use of its for treating respiratory illness in humans e.g. Intravenous administered NAC has been used for the treatment of Acute respiratory distress syndrome (ARDS) [48, 56]. Similarly, clinical trial NCT03364218 and NCT03720483 of nebulized NAC has improved arterial oxygen tension in patients suffering from Bronchiolitis; and has attenuated pulmonary fibrosis [57, 58, 59]. NAC also has a good safety record in clinical trials. Combination of low oral doses of hydroxychloroquine and intravenous NAC in COVID patient with multisystem organ dysfunction, including acute respiratory insufficiency, yielded positive results. There was a measurable decrease in of several of the inflammatory markers [60, 61]. Hence a human clinical trial NCT04374461 for using NAC against COVID-19 virus has been recently started sponsored by Prisma Health-Midlands.

Over the years, several approaches are used in computational drug repositioning. Compared to traditional drug repositioning and discovery strategies, network analysis and systems medicine approaches address both depth and breadth of biological knowledge and help to infer molecular connections to discover therapies [62, 63]. Network based approaches allow widespread, extensive and integrated investigation of multiple biological and molecular datasets to understand vital pathways and can identify functional modules behind multifactorial diseases [64]. These approaches are beneficial to target and potentially help in discovering underlying mechanism responsible for a certain disease pathology and hence may evolve as a potential drug repositioning strategy for complex diseases with varying degree of clinical heterogeneity [65]. Several studies have used different network centric method for drug repurposing e.g. network-based guilt-by-association method (GBA) for drug-disease association prediction-it made suggestions to repurpose drugs based on shared treatment profile from different disease pairs [66]. Another study integrated disease-gene expression signatures, drug targets, disease information, and molecular interaction network to prioritize drug targets for drug repositioning [67]. Some studies have tried to quantify the interplay between the drug targets and the disease-specific proteins in the human interactome by a network-based similarity measure. The index then prioritizes associations between drugs and diseases locating in the same network neighborhoods [68].

The present study tries to explore the effectiveness of Quercetin or NAC or 2-DG as a repurposed drug using shortest path in the drug-gene-disease network to identify the extent these drugs affect the SARS/MERS-associated genes. This extent has been quantified and represented in a vector called DV that helps to prioritise these repurposed drugs.

2. Material and methods

2.1. Collection of disease-genes and construction of disease-gene network

Figure 1 provides a graphical view of the network-based approach to compare Quercetin, NAC, and 2-DG effectiveness against COVID-19 as a repurposed drug. The methodology has been accepted from previous study of Taekeon Lee and Youngmi Yoon et al. [69]. First a directed disease-gene network was constructed. COVID-19 infection is caused by a recently discovered nSARS-CoV-2 that began spreading in December 2019, so there were very few validated-gene-targets available and these were not sufficient to construct a comprehensive disease-gene network. The nSARS-CoV-2 shares an ancestral origin with betacoronavirus like SARS-CoV, MERS-CoV, 229E, HKU1, NL63, and OC43. But SARS-CoV and MERS-CoV are the only two betacoronavirus that cause severe respiratory illnesses in humans. Also, nSARS-CoV-2 shares 82% of its genome with human SARS-CoV and around 43% identity with MERS-CoV as second best [70]. Therefore, SARS and MERS were used to identify the disease associated genes. The disease (SARS and MERS)-gene associations were fetched from DisGeNet [71]. DisGeNet is the largest publicly available repository of genes targets associated with human diseases that have been collected from curated repositories, GWAS catalogs, animal models, and the scientific literature.

The genes associated with these diseases were submitted to Graphite tool to construct a directed disease-gene network [72]. Graphite constructs a directed network by integrating genes and interactions between genes from different pathways repositories such as BioCarta [73], Reactome [74], PID [75], and KEGG [76]. It provides directionality as well as the relationship (positive/negative/neutral) to every interaction between the genes. This relationship was "positive" when activation or expression was recorded, was "negative" when inhibition or repression was recorded, and "neutral" when "binding" was recorded [77]. A directed disease-gene network was thus constructed for further analysis.

2.2. Construction of drug-gene-disease directed networks

For each drug (Quercetin, NAC, and 2-DG) a comprehensive non-redundant list of associated drug-genes was curated by extensive, manual literature-survey. To increase reliability, the search was constrained to docking and experimentally validated in-vivo and in-vitro studies. These curated drug-gene interactions were assigned as positive/negative/neutral as per drug agonist/antagonist/binding effect on its gene target. These drug-gene targets were mapped to the disease-gene network to construct a directed drug-gene-gene-disease network. Thus three such networks corresponding to three drugs namely quercetin, NAC and 2DG were constructed. The drug-gene-gene-disease directed networks were visualized in Cytoscape.3.6.0.

2.3. Shortest paths and DV calculation from a drug-gene to a disease-gene

To calculate the associations between a drug and a disease, the shortest paths from drug-genes to disease-genes were identified. For this, the methodology was adopted from Taekeon Lee & Youngmi Yoon et al which has been validated on 7083 associations between 1163 drugs and 298 diseases [69]. They presume that neutral relationships maintain general actions in the body and drugs affect diseases by regulating disease-related genes either by activation or inhibition Pesca 3.8.0 plugin of Cytoscape was used to identify all the shortest paths between the drug-gene to the disease-gene in the three networks [78].

The extent to which a drug affects the disease-associated genes can be determined using vector DV [69]. To calculate DV, the associations between a drug-gene and a disease-gene in the shortest path were calculated using types and weights of the shortest paths. The type of path (T_k) was calculated by only "positive" and "negative" relationships between genes comprising the path. Thus, 1 and -1 are assigned to each positive and negative edge in the path respectively. The "neutral" path was

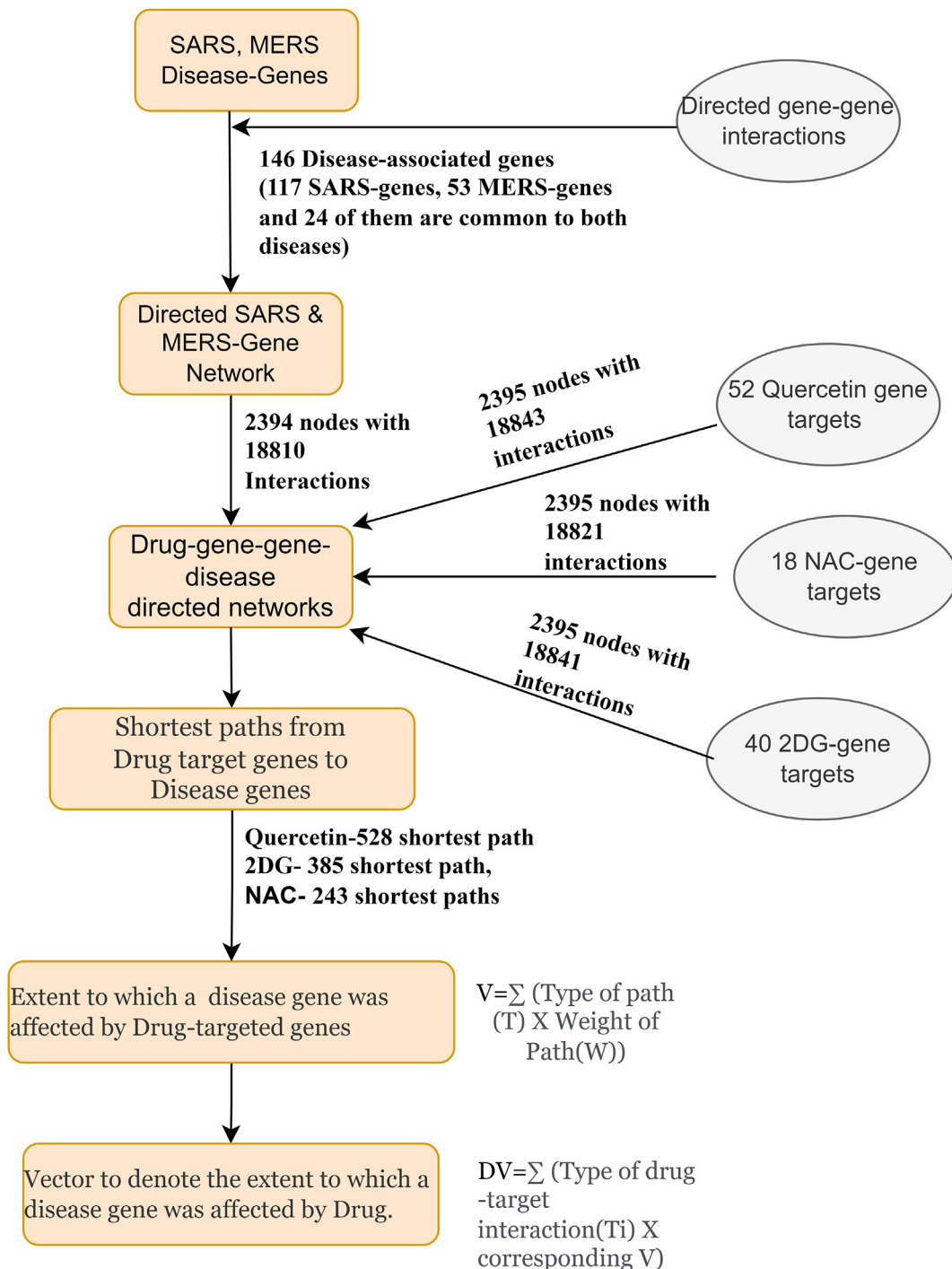


Figure 1. Graphical representation of methodology followed to compare Quercetin, NAC and 2-DG effectiveness against COVID-19 using DV vector.

assigned as 0 to nullify the neutral relationship. The path type (T_k) was defined by multiplying the values corresponding to edges on the path. Path weight (W_k), was calculated as the product of the reciprocals of the out-degree of the corresponding node on a path. To consider the extent to which a disease-gene was affected by a drug-gene, V was calculated. V is the sum of all products of weight (W) and path type (T) on the given shortest path (Equation 1).

$$V = \sum_{k=1}^n (T_k \times W_k) \tag{1}$$

To express the effects of quercetin, NAC and 2-DG on the disease, a vector (DV) for each drug-gene-gene-disease path was calculated. DV is

denoted as the product of (V_k) and the corresponding type of drug-target interaction (Td_k) (Equation 2).

$$DV = \sum_{k=1}^n (\text{sign}(Td_k) \times V_k) \quad \text{sign}(Td_k) = \begin{cases} 1 & \text{if } Td_k > 0 \text{ (positive)}, \\ 0 & \text{if } Td_k = 0 \text{ (neutral)}, \\ -1 & \text{if } Td_k < 0 \text{ (negative)} \end{cases} \tag{2}$$

2.4. Network properties and biological system features to assess efficacy and safety balance

The network properties for each drug-gene in the network were generated by PROFEAT [79]. A total of 31 network properties were

calculated for subsequent analysis (Table 1). These properties are popularly used for analysing complex biological networks.

The number of affiliated pathways associated to each drug-gene was obtained from CTD [80]. To assess for collateral effect, the number of human similarity proteins of the gene were determined. This was done by BLAST similarity screening of human proteome (NCBI) with a cutoff (E-value < 0.005) [81].

A list of 1,580 human core fitness genes or core-essential-genes was obtained [82]. These are the genes that seem to be constitutively essential for human-system irrespective of context. The top 50 direct interactors of each drug-gene were obtained from STRING [83]. For each drug-gene interactor list, the percentage of core-essential-genes was identified. It is assumed that drugs that target core-essential-genes of the human proteome might have lower safety levels.

3. Results

A list of 146 non-redundant disease-genes was identified from the DisGeNet database. The list includes 117 and 53 genes for SARS and MERS respectively with 24 genes common to both the diseases. A directed disease-gene-gene network having 2394 genes connected via 18810 interactions have constructed by graphite tool. These 18810 interactions of the network were classified into 12306 positive, 3055 negatives, and 3449 neutral interactions.

Further 52, 18, and 40 experimentally validated drug-gene interactions of quercetin, NAC, and 2-DG respectively were manually curated by extensive literature survey. Also, with each drug its interaction characteristics with the target gene are considered i.e. positive (+1) for agonist, negative (-1) for antagonist and neutral (0) for just binding interaction.

Further, these genes were mapped to the disease-gene-gene network. This way 33, 11, and 31 genes for quercetin, NAC, and 2-DG were retained in the network. Finally, three networks i.e. quercetin-gene-gene-disease directed network having 2395 nodes and 18843 interactions (Figure 2), NAC-gene-gene disease directed network having 2395 nodes and 18821 interactions (Figure 3) and 2-DG-gene-gene-disease directed network having 2395 and 18841 interactions (Figure 4) were constructed and visualized in Cytoscape.3.6.0.

3.1. Shortest path and DV vector

To calculate the association between a drug i.e. quercetin, 2-DG, NAC, and diseases (SARS and MERS), the shortest paths between drug-genes and disease-genes were identified in all the three networks as explained in detail in the methodology section.

In quercetin-gene-gene-disease network, 528 shortest paths were identified (Figure 5). 1% of the shortest path had common genes interacting with both the drug and the disease directly. These include ESR1, EGFR, ACE, CASP3, BCL2L1 (highlighted as yellow nodes in Figure 5). 7% of the shortest path had no connecting gene between drug-gene and disease-gene pairs. Lastly, 79% of these shortest-path contain 1 connecting gene (highlighted as green nodes in Figure 5) between drug-gene and disease-gene, whereas 13% of these shortest-path contains 2 connecting genes (highlighted as pink nodes in Figure 5) between drug-gene and disease-gene (Figure 5). This shows that quercetin could be an effective drug and would be able to affect many disease-associated genes.

Likewise, NAC-gene-gene-disease directed network contains 243 shortest paths of 11 drug-genes (Figure 6). 8% of these shortest paths have no connecting genes. Whereas, 22% and 79% of the shortest path

Table 1. The 31 network properties calculated for the drug-gene-gene-disease network.

1	Degree	The number of edges linked to a node
2	Scaled Connectivity	The degree of a studied node relative to the most connected node within the same module
3	Number of Selfloops	The number of edges starting and ending at the same node
4	Number of Triangles	The number of triangles that include the studied node as a vertex
5	Z Score	A connectivity index based on degree distribution of a network.
6	Clustering Coefficient	The number of the connected pairs between all neighbors of node
7	Neighborhood Connectivity	The average connectivity of all neighbors
8	Topological Coefficient	The extent to which a node in network shares interaction partners with other nodes
9	Interconnectivity	A connectivity index indicating the quality of the studied nodes being connected together
10	Bridging Coefficient	The extent of the studied node lying between any other densely connected nodes in the network
11	Degree Centrality	The number of links incident upon a studied node
12	Avg Shortest Path Length	The average length of shortest paths between the studied node and all other ones
13	Distance Sum	The sum of all shortest paths starting from the studied node
14	Eccentricity	The maximum non-infinite shortest path length between the studied node and all other nodes in the network
15	Eccentric	The absolute difference between nodes' eccentricities and network's average eccentricity
16	Deviation	The variation between sum of node distances and network unipolarity
17	Distance Deviation	The absolute difference between nodes' distance sum and network's average distance
18	Radiality	The level of reachability of a studied node via various shortest paths within the entire network
19	Closeness Centrality (avg)	The average number of steps required to reach the studied node from any node in a network
20	Closeness Centrality (sum)	The reciprocal of the sum of the shortest paths between the studied node and all other nodes in the network
21	Eccentricity Centrality	The largest geodesic distance between the node and any other node
22	Harmonic Closeness Centrality	The sum of the reciprocals of the average shortest path lengths of each node in network
23	Residual Closeness Centrality	The closeness measured by removing the studied node
24	Load Centrality	The fraction of all the shortest paths that pass through the studied node
25	Betweenness Centrality	The number of times the studied node serving as a linking bridge along shortest path between any two nodes
26	Normalized Betweenness	The fraction of network shortest paths that a given protein lies on
27	Bridging Centrality	The product of the bridging coefficient and betweenness centrality
28	CurrentFlow Betweenness	A centrality index measuring the level of information travels along all possible paths within network
29	CurrentFlow Closeness	The variant of current flow betweenness
30	Eigenvector Centrality	The sum of its neighbors centrality values
31	Page Rank Centrality	An adjustment of Katz by considering the diluted issue

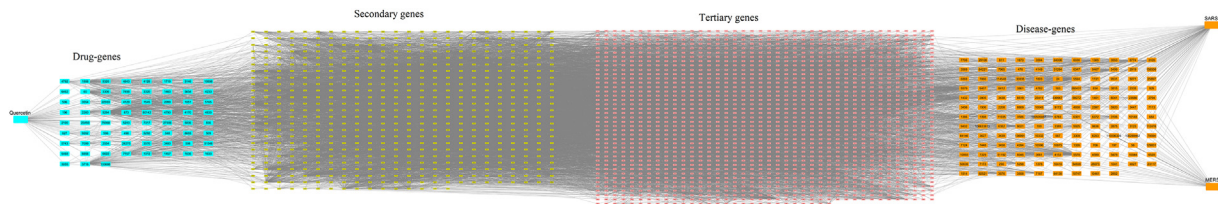


Figure 2. Quercetin-gene-gene-disease directed network. Quercetin direct gene interactors are highlighted as Cyan nodes. Its connecting secondary and tertiary genes are shown as green and pink nodes respectively. SARS and MERS with their direct gene interactors are shown as orange nodes.

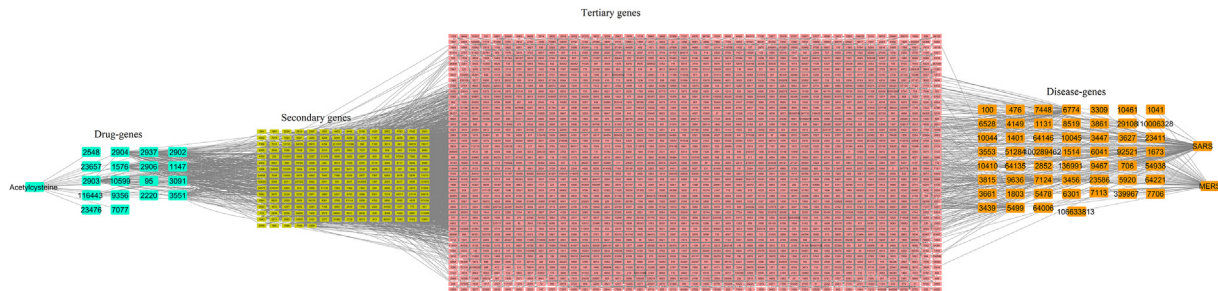


Figure 3. NAC-gene-gene-disease directed network. NAC direct gene interactors are highlighted as Cyan nodes. Its connecting secondary and tertiary genes are shown as green and pink nodes respectively. SARS and MERS with their direct gene interactors are shown as orange nodes.



Figure 4. 2-DG-gene-gene-disease directed network. 2-DG direct gene interactors are highlighted as Cyan nodes. Its connecting secondary and tertiary genes are shown as green and pink nodes respectively. SARS and MERS with their direct gene interactors are shown as orange nodes.

have 1 connecting gene and 2 connecting genes between drug-gene and disease-gene pair respectively (see Figure 6).

385 shortest paths of 31 genes targets were identified in the 2-DG-gene-gene-disease directed network (Figure 7). 14% of the shortest paths in the 2-DG affected network have no connecting genes between drug-gene and disease-gene pair. While 68% and 18% of the shortest path contains either 2 connecting genes or 1 connecting gene respectively.

Thus quercetin has a more number of associations with SARS and MERS disease-genes than that of NAC or 2-DG, as shown by the shortest paths between the drug-gene to disease-gene. In quercetin, 87% of its total shortest paths have at least 1 connecting gene. While NAC and 2-DG have 30% and 82% of their total shortest paths have at least 1 connecting gene respectively. The influence of NAC on disease-genes seem to be complicated as 70% of the shortest paths have atleast 2 connecting genes between drug-gene and disease-gene.

Further, this effect was quantified and expressed as a vector DV. DV is a vector and denoted the extent to which a disease gene was influenced by a drug. The DV values for quercetin, NAC and 2-DG are -70.19, -39.99 and -13.71 respectively. Quercetin has a greater negative influence on SARS and MERS disease-gene with a DV value of -70.19. Whereas NAC and 2-DG show lesser negative influence on SARS and MERS disease-gene target with a DV value of -39.99, and -13.71 respectively.

While analysing the shortest path network of quercetin and 2-DG, some important biological interactions were discovered. Some of these interactions could have therapeutic implications e.g. in quercetin-gene-gene-disease shortest path network, ESR1, EGFR, ACE, CASP3, BCL2L1

are common direct interactors of both SARS/MERS and quercetin. ESR1 can induce classical IFN-induced antiviral genes, which inhibits the virus entry in the cell [84, 85]. Quercetin is an agonist of the ESR1 receptor that could help to boost the antiviral response against SARS-CoV or nSARS-CoV-2 [86]. EGFR dysregulation protects against pulmonary fibrosis development caused by SARS-CoV and nSARS-CoV-2. Quercetin is an antagonist of EGFR and helps to reduce the risk of developing pulmonary fibrosis during SARS-CoV and nSARS-CoV-2 [87]. ACE is an important enzyme activated during SARS that converts angiotensin I (AT I) to angiotensin II (AT-II), which further binds to either angiotensin II receptor 1a (AT1aR), leading to tissue damage and lung edema [88]. Quercetin is the inhibitor of the ACE that can help to reduce the tissue damage and formation of lung edema [89]. The protein encoded by ORF-6 present in both SARS-CoV and nSARS-CoV-2 can induce apoptosis through caspase-3 mediated, ER stress, and JNK-dependent pathway [90]. Quercetin inhibits the CASP3 protein and can decrease excessive apoptosis that reduces the formation of ischemic injuries in SARS-CoV patients [91]. It has found that E protein of the SARS-CoV induces the NFKB1 that triggers the expression of pro-inflammatory cytokines such as IL12RB1, IL6, TNF, IL1B, etc [92, 93, 94]. This increases the infiltration of more neutrophils in lung tissues. The clinical finding of critical nSARS-CoV-2 patients admitted to intensive care units shows consistent high-level pro-inflammatory cytokines in their plasma [94]. Quercetin has also been found as a direct inhibitor of NFKB1 in the shortest path network that further regulates expression of pro-inflammatory cytokines such as IL12RB1, IL6, TNF, IL1B. and it was observed that treatment with drugs that inhibited NF-κB activation led to a reduction in inflammation

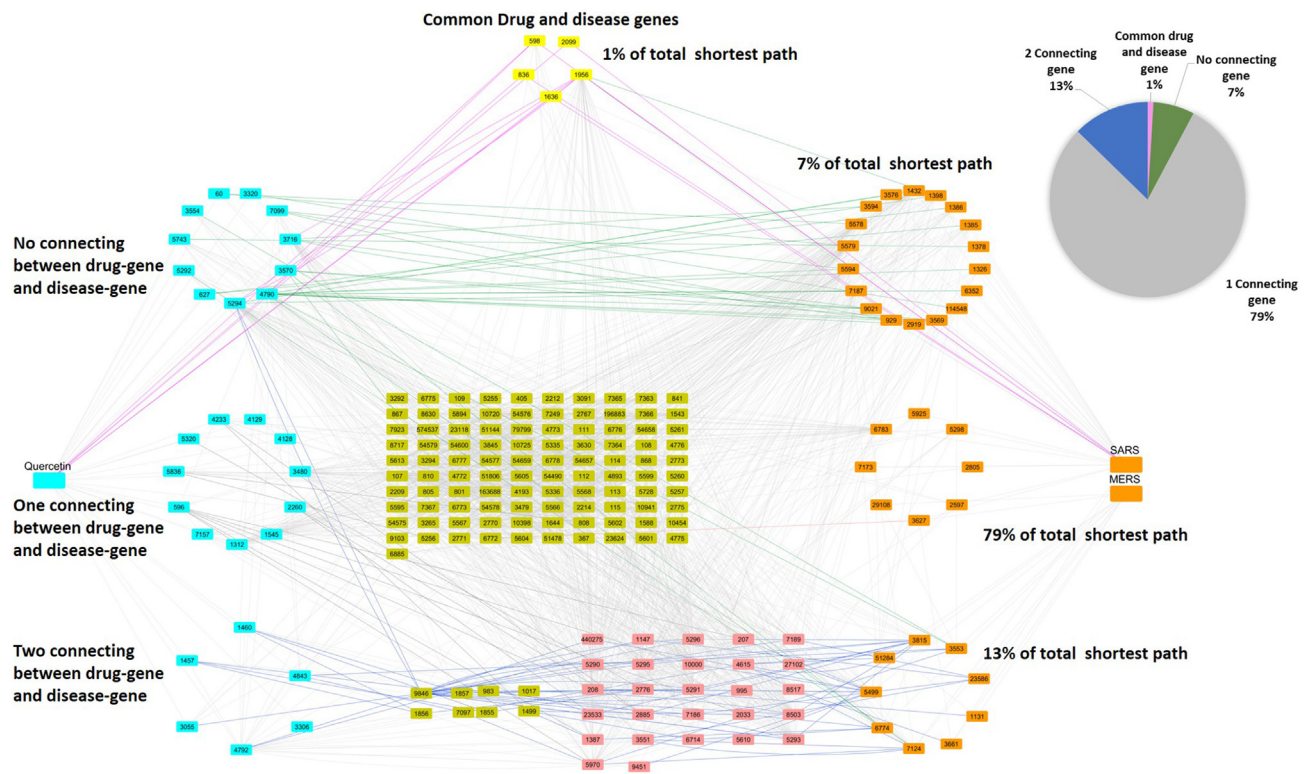


Figure 5. Quercetin-gene-gene-disease shortest path network. The drug and its direct interacting genes are highlighted as cyan nodes. The disease nodes and its direct interacting gene are highlighted as orange nodes. The genes that are common to both drug and disease have been shown as yellow nodes. The primary genes that lie on path having 1 interconnecting gene are shown as green nodes and secondary genes that lie on path having 2 interconnecting genes are shown as pink nodes. The pie-chart depicts the percentage of shortest path in each category of common, zero, one or two connecting genes in pink, green, grey and blue respectively. The respective color scheme is followed for the edge color in the network.

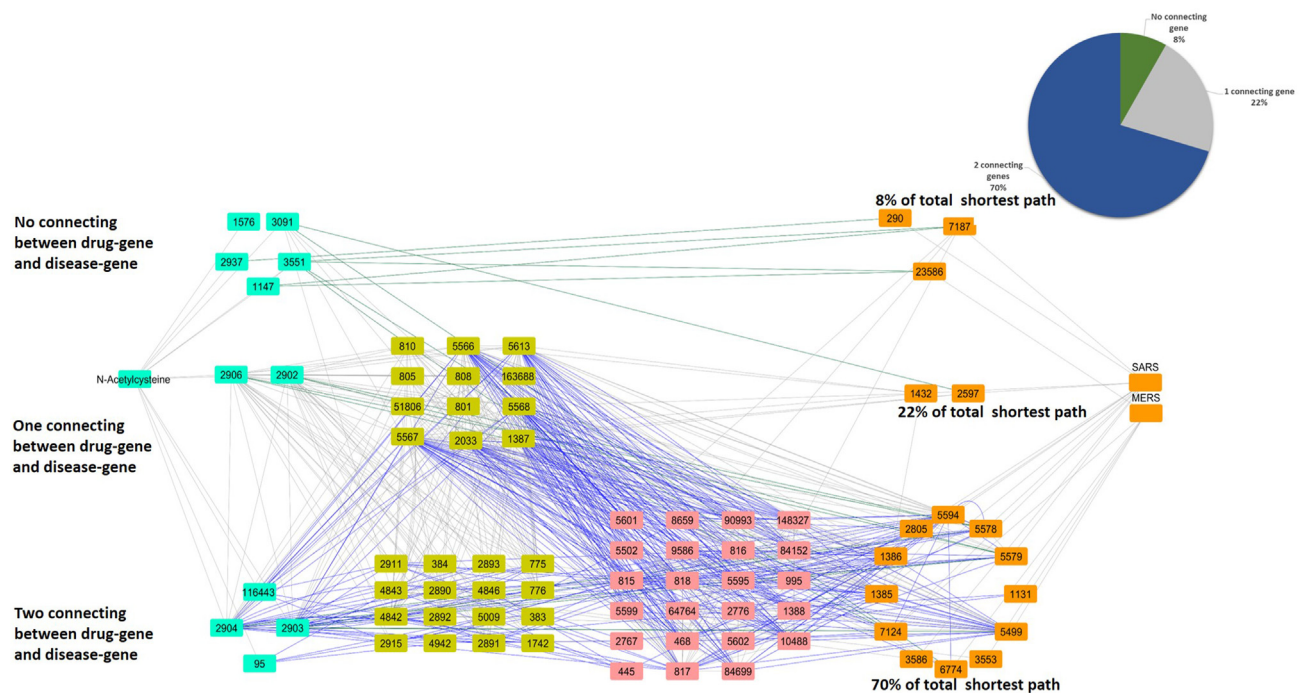


Figure 6. NAC-gene-gene-disease shortest path network. The drug and its direct interacting genes are highlighted as cyan nodes. The disease nodes and its direct interacting gene are highlighted as orange nodes. The primary genes that lie on path having 1 interconnecting gene are shown as green nodes and secondary genes that lie on path having 2 interconnecting genes are shown as pink nodes. The pie-chart depicts the percentage of shortest path in each category of zero, one or two connecting genes in green, grey and blue respectively. The respective color scheme is followed for the edge color in the network.

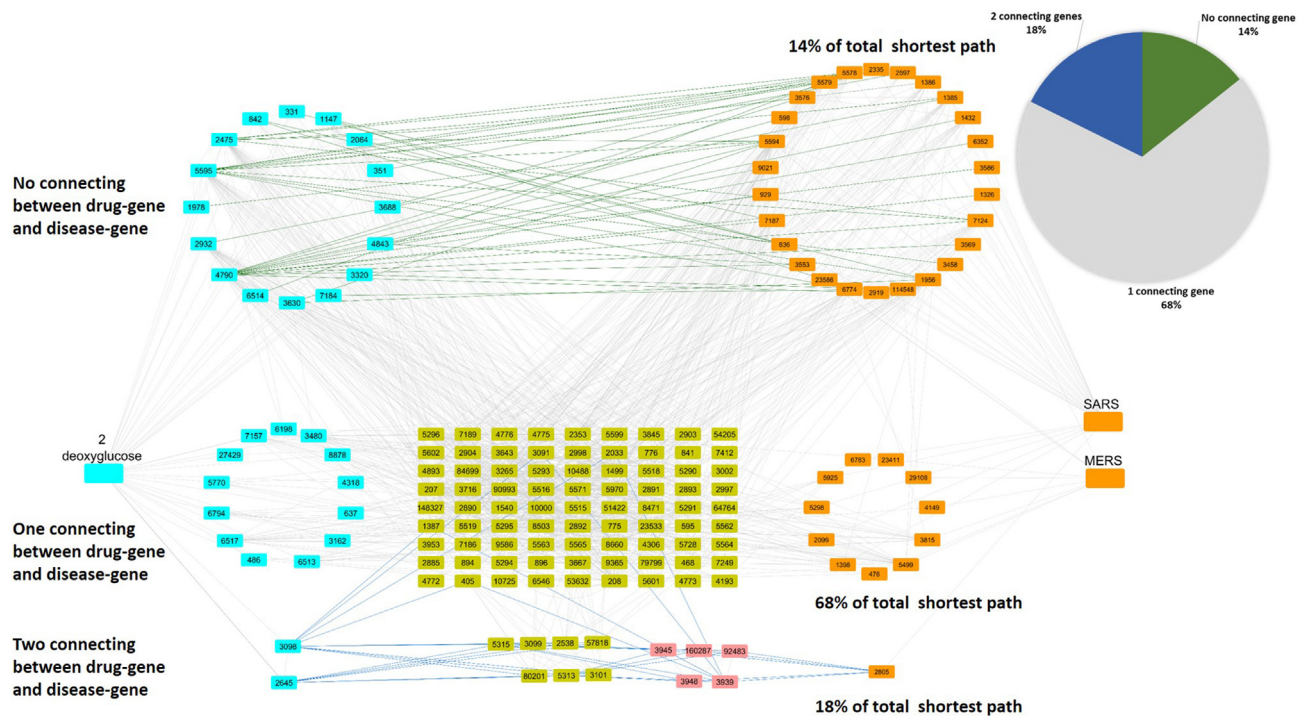


Figure 7. 2-DG-gene-gene-disease shortest path network. The drug and its direct interacting genes are highlighted as cyan nodes. The disease nodes and its direct interacting gene are highlighted as orange nodes. The primary genes that lie on path having 1 interconnecting gene are shown as green nodes and secondary genes that lie on path having 2 interconnecting genes are shown as pink nodes. The pie-chart depicts the percentage of shortest path in each category of zero, one or two connecting genes in green, grey and blue respectively. The respective color scheme is followed for the edge color in the network.

and lung pathology in SARS-CoV-infected cultured cells and also increases the mice survival rate [91]. Literature reports have shown that during both SARS-CoV and nSARS-CoV-2 infection there is an over-production of pro-inflammatory cytokines (TNF, IL-6, and IL-1 β) that results in a cytokine storm [95]. When this high cytokine concentrations get persist over time could lead to an increased risk of vascular hyper-permeability, multiorgan failure, and mortality [95]. Therefore, most of the therapeutical strategies develop until now are directed towards maintaining an adequate inflammatory response for pathogen clearance this includes the use of interleukin-1 inhibitors drug-like anakinra for immune-based therapy of nSARS-CoV-2. Both quercetin and anakinra are antagonists of the human IL-1 receptor [96]. Quercetin and its derivative quercitrin are strong antioxidant agents with anti-inflammatory properties. They are widely used for the treatment of cardiovascular disease, osteoporosis, pulmonary disease, etc [97]. Quercetin reduces the levels of TNF, IL-1 β , and IL-6 helps in maintaining the oxidative and inflammatory balance of the body [97, 98]. Quercetin dysregulating the expression of cytokines pro-inflammatory cytokines like TNF, IL-1 β , and IL-6 etc. can be easily visualized in the quercetin targeted shortest path network. Hence both DV vector calculation and biological implication of quercetin targeted shortest path network show quercetin can be effectively used as a therapeutic intervention against nSARS-CoV-2.

N-acetylcysteine (NAC) is a known mucolytic drug for chronic respiratory diseases and has an established safety profile (oral doses of 600mg/day or 150 mg/kg in the nebulized format in patients with acute bronchopulmonary diseases like pneumonia, bronchitis, tracheobronchitis). It makes bronchial mucous less viscous and being a cysteine derivative, helps in breaking disulfide bridges between macromolecules, which leads to a reduction in mucus viscosity [99]. NAC is also an inducer of Glutathione synthetase (GSS), which is an important enzyme in glutathione biosynthesis. Glutathione (GSH) in the body has an antioxidant effect and it reduces the formation of proinflammatory cytokines, such as IL-9 and TNF- α , and also has vasodilator properties by increasing cyclic GMP levels and by contributing to the regeneration of endothelial-derived relaxing factor. The inhibition of glutamate receptors (GRIN1, GRIN2A, GRIN2D, GRIN3A) also

promotes glutathione synthesis. This interaction can be easily visualized in NAC targeted shortest path network, where NAC promotes the GSS and its downstream signaling and inhibits glutamate receptors (GRIN1, GRIN2A, GRIN2D, GRIN3A). Because of these two properties NAC is already proposed as a potential treatment, preventive, and/or adjuvant against nSARS-CoV-2 [49]. This shows that NAC could be an important drug for the treatment of nSARS-CoV-2 infection but DV calculation shows it presumably would be less effective as an nSARS-CoV-2 therapeutic agent as compared to quercetin.

2-DG is a mimicking agent of glucose and is an antagonist of glucokinase/hexokinase (GCK/HCK), an enzyme that converts glucose to glucose-6 phosphate in the glycolysis cycle [100]. It is also a rate-limiting step of the oxidative metabolism. The inhibition of GCK reduces the ATP levels in the cells. Hence there would be more AMP levels than ATP levels in the cells. This leads to the activation of AMP-Activated protein kinases (PRKAG, PRKAB, PRKAA) [101]. These AMP-activated kinases inhibit Janus kinase (JAK1) and activate STAT transcription factors, especially Signal transducer and activators of transcription-3 (STAT3) [102]. STAT3 regulates cell growth, differentiation, and apoptosis [103]. During SARS infection in Vero cells, it was found that the induction of STAT3 leads to cell apoptosis, thus helping to stop viral replication [104]. This interaction can be easily visualized in 2-DG targeted shortest path network where 2-DG inhibits the GCK and its downstream signalling and leads to the activation of STAT3 through different AMP-activated kinase intermediates. Though 2-DG has a therapeutic effect against SARS, it follows a more complex route to target genes associated with SARS and MERS. Hence as hinted by DV calculation, presumably it might not be as effective as quercetin and NAC as a re-purposed drug for COVID-19.

3.2. Network properties and biological system features to assess safety and efficacy balance

Therapeutic Index is a measure of relative safety of a drug. It is calculated as ratio of the dose that produces toxicity to the dose needed to produce the desired therapeutic response. Drugs with $TI \leq 3$ are

Table 2. The network properties that have been found to be significantly different (p-value<0.05) between the targets of NTI and NNTI drugs. Network properties were grouped based on their innate mutual dependence and are highlighted in similar colors. The values larger/smaller in NTI/NNTI drugs is listed. The corresponding average values of the target genes of quercetin, NAC and 2-DG are also tabulated.

	Parameter	NNTI	NTI	Quercetin	NAC	2DG
1	Closeness Centrality (avg)	Smaller	Larger	0.241	0.264	0.272
2	Harmonic Closeness Centrality	Smaller	Larger	635.697	695.509	715.510
3	Residual Closeness Centrality	Smaller	Larger	180.569	223.146	237.773
4	Avg Shortest Path Length	Larger	Smaller	4.242	3.846	3.709
5	Deviation	Larger	Smaller	3493.062	2473.818	2156.581
6	Distance Deviation	Larger	Smaller	1247.556	1572.156	1789.97
7	Distance Sum	Larger	Smaller	13093.437	9241.818	8910.581
8	Betweenness Centrality	Smaller	Larger	0.002	0.0069	0.0065
9	Current Flow Betweenness	Smaller	Larger	0.0049	0.01	0.0099
10	Load Centrality	Smaller	Larger	0.0020	0.0070	0.0068
11	Normalized Betweenness	Smaller	Larger	0.0121	0.03636	0.03280
12	Eccentric	Smaller	Larger	0.574	0.433	0.593
13	Eccentricity	Larger	Smaller	9.0625	9.1818	8.9354
14	Eccentricity Centrality	Smaller	Larger	0.1111	0.1091	0.1123
15	Degree	Smaller	Larger	21.5312	32.1818	25.9032
16	Degree centrality	Smaller	Larger	0.0088	0.0132	0.0107
17	Z score	Smaller	Larger	0.0180	0.04490	0.0287
18	Radiality	Smaller	Larger	0.7505	0.7810	0.7916
19	Clustering coefficient	Larger	Smaller	0.2505	0.432	0.2311
20	Interconnectivity	Larger	Smaller	0.3883	0.4976	0.3312
21	Neighbour connectivity	Smaller	Larger	35.0512	44.2683	37.0124
22	Topological coefficient	Larger	Smaller	0.3444	0.2276	0.2091
Biological System Features						
1	No. of pathways affiliated by the primary therapeutic target	Smaller	Larger	45.59	54	59.51
2	No. of similarity proteins outside the target family	Smaller	Larger	32.53	53.27	38.45
3	Number of director interactors which are core essential genes	Smaller	Larger	7.6	24.02	9.1

considered less safe and referred to as Narrow Therapeutic Index (NTI) drugs. Drugs with $TI > 3$ are considered better than NTI on safety standards and referred to as Not -Narrow Therapeutic Index (NNTI) drugs. However, determination of TI is very complicated for many drugs and is also highly susceptible to the variations of drug responses.

The efficacy-safety balance of a drug may be inferred from the network properties and biological system profile of the drug-genes [105]. So, an effort was made to assess the safety efficacy balance (i.e. NTI or NNTI) of the three drugs (quercetin, NAC and 2-DG) in the drug-gene-gene-disease network. Several connectivity and adjacency-based network properties,

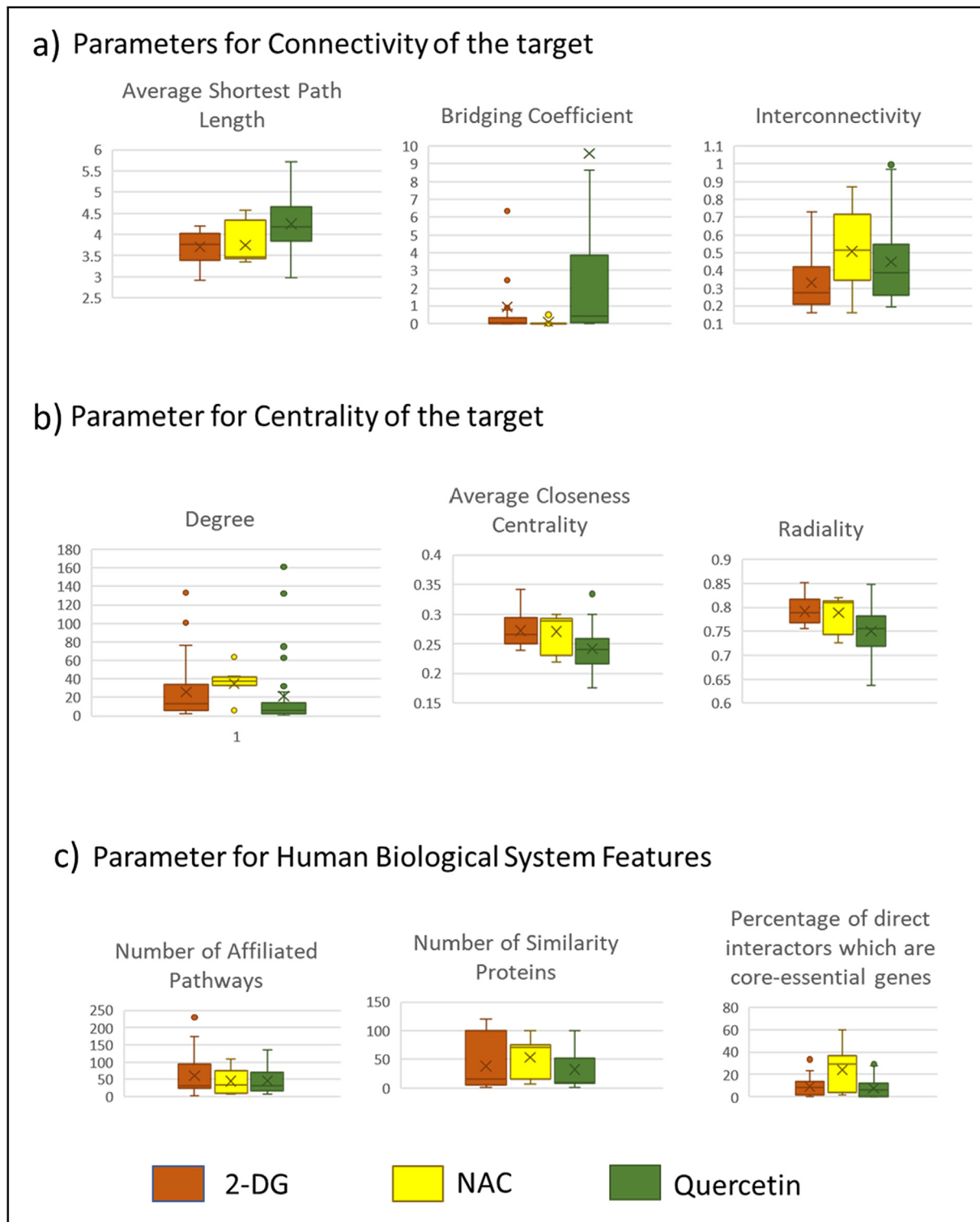


Figure 8. The parameters of connectivity of the target (Panel a), centrality of the target (Panel b) and human biological features (Panel c) of the three drugs. Panel a show that the average shortest path length, bridging-coefficient were highest in quercetin; whereas interconnectivity was highest in NAC. Panel b shows that the average closeness centrality, degree, radiality were lowest in quercetin. Panel c shows that the affiliated pathways; number of similarity proteins and percentage of direct interactors with core essential genes were lowest in quercetin targets.

properties based on shortest path length, have been found to be significantly different (p -value <0.05) between the targets of NTI and NNTI drugs [105] (Table 2). Network properties were grouped based on their innate mutual dependence are highlighted in similar colours.

Some key features such as average shortest path length show increase from the targets of NTI drug to that of NNTI one [105]. Among the three, quercetin showed the highest average shortest path length, followed by NAC and 2-DG (Table 2). Also some others such as average closeness centrality demonstrated a decrease from the targets of NTI drug to that of NNTI drugs [105]. The lower value of average closeness centrality of drug-target has been shown to demonstrate a less lethality risk [106]. The interconnectivity values were lower for lethal diseases like cardiovascular and oncogenic diseases [107]. Quercetin had the smallest average closeness centrality among the three (Table 2).

Based on these parameters, Quercetin and NAC were found to be closer to the profile of NNTI drugs. Properties which determine the connectivity of the target (i.e. average shortest path length, bridging-coefficient, interconnectivity) showed an increasing trend from the targets of NTI to NNTI drugs [105]. These parameters for quercetin, NAC and 2-DG are depicted in Figure 8. Average shortest path length, bridging-coefficient were highest in quercetin; whereas interconnectivity was highest in NAC.

Parameters which determine the centrality of the target in the network (i.e. average closeness centrality, degree, radiality) showed a decreasing trend from the targets of NTI to NNTI drugs [105]. These parameters for quercetin, NAC and 2-DG are depicted in Figure 8. Average closeness centrality, degree, radiality were lowest in quercetin.

Biological system properties (i.e. affiliated pathways, number of similarity proteins) also showed decreasing trend from the targets of NTI to NNTI drugs [105]. The affiliated pathways and similarity proteins were lowest in quercetin targets (Table 2).

It has been proven that the TI-related mechanism could be a result of synergistically effects among these eight features [105]. As per these parameters, among the three, quercetin followed the trends of NNTI drug. Other studies have also shown that number of similarity proteins and affiliated pathways as a good indicator of target drugability [108, 109].

Thus the targets of NTI drugs were highly centralized and connected in network, and the numbers of similarity proteins and target-affiliated pathways were higher than those of NNTI drugs [105].

A list of 1580 core-essential-genes was obtained [110]. These genes without any context-dependence have been important for survival. For each drug-gene, its top 50 direct interactors were obtained from STRING. The percentage of these direct interactors which were part of core-essential-genes were identified. It is presumed that the drugs which target the core-essential-genes would be NTI drugs. It was found that quercetin had the minimum number of core-essential-gene interactors (Table 2).

4. Discussion and conclusions

In this study, we compare three drug candidates i.e. quercetin, NAC, and 2-DG to be repositioned for nSARS-CoV-2 by exploring associations between drugs and disease genes derived from a directed drug-gene-disease network using the shortest path.

In the case of quercetin, 1% of the shortest path had common genes interacting with both the drug and the disease directly; 7% of the shortest path had no connecting gene between drug-gene and disease-gene pairs; 79% of these shortest-path contain 1 connecting gene between drug-gene and disease-gene and 13% of these shortest-path contains 2 connecting genes between drug-gene and disease-gene. In the case of NAC, there were no common genes interacting with both the drug and the disease directly; 8% of the shortest paths have no connecting genes; whereas, 22% and 79% of the shortest path have 1 and 2 connecting genes between drug-gene and disease-gene pair respectively. In the case of 2DG, there were no common genes interacting with both the drug and the disease directly; 14% of the shortest paths in the 2-DG affected network

have no connecting genes between drug-gene and disease-gene pair. While 68% and 18% of the shortest path contains either 2 connecting genes or 1 connecting gene respectively. The shortest path calculation shows that both NAC and 2-DG follow a complex route to effect the SARS and MERS genes as compared to quercetin. Further, the extent of a disease-gene influenced by a drug in the directed drug-gene-disease network was quantified through a vector called DV; it showed that quercetin has a greater therapeutic influence on the SARS and MERS associated genes, followed by NAC and 2-DG. The DV values for quercetin, NAC and 2-DG are -70.19, -39.99 and -13.71 respectively. Thus from the present analysis, quercetin potentially appears to be a better drug that can be repurposed against SARS and MERS than NAC and 2-DG. The shortest path calculation of quercetin revealed five genes ESRL, EGFR, ACE, CASP3, BCL2L1 that are also directly associated with SARS and MERS. As nSARS-CoV-2 shares an ancestral origin and genome with betacoronavirus like SARS-CoV, MERS-CoV, therefore, quercetin could potentially be a more potent drug candidate that could be effective against nSARS-CoV-2 infection.

Detailed biological implications of the three drugs provided enough evidences for their repurposing ability as a therapeutic intervention against nSARS-CoV-2. Further the efficacy-safety balance of these drugs were assessed by connectivity and centrality of the drug-targets in the drug-gene-disease network. The network properties and biological system features were used to assess safety and efficacy balance and hence classify the drugs as NTIs or NNTIs. The network properties that have been found to be significantly different (p -value <0.05) were used for evaluation. Based on these parameters, Quercetin and NAC were found to be closer to the profile of NNTI drugs. Quercetin was found to follow the pattern of a better and safer drug.

The limitation of the study includes the following. Drug repositioning can identify novel indications for existing drugs and is a robust and quick alternative to traditional methods. However, the likelihoods put-forth in the present study need to be validated experimentally. The method relies heavily on the previous knowledgebase that has been established in terms in gene-gene, gene-disease and gene-drug interactions. The method would be more robust as more and more experimentally-validated interactions are incorporated in the drug-gene-disease network. The method may be improvised further if the kinetics of each of these interactions could be added as edge-weights to the network. However, such kinetics data is not available for majority of the interactions.

Thus, in the present study, extent to which a SARS-CoV-2 disease genes were influenced by quercetin, NAC and 2DG was computed and quercetin was quantified as the best among the three drugs, suited for repurposing. Further using biological implications and network properties, the drugs were assessed for their safety and efficacy balance (in terms of therapeutic index). It was found that quercetin was a forerunner than other two drugs.

Declarations

Author contribution statement

Pankaj Khurana: Conceived and designed the experiments; Analyzed and interpreted the data; Wrote the paper.

Rajeev Varshney: Conceived and designed the experiments.

Apoorv Gupta: Analyzed and interpreted the data; Wrote the paper.

Funding statement

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Data availability statement

Data included in article/supplementary material/referenced in article.

Declaration of interests statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

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