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EDITORIAL MESSAGE

It gives us immense pleasure to announce the combines issue of Nirma University Journal of Pharmaceutical Sciences (NUJPS) of the year 2021.

The field of biomedical research stands at the cusp of a revolutionary era, driven by the convergence of artificial intelligence (AI), systems biology, and 3D bioprinting. These ground breaking technologies are not only transforming the landscape of scientific discovery but also reshaping the future of healthcare and medicine.

In this journal, we present a collection of cutting-edge research and review articles that exemplify the synergy between AI, systems biology, and 3D bioprinting. These studies highlight the innovative approaches and interdisciplinary collaborations that are driving the field forward. We are witnessing a paradigm shift in biomedical research, where technology and biology converge to unlock new frontiers of knowledge.

As we navigate this exciting frontier, it is imperative to foster collaboration across disciplines and to support the ethical and responsible use of these technologies. The potential benefits are immense, but so are the challenges. It is our collective responsibility to ensure that these advances are harnessed for the greater good, ultimately improving human health and well-being.

We hope that the insights shared in this journal will inspire researchers, clinicians, and policymakers to continue pushing the boundaries of what is possible. Together, we can shape a future where technology and biology work in harmony to solve some of the most pressing challenges in medicine.

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ARTICLE

THE ROLE OF MACHINE LEARNING IN BIOLOGICAL SCIENCES

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ABSTRACT

In the biological sciences, machine learning (ML) has become an essential technology that is revolutionizing research methods and speeding up discoveries in a variety of fields. A thorough overview of the various uses of ML in biological sciences is discussed in this article, including drug development, protein sciences, vaccines, biosystems, and computational biology. ML models facilitate the rapid discovery of innovative drug candidates with decreased side effects and increased efficacy, hence speeding up the drug development pipeline by utilizing large-scale biological data. ML techniques are improving the prediction of protein interactions, structures, and functions in the field of protein sciences. The design of vaccines, epitope prediction, and antigen selection are all greatly aided by ML techniques. ML models evaluate genetic and proteomic data based on individual immune responses, facilitating the generation of personalized immunizations that are optimal for immunogenicity and vaccine efficacy. Furthermore, by replicating cellular processes, modeling intricate biological networks, and forecasting gene regulatory mechanisms, ML techniques are revolutionizing the study of biosystems. In computational biology, ML is utilized for phenotypic prediction, gene expression profiling, and sequence analysis. ML models facilitate the development of precision medicine techniques, the characterization of medication response patterns, and the identification of disease biomarkers by combining multi-omics data. To fully explore the potential of ML for tackling significant issues in healthcare, computer scientists, biologists, and bioinformaticians must

collaborate across disciplinary boundaries. This review emphasizes the revolutionary impact of ML on biological sciences.

Keywords: *Machine learning, artificial intelligence, biological sciences.*

INTRODUCTION

Machine learning (ML) is the evaluation of algorithms that allow pattern classification, recognition, and prediction based on models created from readily available data. It is important to acknowledge two features of automation when considering ML in a broad sense. First, the categorization and prediction duties ought to be accomplished by a computer system with the proper programming. That is, ML produces a system for classification that might be used practically with existing technology. Moreover, the classification development process is designed to be highly automated with little need for human intervention [1].

ML addresses the problem of building computers that learn automatically from use. Because it lies at the nexus of data science and artificial intelligence (AI), as well as statistics and computer science, it is one of the technical fields with the fastest development rates in the present day. Recent advances in ML can be attributed to the evaluation of new learning algorithms and theories, the continuous development of low-cost processing, and internet data accessibility. The use of data-intensive ML techniques by technology, science, and business has led to a rise in the application of evidence-based decision-making across a range of industries, including financial modeling, manufacturing, marketing, and healthcare. In the previous ten years, there has been a notable growth in the capacity of mobile and networked computing systems to gather and transfer massive amounts of data; this development is commonly referred to as "Big Data." The researchers and engineers who collect these data mostly utilize ML to address the challenge of obtaining meaningful conclusions, producing accurate projections, and making decisions based on such data sets[2]. A few examples of how historical data are used are historical crime statistics, which are used to help distribute police officers to specific places at certain times, and historical traffic data, which are used to enhance traffic control and minimize congestion. Massive experimental data sets are gathered and vetted to progress the fields of neurology, astronomy, biology, and various other data-intensive empirical sciences [3]. The development of machine-learning tools to assess high throughput experimental data in new ways resulted in a correspondingly broad range of effects in the empirical sciences, including social science, cosmology, and biology. Engineers and data scientists are driven by biology, but they also focus on developing ML methods that solve practical problems. Consequently, models constructed using these techniques often neglect acknowledged physiological constraints. Any biological model is an abstraction, one could say, and even while it doesn't

perfectly capture every facet of the living thing, it can still be helpful [4].

Biology is changing to a data-rich field due to the development of high throughput sequencing and "omics" technologies, as well as the consequent exponential increase in the number of measurements of structure, macromolecular sequence, and gene expression. Like physics was before Leibniz and Newton, biology has mostly remained an informative science despite these advancements. ML presently offers some of the most affordable technologies accessible for creating prediction models from biological data. These include techniques for finding genetic markers for diseases, predicting the function of macromolecules, finding functionally important protein sites, classifying new genomic sequences, and figuring out the network of genetic relationships that control important biological processes.

Biology must continue to improve its machine learning (ML) methods to become an engineering discipline. Examples of these methods include learning from highly imbalanced information sets, learning complex structures of class labels (such as labels associated by acyclic graphs that are directed instead of one of multiple mutually exclusive labels), and learning from richly structured data, such as macromolecular DNA sequences along with 3-dimensional molecular structures.[5]

Large-scale genome pattern projects have come out in the availability of hundreds of full genome sequences. More importantly, every 18 months, the amount of nucleic acid patterns in the GenBank database doubles. The structural genomics efforts have also caused a rise in the lot of macromolecular (like proteins) structures. Biologists can currently access over a thousand databases. The advent of high-throughput "omics" techniques, such as those for analyzing the expression of hundreds of genes in response to various perturbations has made system-wide measurements of biological variables conceivable. ML is therefore making discoveries in the biological sciences possible to a greater extent.

A few instances of ML applications in computational and systems biology are as follows: determining a primary (amino acid) sequence of protein, structure, and interaction partners to predict its function(s); identifying the amino acid pattern and if possible its structure to determine functionally significant sites (such as protein-protein, protein-RNA, protein-DNA, and post-translational modification sites); assigning structural classifications to protein sequences (and structures); finding functional modules—groups of genes that act together—and genetic networks using information on gene expression.

ARTIFICIAL INTELLIGENCE REVOLUTION

Recently, deep (multi-layered) neural networks have gained significant prominence in the ML industry. These

networks comprise algorithms that are built into multiple (several to hundreds) functional layers and are roughly modeled after the connectivity of a brain of human [6]. Numerous articles in nearly every branch of science explain how deep learning (DL) could be applicable to address any problem for which sufficient data is provided. There are a lot of reasons behind this. First, the technique itself has become simple to use by a reasonably competent programmer with the accessibility of software that has made it accessible to anyone to try out DL experiments. Only a few years ago, even experienced computer scientists would have found it difficult to carry out these experiments.

The use of inexpensive graphics processors that significantly speed up ML is one example where hardware advancements have also played a significant role. Today, one need not even purchase the hardware because it can be used, sometimes for free, through a variety of cloud services. Lastly, there are a ton more training options available, which has led to an enormous rise in job opportunities for AI [7].

While biology has long employed ML techniques, especially neural networks, there has been a notable upsurge in interest in the field recently [8]. The urge to take on problems whose solutions could enhance the health and happiness of millions of people is strong. The conventional academic publication mechanisms in the biomedical sciences are challenged by the widespread popularization of computer-led biological data science [9]. The problem lies in the fact that many of the papers emerging from AI development are not contributing to the field because these methods are not being applied properly. Often, this means that either their experimental design is flawed or they do not offer any advancement over currently used methods [10]. Table 1 shows examples in which AI is used for prediction modeling.

Table 1 Disease detection and prediction modeling with the use of AI in clinical data modality

Diseases	Algorithm	Outcomes	Reference
AMD	ML-based predictive model	The model was highly reliable to predict AMD progression	[11]
COVID-19	Passive Aggressive (PA)	70–80% accuracy achieved	[4]
Alzheimer's disease	Random Forest, Shapley additive explanations (SHAP)	Accuracy of 93.95% in first layer and 87.08% in second layer was predicted	[12]
Ovarian cancer	Artificial Neural Network	For survival - 93% accuracy In surgical outcomes - 77% accuracy	[13]
Pulmonary cancer	Lung Cancer Prediction-Convolutional Neural Network, Brock model	Compared to the LCP-CNN model was capable to foresee the malignancy of lung nodules with greater accuracy and fewer false-negative outcomes	[14]
Influenza	Innovative Accessible Technology-Back Progression in Neural Network (IAT-BPNN)	For a large population, IAT-BPNN demonstrated high accuracy in predicting influenza-like illness.	[15]

PROBLEM SUITABILITY AND CURRENT DEVELOPMENTS

Initially, one must acknowledge that the efficacy of DL methods has been limited to applications that meet specific data requirements. These requirements include plenty of data, high dimensionality (a sample comprising numerous variables), and well-structuredness (a reference to a graphical interaction among the variables). The images are the perfect sample for DL techniques since they provide a huge number of variables (pixels) that can be precisely categorized into well-defined objects (e.g., the pixels that make up a nose on a face). Text and audio data can also be used. Naturally, a lot of biological data sets—such as text data from sequencing or picture data from microscopes—also satisfy these requirements.

Other, possibly less obvious uses for example, current developments in DL have greatly enhanced our ability to figure out the tertiary structure of proteins from their amino acid sequences. This has been made

possible by the ability to view protein folds as 2D maps of interatomic distances that may be analyzed like that of pictures. However, not all biological data sets lend themselves to DL analysis. One of the examples is the analysis of single nucleotide polymorphisms (SNPs) in genomic data. The existence or absence of known SNPs in a data genome is highly dimensional given the millions of SNPs that are known, although the data remain unstructured.

This kind of information is known as categorical data, and it can only be displayed as tables with an unstructured row order. Although SNP data can still be categorized by tagging them with a specific gene or chromosome, this is insufficient to make them suitable for DL, and other kinds of analysis are probably more useful. Due to this limitation on the data applicability, it is crucial to remember that state-of-the-art status should never be derived from its use of DL in an evaluation. Rather, it needs to begin with the contrary assumption and rely on the research presented in the paper to convince the reviewers of the opposite. [10][16].

MACHINE LEARNING IN BIOLOGICAL SCIENCES

ML in Drug Development

The process of developing new medications requires a long time and investment. Indeed, potential medications must go through a rigorous and competitive process to ensure both patient safety and medical efficacy. Phase 0 to Phase IV are the four main stages that comprise drug development. According to the literature, automating certain significant but monotonous data processing and analysis tasks, particularly with robotics and ML techniques is a rather cheap solution. Certainly, many fruitful collaborations have emerged between AI/ML and companies, universities, research centers, and pharmaceutical laboratories gradually decreasing the gap in bioinformatics between applied mathematics, computer sciences, and biology. This would facilitate the drug development pipelines to go more quickly because they could be carried out computationally, autonomously, and with a lower risk of human error [17]. By providing novel approaches to enduring problems, ML is a key factor in transforming the drug development process.

Fig. 1 ML in drug development

Fig. 1 lists the various stages where ML is used in drug development. ML algorithms aid in target identification during the early phases of drug discovery by analyzing large biological datasets to identify targets linked to disease and rank possible therapeutic candidates. Then, by precisely predicting structure-activity relationships (SAR), ML algorithms facilitate lead optimization by accelerating the identification of molecules with favorable pharmacokinetic characteristics. Moreover, ML algorithms improve medication safety profiles by predicting adverse drug responses (ADRs) and identifying off-target effects, which helps with toxicity prediction. Also, ML-driven drug repurposing techniques find new therapeutic indications for authorized medications by utilizing large-scale data integration and current biological knowledge, greatly cutting down on the time and expense of conventional drug discovery procedures. Additionally, by customizing treatment plans to fit each patient's unique profile, anticipating a patient's reaction to a particular therapy, and streamlining treatment schedules, ML facilitates precision medicine approaches. By way of publicly funded programs such as the US National Institutes of Health's Molecular Libraries Screening Centres, these technologies greatly boost the rate and volume of information that can be obtained regarding the influence of chemical compounds, paving the way for the development of massive databases such as PubChem. These databases frequently comprise scores for many compounds on an assay, which represent the outcomes of multiple screens, in addition to details on the targets of particular assays. To evaluate the influence of perturbagens on certain molecular targets and cell behaviors, high content screening and high throughput microscopy are widely used techniques. Classifier training to identify predicted patterns and feature computation to define visual elements are common analysis techniques for high-content displays. One method involves employing clustering algorithms, which don't necessitate prior knowledge of the patterns, to determine compounds with comparable biological effects. Machine-vision techniques, which can extract more accurate data, could be applied to high-content assays. Pattern-unmixing techniques try to cope with the continuous nature of relocation events by calculating the proportion of a target that exists in each subcellular place [18]. Ultimately, there is a lot of potential for the use of ML in drug development to hasten the identification of safe and effective treatments, bringing about the era of precision medicine and better patient outcomes. On the other hand, the integration of multi-view data may allow for the deployment or improvement of precision medicine procedures, which could reduce the cost and time associated with drug discovery while simultaneously making medicines more patient-oriented.

ML in Protein Sciences

Essentially, intermolecular interactions are necessary for proteins to create complexes that regulate biological functions in living

cells. Such interactions between proteins that occur in a wide range of dynamic conformational states and levels of inherent disorder are becoming more recognized. Furthermore, the structures' size varies from tiny dynamic biomolecular condensates of 100 nm or more to simple binary complexes. Some of the major issues in molecular biosciences include how such interactions are governed, how they arise, how they influence function, and what happens when they occur inadvertently and cause disease [19]. ML is revolutionizing protein sciences by offering powerful computational tools to predict the actions of proteins shown in Fig.2. ML algorithms excel in predicting protein structures, functions, and interactions, thereby accelerating the pace of biological research (8). By using massive repositories of known protein structures, ML methods predict protein structures accurately from amino acid sequences, offering important information on the folding patterns and functional characteristics of the resulting proteins (9).

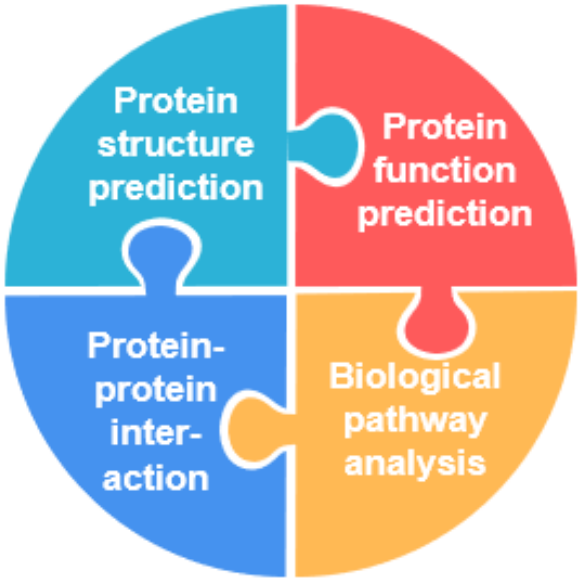

Fig.2 ML in protein sciences

Predictions of structural attributes of many proteins, like those related to solvent accessibility, secondary structure, disordered regions, binding sites, functional sites, protein domain boundaries, and transmembrane helices, are 1-D prediction problems. Basic correlation techniques can achieve accuracy levels substantially above random fluctuations, with up to 50% accuracy and a specific amount of data captured. The development techniques have been improved upon. To further enhance secondary structure prediction, PSI-PRED,

for example, leverages PSI-BLAST to generate additional profiles based on position-specific score matrices. In an attempt to increase the reliability of prediction by integrating data that exceeds the fixed-size window input of conventional feedforward neural networks, more complex recursive neural network architectures have been created through new algorithmic advancements motivated by the theory of probabilistic graphical models. Numerous neural network ensembles in size have also been employed. Secondary structure prediction accuracy has increased to between 78% and 80% because of new technologies and an increase in protein sequence databases used to create profiles. Furthermore, to enhance secondary structure prediction, hybrid techniques that incorporate homology searches and neural network approaches have been created [20]. Many facets of predicting the 3D structures of proteins, including the development and assessment of the fold recognition model, have been addressed using ML techniques. Fold recognition aims to identify a known protein with a structure believed to be similar to that of an unknown protein. The most efficient methods for predicting 3D structures using templates necessitate the initial identification of structural homologs. First, neural networks were combined with threading for this task. Recently, a universal ML framework based on pairwise similarity characteristics between query and template proteins has been suggested to enhance fold recognition sensitivity and specificity. The framework can be expanded to any other supervised learning technique, even though its current implementation employs support vector machines to find folds. Moreover, by analyzing structural properties, evolutionary conservation patterns, and sequence motifs, ML approaches allow the prediction of protein activities. This makes it easier to identify new drug targets and annotate proteins whose functions are unknown. Additionally, ML-driven approaches play a crucial role in predicting protein-protein interactions [22,22] (shown in Fig. 3), elucidating the intricate networks of molecular interactions underlying various biological processes. Researchers can obtain more detailed knowledge of disease mechanisms, signaling pathways, and protein dynamics by combining multi-omics data and utilizing modern ML techniques [23]. This can facilitate the development of innovative therapies and precision medicine tactics. In general, applying ML to the field of protein sciences has great potential to improve our knowledge of biological systems and hasten the development of novel therapeutics for human illnesses.

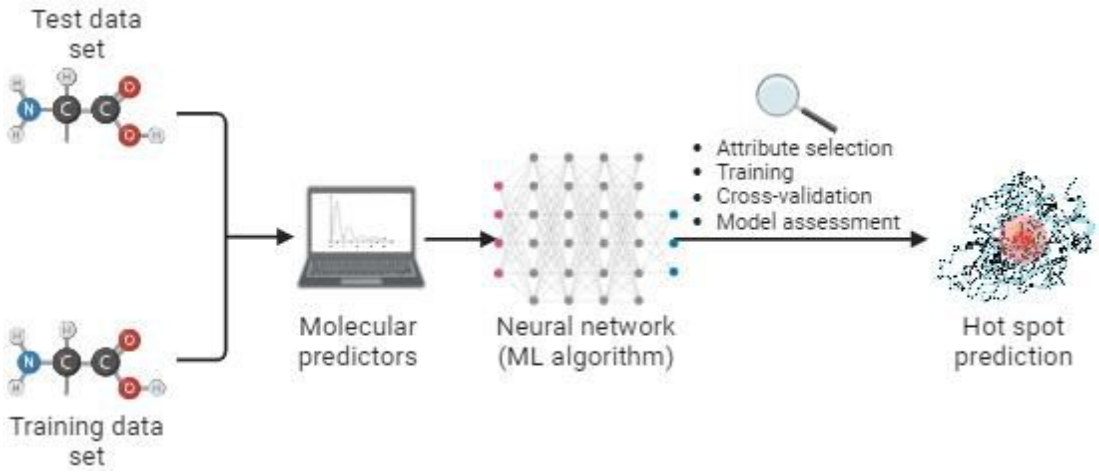

Fig.3 Hotspot prediction in protein-protein interactions

Improving Reverse Vaccinology

ML is increasingly being utilized in various aspects of vaccine development, from antigen selection to vaccine design and optimization as shown in Fig.4.

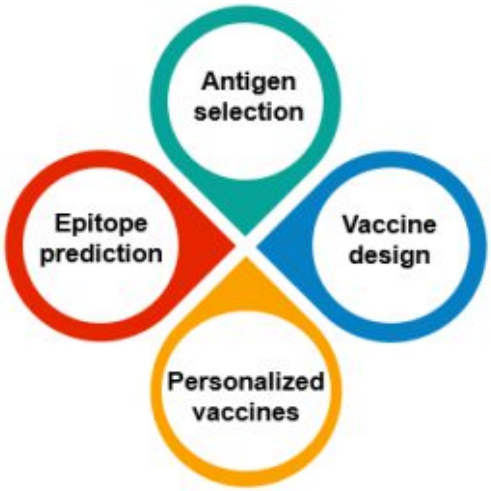

Fig.4 Use of machine learning in vaccine development

Reverse vaccinology (RV), a technique that utilizes bioinformatic algorithms to find putative protective antigens in bacterial proteomes that could serve as prospective vaccine candidates, has gained popularity during the past ten years. This rise has been fueled by the quick collection of information from the full genome sequencing of more than 4000 bacteria, which is utilized to pinpoint the protein-coding genes that serve as the foundation for RV methods. Since there is no need to

cultivate bacteria, promising vaccine candidates can be identified quickly and at a lower cost using RV than with conventional vaccinology. Additionally, RV can discover all of a bacterial species' putative protective protein antigens rather than only the most prevalent antigens that are conventionally extracted from bacterial cultures [24]. The most reliable strategy to address the urge for future vaccinology has been described as using recombinant proteins as immunogens. RV has been used to limit the number of vaccine candidates whose immunogenicity is tested in experimental animals before vaccine development and human trials. Comparing subunit vaccines to live vaccines, which have issues with attenuation and reversion to virulence, the benefits encompass improved safety, decreased cost, minimized competition between antigens, and targeted delivery to infectious sites. Delivery methods for subunit vaccinations that align with the RV-predicted antigens include non-pathogenic vectors, DNA vaccines, and non-living antigen delivery systems. The process of choosing potential antigens for immunogenicity tests and the creation of subunit vaccines can be expedited quickly using RV methods [25]. The first supervised, web-based technique for predicting bacterial protective antigens (BPAgs) is Vaxign [26]. Fig.5 shows the use of ML in vaccine development [27]. Overall, ML holds promise for revolutionizing vaccine development by facilitating antigen discovery, epitope prediction, vaccine design, immunogenicity assessment, and personalized vaccine strategies, thereby advancing efforts to combat infectious diseases and emerging pathogens [28].

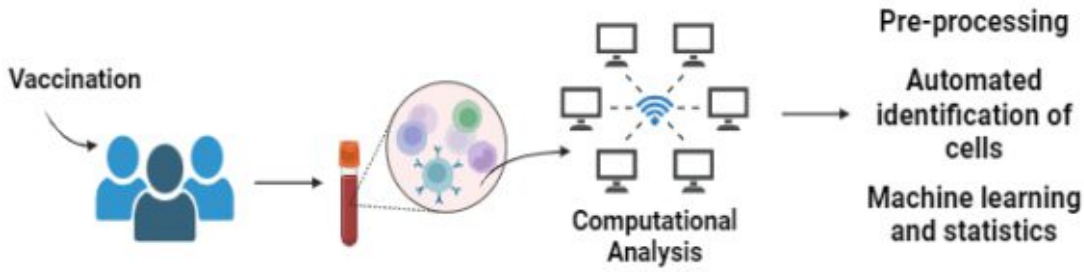

Fig.5 Computational analysis of vaccination data

Biosystems Design by ML

More and more research is being done on biotechnological applications of biosystems, comprising enzymes, pathways, and entire cells. However, because of the enormous interdependence of biosystems, it is very challenging to design biosystems with the necessary characteristics. The development of high-throughput phenotyping technology has made ML an efficient method for predicting biological system behavior and enabling the learning phase [29]. Designing biosystems with ML techniques integrated has become possible since high

throughput technologies like as-omics have developed quickly. By identifying new candidates for the best performance, ML models can improve biosystem design applications by identifying patterns in complicated biological data at various scales of investigation [30]. ML is being used throughout the entire design process for biosystems to find novel technical solutions with fewer variations in design. By seeing trends and patterns in systems with a lot of data, ML has become a promising tool for accelerating success in biosystems design. We want to further close any gaps between computer science and biology researchers because effective research in this field requires their cooperation. The biological audience will gain from a brief explanation of the fundamentals of ML required to comprehend the technical aspects of pertinent work and understand the advantages and disadvantages of ML approaches and the potential applications of ML in other fields [31]. The objective of ML from a computing perspective is to create a function that can convert a specific instance of input data into a desired output. The ML paradigm assumes that the values of input and results of the training data are associated. By using ML algorithms to identify these correlation patterns, instances of unknown input data from the training set can be processed to get the intended output value. The more training data that is provided, the more accurate the learned function will be. This function, which is usually referred to as a model since it may be considered as establishing a model of the data being investigated, can be created in a variety of ways, which is why ML is closely related to statistical models [32].

ML in Computational Biology

Important biological elements of the process of controlling gene expression are transcription factors. To precisely control the spatiotemporal regulation of genes, transcription factors or chromatin regulators bind to specific DNA sequences at specified locations known as transcription factor binding sites. Transcription factors are ubiquitous proteins that are essential for many biological processes. It is vital to anticipate the function and structure of proteins effectively given the rise in protein sequences [33]. Currently, techniques for predicting protein-DNA binding sites are based on both DL and conventional ML algorithms. To anticipate protein-DNA binding sites in the early stages, we typically employ a development method based on a conventional ML algorithm. DL-based techniques for predicting protein-DNA binding sites from sequence data have been incredibly successful in recent years [34]. The function of DNA-binding proteins can be predicted using a variety of statistical and ML techniques, and these techniques are constantly being refined. Convolutional neural networks (CNN), recursive neural networks (RNN), and combined neural networks based on CNN-RNN can be used to classify the current state of deep-learning techniques

for protein-DNA-binding site prediction [35].

CHALLENGES AND OPPORTUNITIES

One significant challenge of ML is data integration and quality. ML models heavily rely on large and diverse datasets, and integrating disparate sources of data while ensuring data quality and consistency can be complex and time-consuming. Additionally, maintaining data privacy and security remains a critical concern, particularly in sensitive domains like healthcare. Another challenge is the interpretability and transparency of ML models. As ML algorithms become increasingly complex, understanding how they arrive at decisions or predictions can be difficult. Furthermore, ethical considerations such as bias and fairness in algorithms, unintended consequences of automated decision-making, and the ethical use of data are central concerns. Despite these challenges, ML also presents numerous opportunities. ML algorithms have the potential to uncover patterns and insights in data that humans may overlook, leading to breakthroughs in areas such as drug discovery, disease diagnosis, and personalized medicine. Moreover, ML enables automation and optimization of processes, improving efficiency and productivity across industries. By tackling issues related to data integration, interpretability, and ethics, we can harness the full benefits of ML while minimizing risks and ensuring equitable and ethical outcomes for society as a whole.

SUMMARY AND IMPLICATIONS FOR THE FUTURE

The review article delves into the transformative impact of ML on biological sciences. It highlights ML's pivotal role across various domains, including drug development, protein sciences, vaccine design, biosystems, and computational biology. ML techniques analyze vast biological datasets to identify novel drug targets, predict protein structures and functions, design personalized vaccines, model biological networks, and integrate multi-omics data for diagnosis and treating illness. The future of ML in biological sciences holds huge potential to improve our knowledge of diverse biological systems and address key challenges in healthcare. By continuing to innovate in algorithm development, data integration, and model interpretability, researchers can unlock new insights into disease mechanisms, develop more effective therapies, and optimize bioproduction processes. By fostering interdisciplinary partnerships and promoting accessibility to data and computational resources, ML promises to revolutionize biological sciences, ushering in a new era of innovation and discovery for the betterment of society.

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ARTICLE

IDENTIFICATION OF NOVEL SARS-COV-2 ENTRY INHIBITORS VIA STRUCTURE BASED HIERARCHICAL VIRTUAL

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ABSTRACT

A novel coronavirus (2019-nCov) is a pneumatic infectious disease caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has declared pandemic by the World Health Organization (WHO). However, there is no efficient drug therapy available to combat with this deadliest disease. By considering the public-health emergency, most of the SARS-CoV inhibitors and antiviral drugs are utilized for the treatment of COVID-19 infection. Herein, we presented integrated drug design strategies using E-pharmacophore modeling, molecular docking and molecular dynamic simulation studies based on the recently published SARS-CoV-2 RBD protein structure. Structure-based pharmacophore model (ADHRR) and high-throughput virtual screening (HTVS) were used to screen ZINC and ChEMBL molecular databases for identifying novel SARS-CoV-2 entry inhibitors. The retrieved potential hits were taken for the comparative molecular docking against SARS-CoV-2 and SARS-CoV enzymes to understand the different binding interactions. Further, the stability of receptor-ligand complex and specific amino acid interactions was evaluated by performing molecular dynamic simulation of 30 ns in solvated model system. This study

identified ZINC00662497, ZINC00669387, ZINC08426008, ZINC0660155 and ZINC0844005 as promising leads for inhibiting the entry of 2019-nCoV virus.

Keywords: *Corona virus, E-pharmacophore modelling, Molecular docking, Molecular dynamic simulation, SARS-CoV-2 entry inhibitors*

INTRODUCTION

Several members of corona viridae family are constantly circulating in human population and causing acute/ mild respiratory diseases. The mild and asymptomatic infections are caused by alpha-coronavirus, whereas severe acute respiratory syndrome coronavirus (SARS-CoV) and Middle East respiratory syndrome coronavirus (MERS-CoV) are beta-coronaviruses caused serious epidemics [1]. Severe acute respiratory syndrome coronavirus (SARS-CoV) and Middle East respiratory syndrome coronavirus (MERS-CoV) are transmitted from animals to humans. The SARS emerged in 2002 from horse shoe and bats, natural reservoir hosts for SARS-CoV [2]. Human transmission was facilitated by intermediate hosts like civet cats and raccoon dogs [3]. In 2012, it recurrence in the form of MERS-CoV; HCoV-229E, HCoV-OC43, HCoVNL63 and HCoV-HKU1 are few other coronaviruses responsible for human infections [4]. The re-emergence of coronavirus (2019-nCoV), officially named as SARS-coronavirus 2 (SARS-CoV-2) has created alarming situation and demanding potential therapeutic to preclude the death of infected patients [5]. However, there is no approved drug therapy is available for the treatment of COVID-19 disease. Recent treatments are focused on quarantine and containment of infected patient to minimize the human transmission [6]. The cellular entries of coronaviruses facilitate by viral surface spike protein (S-protein). Their attachment depends on the interactions between envelope-anchored spike glycoprotein (SARS) and angiotensin-converting enzyme 2 (ACE2) receptor on the surface of target cells [7]. The S-protein is homotrimeric in which each monomer comprised of two subunits, S1 contains receptor binding domain (RBD) and S2 implicates viral fusion on cell membrane [8]. Sequence similarity between SARS-CoV and SARS-CoV-2 spike protein is about 80% [9]. Moreover, amino acid residues S-RBD of SARS-CoV-2 are highly conserved with S-RBD of SARS-CoV of bats, human, and civet. The binding affinity of S-RBD of SARS-CoV-2 and ACE2 is approximately ten times higher than SARS-CoV RBD, indicating that ACE2 is the specific receptor responsible for the SARS binding to the host cell [10]. Thus, virus specific molecular interaction of SARS-S/ACE2 proteins is considered as a promising therapeutic target for discovery of newer SARS-CoV-2 entry inhibitors. Furthermore, many research reports are available for targeting viral entry

inhibitors, such as hydroxychloroquine [11], camostat [12], umifenovir [13], etc for minimizing the COVID-19 infection. Discovery of newer entry inhibitors can be considered as potential cure for minimizing the exponentially expending of COVID-19 infection [14]. Molecular modelling methods such as structure-based pharmacophore mapping is a useful tool in order to describe structural and functional requirements of the molecules for biological activity. Pharmacophore model when search against molecular data base as a 3D search query retrieves molecules, which possess almost similar features as per the requirement to achieve desired biological activity [15,16] . Herein, we used receptor structure-based E-pharmacophore model for database screening, followed by high-throughput virtual screening using recently published receptor-binding domain (S-RBD) of SARS-CoV-2. Firstly, the database comprised of molecules from ZINC and ChEMBL libraries was screened by receptor-based energy-optimized pharmacophore model. Further, the identified hits comprising of essential pharmacophoric features were considered for molecular docking based high-throughput virtual screening to recognize their essential binding interactions and binding affinities. Finally, the most promising retrieved hits were taken for comparative docking study against both SARS-CoV and SARS-CoV-2 RBD proteins to gain the different binding insights. The essential residual interactions were validated and dynamic behaviour of protein-ligand complex was determined by performing the molecular dynamic simulation for 30 ns. These identified leads may be developed as potential antiviral agents for preventing the spread COVID-19 infections (Fig.1).

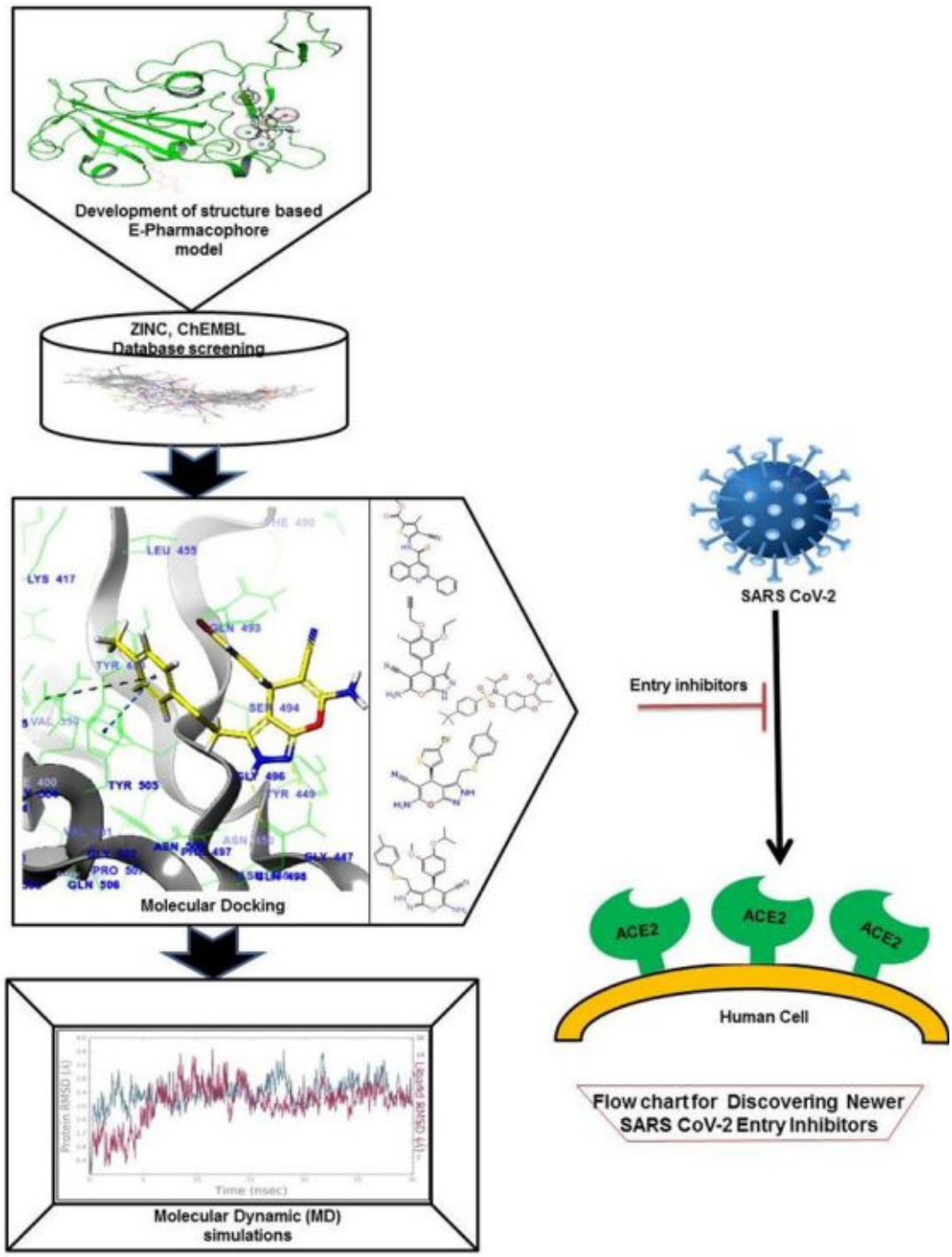

Figure 1. Overall Study Workflow.

MATERIALS AND METHODS

Protein preparation and receptor grid generation

The crystal structure of SARS-CoV and SARS-CoV-2(COVID-19) spike receptor-binding domain proteins (PDB ID: 6M0J, 2AJF) complexed with human ACE-2 was downloaded from Protein Data Bank [17,18]. The proteins were prepared by assigning bond orders, adding H- atoms and removing water molecules using Protein preparation Wizard (Maestro, v11.4, Schrödinger, LLC, NewYork, NY, USA). The atom types were assigned and charge state was optimized. Finally, the structures were minimized by OPLS-2005 force field by converging the heavy atoms to RMSD of 0.3 Å. As, there was no co-crystalized ligand available in the protein structure, 20 × 20 × 20 Å grid was generated by selecting amino acid residues (Lys 417, Tyr 449, Tyr 453, Phe 486, Asn 487, Gln 493, Gly 496, Gln 498, Thr 500, Asn 501, Gly 502 and Tyr 505) [9] using Receptor Grid Generation module (Maestro, v11.4, Schrödinger, LLC, New York, NY, USA).

Molecular database construction and ligand preparation

We performed virtual screening against two molecular databases for the identification of novel entry inhibitors. ZINC [19] includes 2500 molecules and ChEMBL [20] includes 160 molecules. Both the libraries were screened using refined 3D search query for drug like properties, such as molecular weight (150-500); xlogP (-4to5); rotatable bond (8); polar surface area (150); hydrogen-bond donar (10); hydrogen-bond acceptor (10). The database was constructed by merging both molecular libraries followed by ligand preparation and ADMET filtration using phase module. Ligand preparation involved addition of hydrogen atoms, removal of salt, and generation of stereoisomers, ionization at pH (7 ± 2) and determining valid 3D conformation. All the compounds were filtered based on their drug-like properties.

Protein sequence similarity

The SARS-CoV and SARS-CoV-2 (COVID-19) spike proteins and their sequences were downloaded from the protein data bank (PDB). The sequence similarity was assessed by superimposing Chain E of proteins using protein sequence similarity tool of Schrödinger software.

E-pharmacophore generation and validation

Three promising entry inhibitors were docked against previously prepared receptor grid of SARS-CoV-2 protein using glide module. Energy-optimized structure-based pharmacophore (E-pharmacophore) was built by retrieving glide XP descriptors information and mapping the energy terms on the atoms [21]. E-pharmacophore was built by mapping the Glide XP energies for the best

docked complex using phase module (Schrödinger, LLC, NY, USA, 2017). The pharmacophore hypothesis ADHRR was generated based on the identified pharmacophoric features of hydrogen bond acceptor (A); hydrogen bond donor (D); hydrophobic (H) and ring aromatic (R) using develop pharmacophore from receptor-ligand complex' option in Phase module. The performance of pharmacophore hypothesis was evaluated by calculating Enrichment Factor (EF) calculation. For that, 20 actives and dataset of 1000 decoys was downloaded from Schrödinger (http://www.schrodinger.com /glidedecoyset) [22]. The enrichment factor at 1% indicated the enhanced recovery of known actives over the decoys. Boltzmann-enhanced discrimination of receiver operating characteristic curve (ROC) i.e., BEDROC (α=20.0) metrics exhibits enhanced recognition of known actives over decoys from the internal database [23].

E-pharmacophore database screening

A five-point pharmacophore model ADHRR was used to screen molecular database containing 2660 compounds. The database screening was performed by adjusting minimum features match of 4 out of 5 sites in ligand and one inter site distance constrain in Phase module [24,25]. Screening was processed by generating conformers for each ligand, matching the excluded volumes and inter site distance tolerance was set at 2.0 Å. The screened compounds were ranked based on their fitness score, alignment score, volume and RMSD value. The compounds with the best fitness score were subsequently subjected for high throughput virtual screening. The HTVS docking is quick and more tolerant to suboptimal fits than standard precision (SP) and extra precision (XP) docking study [26]. For docking study, ligands were prepared, filtered based on their drug like properties and receptor grid was defined by selecting the reported amino acid residues. Compounds with the best docking score and glide score were subjected to XP docking.

Comparative molecular docking studies

The retrieved compounds from HTVS screening were taken for the rigid XP (extra precision) docking against 6M0J and 2AJF proteins [27]. The shape and properties of receptors were represented by computing a grid box by selecting protein residues using receptor grid generation panel. Ligands were prepared and chemical correctness was achieved by protonation, ionization variations, energy minimization and tautomerization at pH 7.0 using Ligprep module. Comparative docking study was performed using XP docking algorithm in glide module by keeping van der Waals scaling factor at 1.0. Further, the protein-ligand interactions were identified and docking poses were visualized by Maestro interface 11.4. The best docked ligand with the highest XP score was

considered for molecular dynamic simulation study.

In silico ADMET prediction

The ADMET properties of retrieved hits, mainly partition coefficient (QPlogPo/w), water solubility (QPlogS), cell permeability, (QPPCaco-2), % human oral absorption and HERG K^+ IC_{50} (QPlogHERG) were predicted using the QikProp module. It predicted 44 physicochemical and pharmacokinetic descriptors values based on 95% of known drugs in the normal mode with default setting [28].

Molecular dynamics simulations

A molecular dynamic simulation (MD) of SARS-CoV-2-ZINC00662497 complex was performed using Desmond module (Schrödinger, LLC, NY, USA, 2017). For that, protein-lead complex was inserted in to SPC water model and minimized using OPLS-2005 force field [36]. The three-step process included system builder, minimization and molecular dynamics. A system was built on the receptor using SPC system builder having 10 Å buffered orthorhombic boundary box. The system was solvated using TIP3P water molecules and neutralized using 0.15M NaCl [22]. The energy of neutralized system was minimized by steepest descent method having maximum 2000 steps with or without solute restrains. Particle mesh Ewald (PME) method was used to calculate long range electrostatic interactions and van der Waals and short-range electrostatic interactions were truncated at 9.0. The SHAKE algorithm was applied for limiting the movements of hydrogen atoms of covalent bonds. Nose–Hoover thermostats were utilized to maintain constant simulation temperature, and Martina–Tobias–Klein method was used to control pressure throughout simulation. The simulation was carried out at 300 K and 1.0 bar pressure using Berendsen thermostat and barostat [29]. The system was relaxed for 2 ns before productive simulation of 30 ns having recorded trajectory at time interval of 5.0 ns. The intermolecular hydrogen bond interactions, energy potential and root mean square deviation (RMSD) were examined to exhibit stability of receptor-ligand complex.

RESULTS AND DISCUSSION

Generation of E-pharmacophore model

As, there is no co-crystalized ligand available in SARS-CoV-2 protein structure; the residual based receptor grid was generated by performing docking of known antiviral entry inhibitors (umifenovir, camostat and hydroxychloroquine) into the identified binding site and represented in Fig. 2. The various receptor-ligand complexes were evaluated based on their binding interactions and docking poses.

Further, the best dock complex was considered to generate energy-optimized

pharmacophore model using development of common pharmacophore hypothesis option available in phase module. The E-pharmacophore model was developed by mapping Glide XP energetic terms on the pharmacophoric sites, which were evaluated from structural and energy information of protein-ligand complex. We developed five-point pharmacophore hypothesis ADHRR comprised of one hydrogen bond acceptor, one hydrogen bond donor, one hydrophobic and two ring aromatic features. The structure-based pharmacophore model was generated in such way, that it could effectively map all the structural features, which are responsible for biological activity.

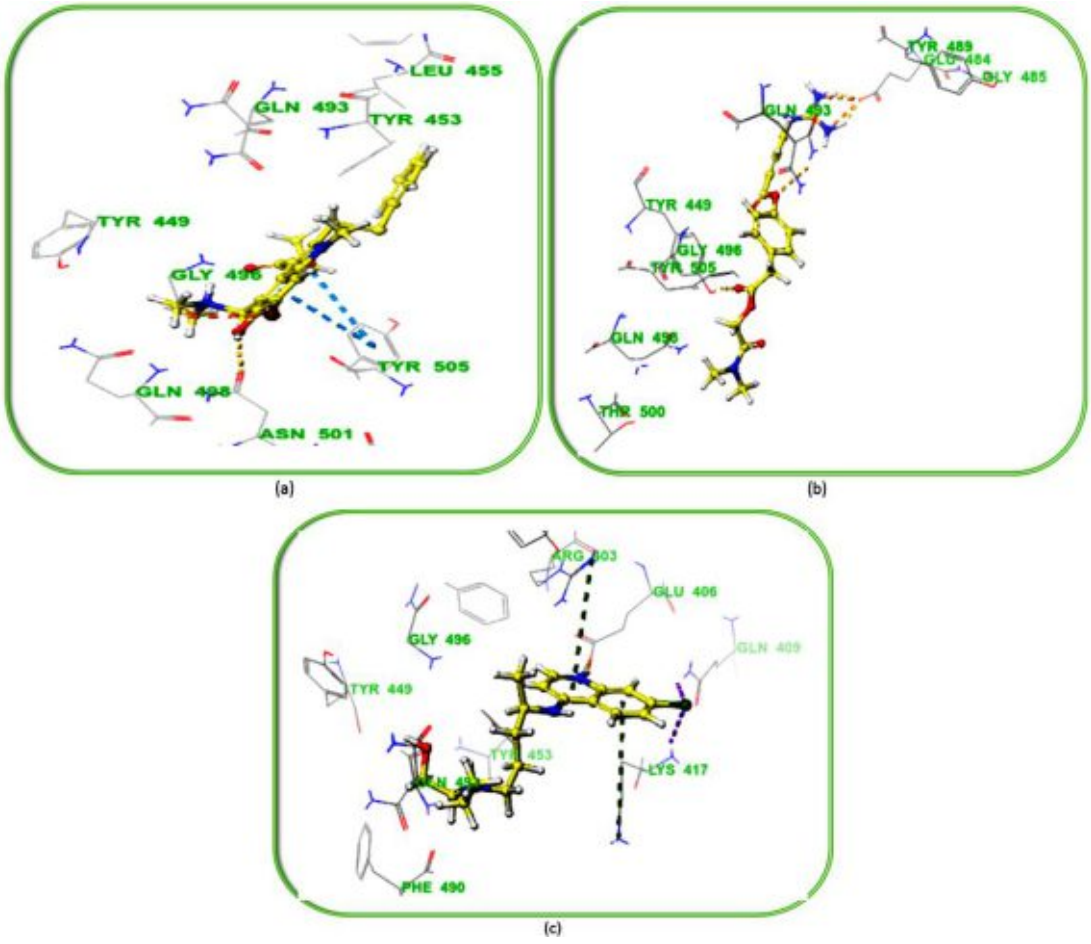

Figure 2. Residual interactions of a) Umifenovir; b) Camostat and c) Hydroxychloroquine at the binding site of SARS CoV-2 RBD (PDB ID: 6M0J)

Validation of energy-optimized pharmacophore

The performance of generated E-pharmacophore model was evaluated by screening the molecular library using validation of pharmacophore hypothesis module in Phase. The internal molecular library composed of 20 known actives obtained from literature and 1000 decoy molecules downloaded from Schrödinger. After screening statistical parameters, mainly total hits (H_t); % ratio of actives; % yield of actives; model enrichment factor (EF); false negative; false positives; goodness of hit score (GH), N% of sample size and BEDROC (α=20) were calculated (Table 1).

Table 1: Goodness-of-hit (GH) Scoring Parameters of ADHRR

Serial No.	Parameter	Values
1	Total number of decoy molecules (D)	1000
2	Total number of Active molecules (A)	20
3	Total hits (Ht)	26
4	Active hits (Ha)	18
5	% Yield of Actives [(Ha/Ht)X100]	70
6	% Ratio of Actives [(Ha/A)X100]	90
7	Enrichment factor (EF)*	34.61
8	False positives, FP [Ht-Ha]	08
9	False negatives, FN [A-Ha]	02
10	Goodness of hit (GH)*	0.72

EF= [(Ha X D)/(Ht X A)]; GH= [$Ha(3A+H_t)/(4H_tA)$]*[1-[H_t-H_a)/(D-A)]

The enrichment factor matrices measure the actives in the top ordered list compared to decoys from the internal library. Further, the GH score ranges from 0 to 1, where 0 exhibits the null model and 1 represents the ideal model. The receiver operative characteristic curve (ROC) metric represents the linearly scaled average of actives positions and ranked within the library. The ROC is ranging from 0 to 1; Truchon and Bayly suggested ROC e" 0.7 as a desirable performance value.

BEDROC denotes the early identification of actives from the database ranging from 0-1 in which α=20.0 exhibits 80% of the BEDROC results come from the first 8% of ranked molecules. The EF (34.61), ROC (0.93), AUC (0.95), RIE (14.69) and BEDROC (α=20) (0.939) indicated efficiency of generated pharmacophore hypotheses to identify actives from the ranked molecules in the internal library (Table 2). Finally, this statistically significant five features E-pharmacophore hypothesis was considered for the database screening.

Table 2: Enrichment Matrices for Energy-Optimized Pharmacophore (ADHRR) Validation

Serial No.	PARAMETERS	VALUES
1	ROC	0.93
2	AUC	0.95
3	RIE	14.69
4	EF	34.61
5	BEDROC(α=8)	0.923
6	BEDROC(α=20)	0.939
7	BEDROC(α=160)	0.980

ROC= receiver operating characteristic; AUC = Area under curve; RIE = Robust initial enhancement; EF = enrichment factor; BEDROC = Boltzmann-enhanced discrimination of receiver operating characteristic.

Database screening

The validated E-pharmacophore hypothesis (ADHRR) was employed to screen the constructed database containing 2500 molecules from ZINC and 160 molecules from ChEMBL library using database screening option in Phase module. The database was screen by matching maximum number of features is 4 out of 5 and applying one inter site distance constrain. E-pharmacophore based screening identified 350 molecules based on their highest fitness and alignment score from the database. The retrieved molecules were further filtered by hierarchical structure based molecular docking protocol of high throughput virtual screening (HTVS) in Glide module. These hits were pre-filtered by calculating ADMET

properties within the module. Subsequently, they were subjected for the Glide HTVS/SP/XP docking in the designated binding site of SARS-CoV-2 protein (PDB ID: 6M0J). Total 250 compounds were obtained from HTVS; 158 compounds resulted from SP and 135 compounds retrieved from XP docking studies. Finally, the resulted hits with better Glide XP score and docking pose were analysed.

Comparative docking analysis

The retrieved hits from high throughput virtual screening having SP docking score e" -7.0 kcal/mol were subjected for the comparative molecular docking analysis against SARS CoV-2 and SARS CoV. The extra precision (XP) rigid docking was performed in the constraint grid of 6M0J and 2AJF proteins to analyse the different binding interactions. Total 75 hits were docked into the active site of receptor binding domains of SARS CoV-2 and SARS CoV.

Top15 virtual hits with their fitness score, glide score and XP scores are summarized in Table 3.

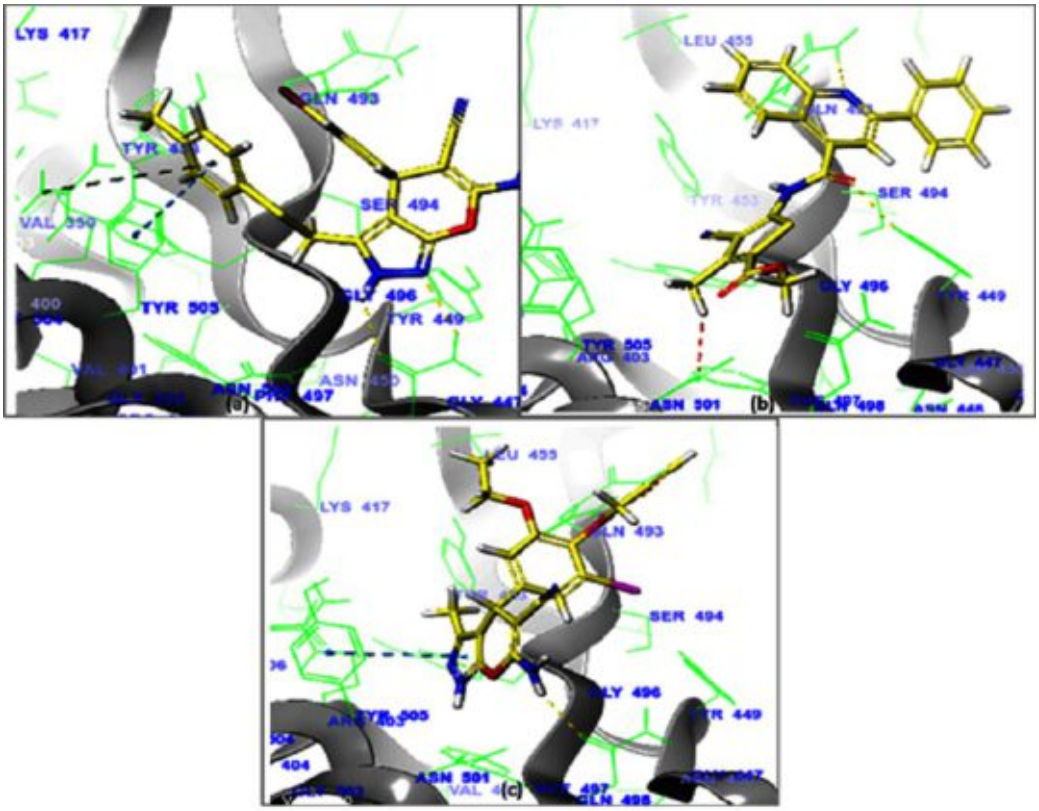

Figure 3. Residual Interactions of a) ZINC00662497, b) ZINC00669387 and c) ZINC08426008 at the Binding Site of SARS-CoV-2 (PDB: 6M0J)

The greater binding affinities for SARS-CoV-2 was identified by the higher docking score of -8 to -7 kcal/mol, whereas the lowest docking score of -6 to -3 kcal/mol indicated less binding affinity for SARS–CoV. The best docked compounds were ZINC00662497, ZINC00669387, and ZINC08426008 against the both the proteins are presented in the Figure 3 and 4. ZINC00662497 has docking score of -8.197 kcal/mol at the binding site of 6M0J protein; the amino group of 2,4-dihydropyrano pyrazole formed H-bonding with Gly 496 and Tyr 449; phenyl ring exhibited pi–pi stacking with Tyr 505 and formed hydrophobic contacts with Val 350.

Table 3: Top 15 Virtual Hits with Their Highest Fitness Score and Binding Free Energy to SARS- Cov-2 and SARS-Cov Enzymes.

Sr No.	Compound Name	Fitness Score	SARS CoV-2		SARS CoV	
			glide gscore	XP GScore (kcal/mol)	glide gscore	XP GScore (kcal/mol)
1	ZINC00662497	1.754	-8.197	-8.197	-5.832	-5.836
2	ZINC00669387	1.577	-8.101	-8.101	-5.539	-5.539
3	ZINC08426008	1.733	-8.057	-8.057	-5.457	-5.457
4	ZINC0660155	1.749	-7.975	-7.975	-5.765	-5.775
5	ZINC0844005	1.624	-7.924	-7.924	-5.289	-5.289
6	ZINC00688318	1.638	-7.870	-7.875	-6.095	-6.095
7	ZINC00688321	1.571	-7.844	-7.844	-4.857	-4.857
8	ZINC02497152	1.553	-7.785	-7.788	-3.933	-3.933
9	ZINC10009832	1.476	-7.698	-7.698	-3.457	-3.457
10	ZINC02170042	1.442	-7.671	-7.671	-3.539	-3.539
11	ZINC02497224	1.429	-7.503	-7.505	-3.766	-3.764
12	ZINC08426766	1.377	-7.415	-7.415	-4.888	-4.888
13	38	1.418	-7.371	-7.371	-3.325	-3.325
14	13	1.315	-7.351	-7.351	3.623	-3.623
15	55	1.626	-7.258	-7.258	-3.317	-3.317

ZINC00669387 possessed docking score of -8.101 kcal/mol; the nitrogen of quinoline ring formed H-bonding interaction with Gln 493 and amidic oxygen formed H-bonding with Tyr 449; 3-CH_3 group of 5-amino-4-cyano-3-methylthiophene ring showed hydrophobic contacts with Asn 501 of SARS –CoV-2 protein. The binding affinity of ZINC08426008 for SARS–CoV-2 spike glycoprotein was demonstrated by docking score of -8.057 kcal/mol; 6-NH_2 group of 6-amino-3-methyl-1,4-dihydropyrano pyrazole ring formed H-bonds with Gly 496 and 2-propylnyoxy substituent of 1-ethoxy-3-iodo-2-(propynyloxy)phenyl ring also exhibited H-bonding interaction with Gly 493; pi–pi interactions were observed between 1,4-dihydropyrano pyrazole and Tyr 505. Residues Tyr 449, Gln 493, Gly 496, Asn 501 and Tyr 505 are the most prominent for the binding interactions in SARS-CoV-2 protein (Figure 3).

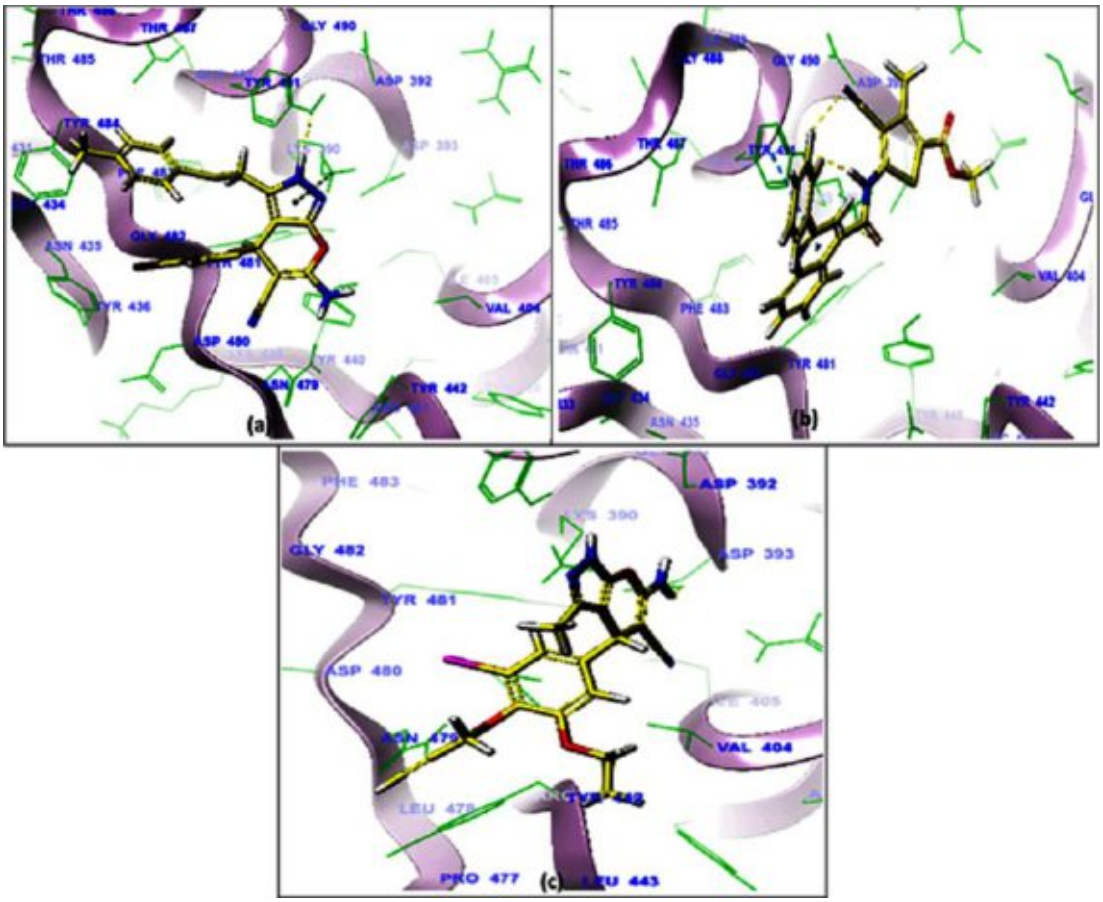

Figure 4. Residual Interactions of a) ZINC00662497, b) ZINC00669387 and c) ZINC08426008 at the Binding Site of SARS-CoV-1(PDB: 2AJF).

In comparative docking study ZINC00662497 exhibited the lowest binding energy of -5.836 kcal/mol at the binding site of SARS-CoV protein. The 2, 4-dihydropyrano pyrazole formed H-bonding interaction with Tyr 491 and hydrophobic contacts with Lys 390. The ZINC00669387 represented minimum docking score of -5.539 kcal/mol against 2AJF protein; -NH of amide bond and 5–NH_2 of 5-amino-4-cyano-3-methylthiophene showed H-bonding interactions with Tyr 491; pi-pi stacking was observed between the phenyl ring and Tyr 491. Further, ZINC08426008 exhibited relatively less binding affinity of -5.457 kcal/mol for SARS-CoV protein; 6-NH_2 group of 6-amino-3-methyl-1, 4-dihydropyrano pyrazole ring formed H-bond with Asp 393. As shown in the Figure 4 residues, Lys 390, Asp 393 and Tyr 491 are important for interactions at the active site of SARS-CoV. Overall, the highest binding energy indicated that identified leads showed good binding affinities for SARS-CoV-2 compared to SARS-CoV protein.

Molecular dynamics (MD) simulation

The best docked 6M0J- ZINC00662497 complex was subjected to MD simulation to gain the dynamic comprehension of binding interactions for time period of 30ns. The stability of protein-ligand complex was studied by analysing RMSD and RMSF plots of protein backbone compared to its initial frame structure. The protein trajectory fluctuated between the RMSD of 2.4 Å to 3.6 Å as displayed in Fig. 5a. The ligand RMSD between 1.2 Å to 3.0 Å indicated the stability of ligand within binding pocket of protein during the simulation. The difference between the RMSD values of ligand and protein reflected the stability of ligand throughout the simulation. Further, the RMSF analysis of protein backbone exhibited fluctuation of N- and C-terminal residues as shown in Fig.5b. Most of the protein residues fluctuate below 2.0 Å and larger fluctuation up to 5.0 Å. As shown in the Fig. 5c the RMSF value of ligand was also about 2.5 Å. The favourable amino acid interactions with ligand atoms were also analysed. Residues Lys 417, Tyr 449, Asn 487, Gln 493, Gly 496, Gln 498, Asn 501 and Gly 502 formed hydrogen bonding interactions during the simulation. The Lys 417 also exhibited water bridges and ionic interactions with the inhibitors. Tyr 449, Phe 486, Thr 500 and Tyr 505 displayed hydrophobic contacts and Pi-Pi interactions with ZINC00662497 (Fig. 5d).

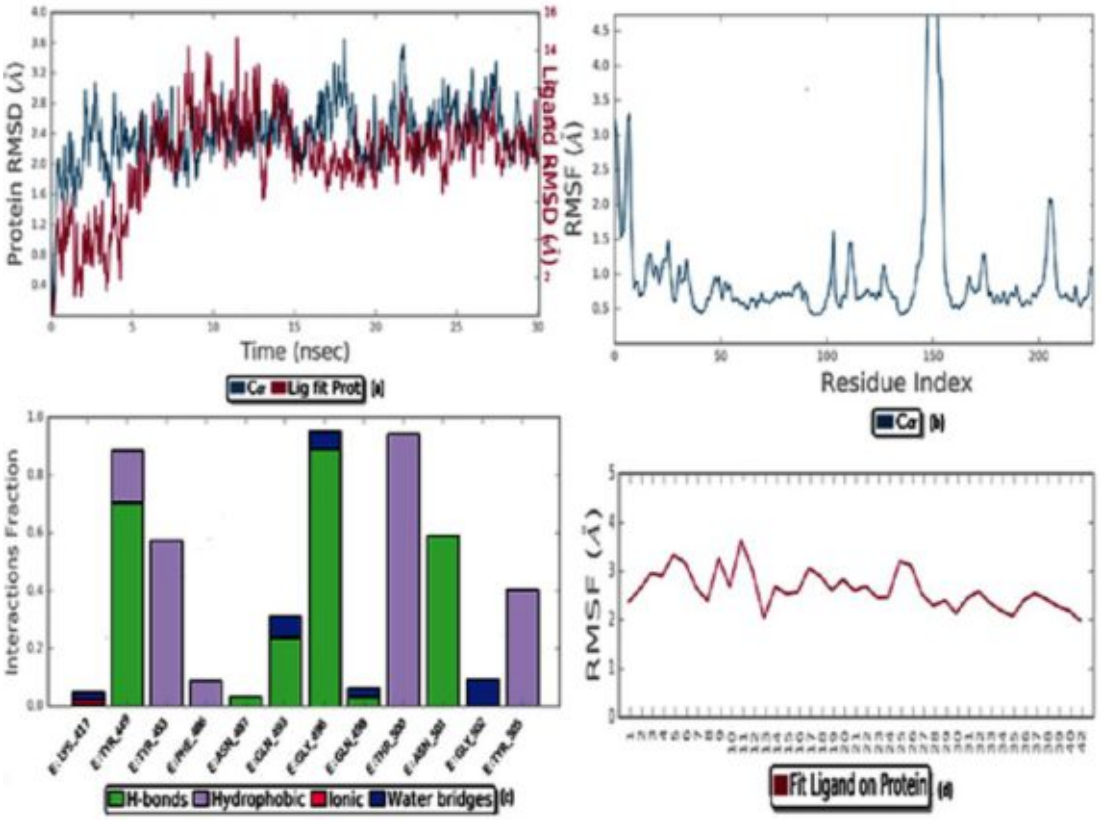

Figure 5. Analysis of MD Simulation Results of 6M0J- ZINC00662497 a) RMSD Plot of 6M0J ZINC00662497 Complex b) RMSF Plot of 6M0J Protein c) RMSF Plot of ZINC00662497 with Respect to 6M0J Protein d) Fraction of Interactions Between 6M0J and ZINC00662497 over the Simulations of 30 ns.

CONCLUSION

In this study, we used combined E-pharmacophore modelling and molecular docking based high throughput virtual screening (HTVS) to identify newer SARS-CoV-2 entry inhibitors. The receptor structure-based pharmacophore model (ADHRR) was developed using the energetic results of docked fragment in to 6M0J protein. Further, the validated pharmacophore hypothesis detailing essential pharmacophoric features was used to screen ZINC and ChEMBL molecular databases to identify potential hits. The resulted 350 hits were further taken for molecular docking based high throughput virtual screening using SARS-Cov-2 entry protein. The retrieved 75 potential hits were again taken for the comparative extra precision (XP) glide docking in to the receptor binding domain of SARS-CoV and SARS-CoV-2. Top 15 promising hits were analysed based on

their fitness scores, docking scores, binding interactions and ADME profiles. The stability of protein-ligand complex and their binding interactions were determined by performing MD simulation of best docked complex for 30ns. Finally, ZINC00662497, ZINC00669387, ZINC08426008, ZINC0660155 and ZINC0844005 were identified as promising leads for the treatment of Covid-19 infections with the highest fitness score and lowest binding free energies. In future, these potential hits will be acquired from vendors and can be used for further in vitro and in vivo studies to evaluate their effectiveness as newer SARS-CoV-2 entry inhibitors and also to validate these combined in silico findings.

CONFLICT OF INTEREST

The authors disclose there is no conflict of interest

ACKNOWLEDGMENT

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ARTICLE

CASE STUDIES ON APPLICATIONS OF COMPUTATIONAL TECHNIQUES IN DRUG DESIGN

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ABSTRACT

Computer-aided drug design (CADD) along with Artificial Intelligence (AI) based machine learning technologies have a powerful impact in the field of drug discovery as it can handle vast biological data which in turn reduces the cost and time of drug discovery and development process. Identifying hits through virtual screening and its further optimization for the development of lead molecule through ligand- or structure-based drug designing have played a vital role. Docking and molecular dynamics studies highlighted the binding of ligands with targeted proteins and their binding affinity. ADMET prediction studies prevent the failure of many drugs in clinical trials and thus prevent loss of time and money. The availability of open-source big data has facilitated the screening of vast libraries intending to come up with novel potent and target-specific drugs. Present review has given an overview of the technology used in drug discovery by highlighting some of the case studies.

Keywords: *Artificial Intelligence (AI), Machine Learning (ML), Ligand- based drug design (LBDD), and Structure-based drug design (SBDD)*

INTRODUCTION

Drug discovery is an interdisciplinary multiple-step process that is time-consuming and expensive [1]. It requires 15-20 years for a drug to reach the market from the concept; which includes phases like target identification and validation, lead identification and its synthesis, pre-clinical screening, clinical trials and regulatory approval [2,3]. Although a huge amount of money and time are involved in the drug discovery process, the success rate is as low as 15-20%. The major cause of the failure is the poor ADMET profile of the lead [4].

To accelerate and elevate the success rate in the drug discovery process, the computational approach is harnessed to a tremendous extent [5]. With the swift evolution of diverse computer hardware and software, various structure based and ligand-based drug design techniques or algorithms are being utilized nowadays in drug screening and design [6]. For the advanced *in silico* drug design, chemoinformatic is considered as a prime tool [7]. Bioactivity spectra-based algorithms, data mining, chemical structure similarity searching, panel docking etc. are some representative examples of cheminformatics tools [8]. Target identification which is the first step in rational drug design; can be studied through network pharmacology, a field assimilating varied information in protein-disease, protein-protein and drug-protein networks [9]. Network pharmacology is very helpful in study of inter-correlations across different biological pathways, genomics, proteomics, metabolomics, transcriptomics etc. that rely on computational data and its interpretation [10]. Other approaches are; protein ligand interaction fingerprints method, ligand-based interaction fingerprint method, MetaboAnalyst approach for pathway analysis etc. [11,12].

Present review summarizes the published case studies on the basis of type of application of computational techniques and artificial intelligence in the drug design as summarized in **Fig. 1**.

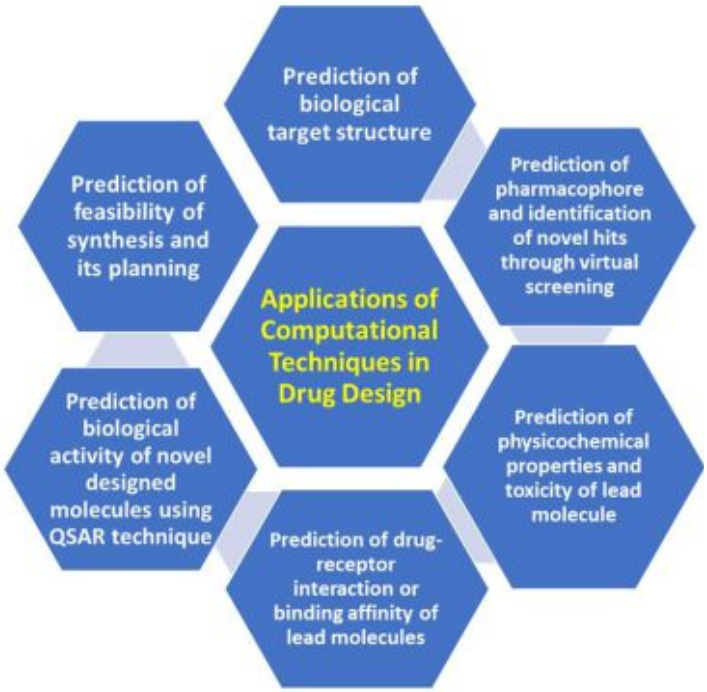

Fig. 1 Diverse Applications of Computational Techniques in Drug Design

APPLICATIONS OF COMPUTATIONAL TECHNIQUES IN DRUG DESIGN

Prediction of biological target structure

For structure-based drug design (SBDD), availability of 3D crystal structure biological macromolecular target is crucial. Through the advancements in the structure elucidating experimental techniques like X-ray, NMR, electron microscopy etc., 3D structure of proteins is resolved and then they are made available in public for the users [13]. However, in cases where 3D structure of the target protein is not known, computational techniques like homology modeling or ab initio modeling can be used to predict the 3D protein structure [14].

Homology modeling is a computational technique to predict protein structure based on its sequence identity and similarity with the template protein of known structure. For the reliable prediction, minimum 30% sequence identity should be possessed between the query sequence and template sequence [15].

Recently in 2024, Goswami, V. et al. [16] have reported the homology modeling of Porcupine protein which an enzyme involved in the Wnt signaling pathway. Various disorders like osteoporosis, cancer,

Alzheimer's disease etc. are associated with the dysregulated Wnt signaling. To design novel Porcupine inhibitors, there was a need of 3D structure of Porcupine. Goswami, V. et al. have predicted its 3D structure through homology modeling using an online server, I-TASSER and commercial software, Molsoft ICMPro. They compared and validated both the homology models through Ramachandran plot, Protein health tool of Molsoft ICM and other tools available on metaserver, SAVES v6.0. Molsoft model was found to be better with 84.6 % residues in most favored region and only 0.3 % residues in disallowed region in Ramachandran plot in comparison to 75.9% and 1.7% residues respectively in I-TASSER model. Finally, the predicted model using Molsoft ICMPro was considered for molecular docking of known porcupine inhibitors and future designing of novel Porcupine inhibitors. Later, authors have also compared the homology model binding site with that of the actual binding site of Porcupine structure deposited in the protein databank; PDB ID: 7URD and found high similarity between them. Thus, Computational technique, homology modeling was proved crucial in the designing of novel inhibitors.

Prediction of physicochemical properties and toxicity of lead molecule

Prediction of pharmacokinetic properties is an integral part of drug discovery that uses diverse AI-based tools which can analyze the behavior of drugs in the body. Few structural parameters being used in drug designing are molecular descriptors like SMILES strings, electron distribution of the molecule, energy calculation and bonds etc. These parameters are used in deep neural networking (DNN) for drug designing [17]. (Yukawa & Naven, 2020).

In 2019, Han et. al. [18] assessed the *in silico* ADMET profile of ceftazidime as well as its impurities A-I using Discover Studio 4.0 (DS4.0) software package and pkCSM ADMET descriptors algorithm protocol. Ceftazidime has been in clinical use since 1990s to treat various infections. There are many adverse effects related to its use which are not only related to the toxicity of API but also due to impurities in drugs. To identify the type of impurity and its structural features responsible for toxicity, prediction of ADMET properties of all the known impurities A-I have been carried out by Han et. al. Through structure toxicity relationship study, they could identify specific functional groups and stereochemistry of the drug, impurities and its metabolites responsible for various types of toxicity like neurotoxicity, genotoxicity etc. This study overall provided the base for experimental validation of predicting the toxicity of all other cephalosporins and their impurities, and for the quality control of these impurities.

Prediction of drug-receptor interaction or binding affinity of lead molecules/drugs

Molecular docking is an interesting in silico technique to determine the interaction of ligands with macromolecules free binding energy. It requires an input of 3D structure of an unbound macromolecule either directly obtained from protein data bank (PDB) or through homology modeling to predict the covalent/non-covalent binding of ligand molecules.

In 2021 Dassi et al. [19] reported drug repurposing for Leishmaniasis through hybrid approach. They blended representation learning, a deep learning approach with molecular docking to predict ligand-target interaction. Initially, an online deep learning-based tool, DeepPurpose, was used for docking based virtual screening of large dataset total 2,058,752 protein-ligand pairs which shortlisted 3400 pairs based on the affinity scores. These pairs were then processed for computing interface energies using molecular docking by Autodock Vina. The deep learning assisted hybrid approach proved to be 50 times faster than conventional docking based virtual screening without losing valuable drug candidates. The model predicted alpha-glutamicin, a non-steroidal anti-inflammatory drug, as a promising repurposed drug for the treatment of leishmaniasis.

Prediction of pharmacophore and identification of novel hits through virtual screening

According to the IUPAC [20], "A pharmacophore is the ensemble of steric and electronic features that is necessary to ensure the optimal supra molecular interactions with a specific biological target structure and to trigger (or to block) its biological response". Various pharmacophoric features are hydrogen bond donor (D), hydrogen bond acceptor (A), ring aromatic (RA), hydrophobic (H), Positive ionizable (PI), negative ionizable (NI) etc.

Babu et al. in 2022 [21] reported a study for identification of novel lead molecules as Janus kinase 1 (JAK1) inhibitors which can be used for the treatment of autoimmune diseases and cancer. They adopted hybrid approach and performed ligand-based pharmacophore modelling, virtual screening and molecular docking studies. Using 52 reported C-2 methyl/hydroxyethyl imidazopyrrolopyridines derivatives, ligand-based pharmacophore models were generated by Phase 4.3 module of a software, Schrodinger. The top 8 models were selected on the basis of validation techniques; Guner-Henry score and selectivity check for virtual screening of

various chemical libraries like Chemdiv, Asinex, Maybridge, Zinc, Enamine and Lifechemicals. ADMET study was performed on the identified hit and finally 2,856 hits which showed acceptable drug-like properties were selected for induced fit docking, MM-GBSA calculation and cross docking using GLIDE. Later, molecular dynamics (MD) simulation study was performed for the top 5 candidates using the GROMACS and DFT calculations using Gaussian. At last, best 2 hits, T5923531 and T5923555 were concluded as the best molecules for experimental validation using *in vitro* and *in vivo* techniques.

Prediction of biological activity of novel designed molecules using QSAR technique

Quantitative structure activity relationship (QSAR) is a ligand based drug design technique that establishes the relationship between different 2D/3D physico chemical descriptors of ligands with biological activity quantitatively using statistical methods and predict the activity of designed molecules before their synthesis [22,23]. Recently AI techniques are incorporated to develop SAR/QSAR predictive models which not only reduces the time but also allows the input selection to extract the features that affect the generated model. It improves the prediction accuracy and efficiency of QSAR [24].

Tsou L.K. et al. [25] in 2020 reported a comparative study between the deep neural networks (DNN) based QSAR model and PLS based QSAR model for their hit prediction efficiency thorough virtual screening. They proved DNN as well as random forest (RF) techniques superior. A database of 7130 molecules reported for their MDA-MB-231 inhibitory activities were collected from ChEMBL website and divided into training set (85%, 6069 compounds) and test set (15%, 1061 compounds) to develop the QSAR models. The results showed that DNN based method could achieve higher predicted r^2 value with only fewer compounds (Only 63) required in the training set than traditional QSAR method. Using this model, a potent (~ 500 nM) mu-opioid receptor agonist was identified as a hit from the in-house database of 165,000 compounds. This study proved the efficiency of AI based QSAR techniques in novel hit identification and prediction of their activity.

Prediction of feasibility of synthesis and its planning

The traditional path of drug discovery is organic synthesis but it is often accompanied by synthetic challenges which restrict chemical space available for drug designing [26]. Varied computational

approaches have been developed for doing systematic synthetic planning. Three aspects of the synthetic scheme are emphasized in computational studies, predicting the compound structure based on the starting chemical structure; predicting practical yield; and retrosynthetic planning [27]. In retrosynthetic planning, a knowledge-based system follows the rule from the reaction database. Forward synthesis prediction ranks the synthetic routes with a Monte Carlo tree search from retrosynthetic analysis using ML-based approach for an excellent performance. One of the open-source software is AiZynthFinder for retrosynthetic planning. In this software, Monte Carlo tree search system guided by an artificial neural network approach develops an algorithm that breaks down a molecule to purchasable chemical reagents and starting materials [28].

Thakkar A et al. [29] developed a basic retrosynthetic tool based on single neural network to investigate the role of the machine learning template prioritization method in the tree search algorithm. They trained USPTO dataset and developed a deep learning-based model and predicted the retrosynthesis route for a drug, Amenamevir. The route suggested by the model was compared with the literature routes. The model predicted the route of synthesis in just 4.26 seconds.

CONCLUSION:

With the case studies discussed above we can conclude that use of computational technology in drug discovery is a vital and appreciated tool that reduces the cost and time for research in the field of drug discovery. In the Big Data Era, hybridization of Artificial intelligence, machine learning, and deep learning approaches result in dealing with massive amounts of data and enable us to make quick decision. As we understand the application of AI for handling big data, we need to take care to avoid the access and utility of poor-quality data as input which hampers those final results of drug discovery related computational studies. The validation the hypothesis generated from computational approach is essential and inevitable steps. As the same time precaution of should be taken for distinguish false positive result from true positive results.

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ARTICLE

APPLICATION OF 3D BIOPRINTER GENERATED ORGANS TO OVERCOME SHORTAGE OF ORGAN DONATION

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ABSTRACT

Reliability of human organs for transplantation remains severely compromised, even with the remarkable advancements in transplant technologies. Of the 154,324 individuals in need of organs in 2009, just 18% were able to receive them; there were 8,863 deaths on the waiting list, or an average of 25 deaths every day. About 120,000 people in America were on the waiting list for organ transplants at the start of 2014. A fresh approach to the production of 3D organs is presented by 3D bioprinting, which appears to be a viable remedy to this grave situation. Using this innovative technique, three-dimensional structures can be produced which can mimic tissues by layering cells onto a biocompatible substrate using a combination of tissue engineering and 3D printing. Through the utilization of modern computer systems, powerful computer programming, and CAD file instructions, 3D bioprinting presents a promising solution to reduce the gap between organ supply and transplantation requests. In addressing the important gap in organ availability for transplantation, it stands as a ray of hope. In this review the current state of scientific research on 3D bioprinting, tissue engineering, and epithelialized organs will be examined. Additionally, it will also examine the practical applications for these exquisitely crafted, three-dimensional printed organs.

Keyword *Organ transplantation, 3D bioprinting, Tissue engineering.*

INTRODUCTION

The process of precisely dispensing biomaterials containing cells enables three-dimensional (3D) bioprinting, which can be used to build artificial organs or functional 3D intricate living tissues. The production of biomedical components by 3D bioprinting often aims to replicate the characteristics of genuine tissue by combining cells, biomaterials, and/or growth hormones. 3D bioprinting generally allows for the layer-by-layer deposition of materials known as bioinks, thereby enabling the creation of tissue-like structures for use in a range of medical and tissue engineering applications. [1,2]. A wide variety of biomaterials and bioprinting methods fall under the umbrella of 3D bioprinting. Currently, tissue and organ models may be printed using bioprinting to aid in the study of medications and prospective therapies [3]. The implementation of bio-printed live cellular structures into clinical application is hampered by the complexity and quantity of cells needed to build functional organs [4]. On the other hand, innovations include the bioprinting of extracellular matrix and the fusion of cells with hydrogels, which are subsequently applied one layer at a time to generate the necessary tissue. [5]. Scaffold printing has also started to be used in 3D bioprinting. It is possible to rebuild ligaments and joints using these scaffolds [6].

STEPS INVOLVED IN 3D BIOPRINTING

Typically, three phases are involved in 3D bioprinting, which include pre-bioprinting, bioprinting, and post-bioprinting [7], These is elaborated in fig.1 as given below.

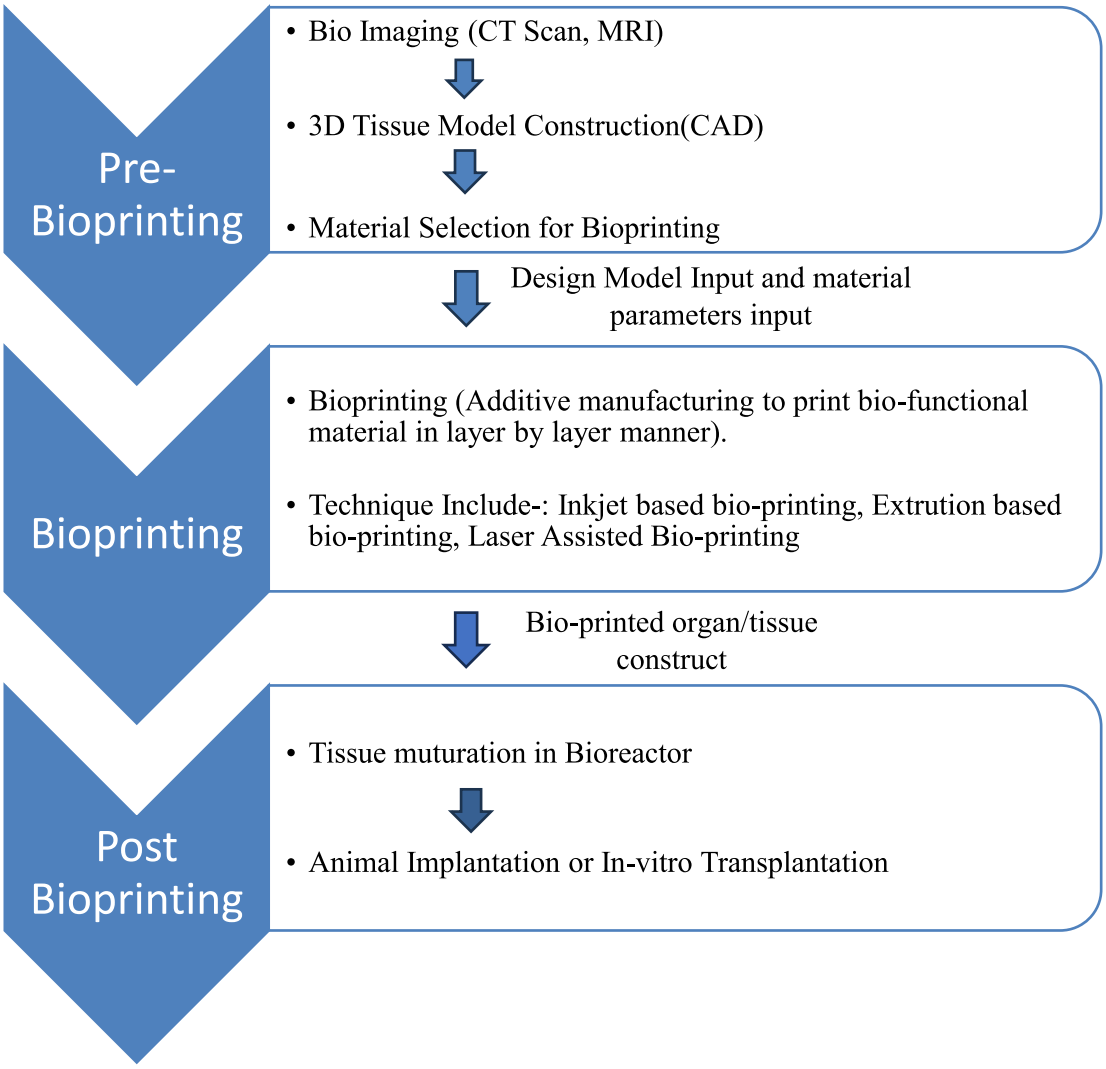

Figure 1: A schematic flow chart for 3D bioprinting procedure.

Pre-bioprinting

The pre-bioprinting process involves selecting the best material, in order to prepare a model that the printer will use to produce the final product. Initially, organ biopsy was typically used to design the model. Pictures collected by patients' computed tomography (CT) undergo tomographic reconstruction, x-ray images and magnetic resonance imaging (MRI) are often utilised for model development for bioprinting. Once 3D model is been created, certain cells are separated and multiplied [8]. The specialized liquid fluid that feeds these cells with oxygen and other nutrients is then blended with the cells. In some procedures, cells are encircled by 500 m-diameter cellular

spheroids. This cell aggregation, which does not require a scaffold, is crucial for embedding in the tubular-like tissue fusion for procedures like extrusion [9].

Bioprinting

Second phase involves placing the liquid bioinks—a mixture of matrix, cells, and nutrients in a cartridge of printer and depositing them using 3D model developed during the initial stage [10]. This cell-based pre-tissue when placed in an incubator develops into a tissue. Typically, 3D bioprinting fabricates the biological constructs by dispersing cells layer-by-layer onto a biocompatible scaffold in order to build three-dimensional tissue structures. Photolithography, stereolithography, direct cell extrusion and magnetic 3D bioprinting are a few of the techniques utilised for 3D bioprinting of cells. The capacity to maintain the stability and survival of the cells during the manufacturing process varies across the technologies used to design these tissues.

Post-bioprinting

Post-bioprinting is necessary in order for the biological material to become a stable structure. Both chemical and mechanical stimulations maintain the mechanical integrity and functionality of the 3D-printed product [7]. Cells get signals from these stimulations, which also control cell division and tissue remodeling. Bioreactor technologies have been developed recently [11]. The advancement of bioreactor technology increases the likelihood of tissue vascularization, rapid maturation, and long-lasting transplants [8]. Bioreactors function through many methods such as convective nutrition transfer, microgravity conditions, pressure changes-induced solution flow across the cells, or the addition of compression for either static or dynamic loading. Certain bioreactor types are better suited for particular tissue types; cartilage tissue is best served by compression bioreactors, for instance [9].

BIOPRINTING TECHNIQUES

Inkjet, extrusion, and laser-assisted bioprinting are the three main methods used in 3D bioprinting. There is currently no single bioprinting process that can be used to create synthetic tissues of all sizes and complexity. Each approach has unique advantages, disadvantages, and restrictions.

Inkjet Printing

First-generation 3D bioprinting technique is called 3D Inkjet bioprinting. It resembles a conventional 2D inkjet printer, in that bioink (hydrogel with suspended cells) is put into the cartridge. The cartridge is then attached to the printer head. During this process, piezoelectric actuator or thermal provides pressure and deforms the printer heads to produce/or to create droplets of a predetermined size.

Because of its structural resemblance to commercial printers, inkjet printing offers several advantages which include inexpensive cost, fast printing speed: parallel work mode and comparatively high cell survival are made possible by the printer heads., as determined by various experimental findings [12–14].

Laser-assisted printing

Laser printing originated from laser-induced transfer technologies [15,16] and laser direct-write [17]. The process involves two layers: a top donor layer and a bottom bioink layer. The top donor layer is critical as it is stimulated by a laser. This layer is structured like a "ribbon" with an energy-absorbing layer inside, often made of materials like titanium or gold. During printing, a focused laser pulse stimulates a portion of the absorbent layer, vaporizing it and generates a bubble of high pressure when it meets the bioink layer. This bubble launches the suspended bioink, which is later caught on the receiving substrate and crosslinked. Laser-assisted printing differs from inkjet printing by avoiding direct interaction between the dispenser and the bioinks, thus reducing mechanical stress on the cells and achieving high cell survival rates (typically over 95 percent).

Furthermore, laser-assisted printing offers more flexibility in bioink selection, accommodating a wider range of materials including highly viscous ones. However, the long-term effects of laser exposure on cells remain unclear. Furthermore, operating the laser printing system might be difficult, which prevents the mass adoption of this technology. High-resolution and powerful laser diodes are more expensive than nozzle-based printing processes.

Extrusion printing

Extrusion printing has enhanced and changed inkjet printing. To print materials that inkjet printers cannot deposit due to their viscous nature, bioinks are distributed using either mechanical screw plunger or an air-force pump in extrusion printing. Applying force continually during extrusion printing may result in continuous cylindrical lines rather than just one bioink droplet. Extrusion bioprinters are capable of printing aggregates with high cell densities and nearly all types of hydrogel pre-polymer solutions with varying viscosities. Although extrusion bioprinters can print a larger range of materials, they also put the cells inside under increased mechanical stress, which is thought to reduce the likelihood of cell death [18,19].

MATERIALS FOR BIOPRINTING

The hydrogel pre-polymer solution and cells which are covered in detail below are the two primary components of bioinks.

Hydrogel

The use of hydrogels is essential to the 3D bioprinting process. In addition, not only do they stay in close proximity to the cells to offer structural reinforcement, but they also control the chemical and physical characteristics of bioinks [20]. Ideal hydrogels for bioprinting should be able to provide mechanical support, be printable and crosslinkable, and create an environment that encourages attachment, proliferation, and differentiation [21, 22]. It should have the quality of being biocompatible as well as biodegradable. As far as hydrogel design is concerned, there are essentially two kinds of hydrogels: those based on synthetic and natural polymers [23].

Cells

Bio-printed cells must multiply in order to develop into a large-scale tissue or organ that is well mimicked. When choosing cells for bioprinting, two primary criteria are taken into account: how successfully the bio-printed cells can mimic the typical state of cells in vivo and how well they can develop or maintain their roles in vivo in ideal microenvironments [19].

APPLICATIONS OF 3D BIOPRINTER GENERATED ORGANS TO OVERCOME SHORTAGE OF ORGAN DONATION

3D organ printing and transplantation, regenerative medicine and tissue engineering, high-throughput screening, drug printing, toxicity and drug screening, and clinical research are among the primary uses of 3D bioprinting. Nevertheless, among these uses, the use of organs created by 3D bioprinters to address the organ donor shortage is particularly very desirable.

Every 15 minutes, a new name joins the waiting list for organ transplants in America [24]. Despite this list growing swiftly, less than one-third of patients awaiting transplants are able to receive matching organs from donors [25]. Over the previous ten years, there has been no increase in the supply of transplantable organs which raise doubts about the possibility of bridging this widening gap [26]. Tissue engineering, which involves the creation of new tissues and organs through a combination of cellular, engineering, and material techniques, emerges as one of the best solutions to address the scarcity of organs [27]. 3D bioprinting enables the reconstruction of tissues from various parts of the body. Recent progressions in 3D bioprinting have led to the production of ear, windpipe, bone, exoskeleton, jawbones,

stem cell, cell culture, eyeglasses, vascular networks, blood vessel, organs and tissue, as well as innovative dosage forms and drug delivery devices. Below is a discussion of a few of these:

Liver Tissue

Organova, a bioprinting pioneer known for successfully fabricating heart tissues, blood vessels and bones that recently achieved a significant milestone in the field: the development of 3D liver tissue. This tissue consists of three distinct types of cells typically found in the human liver. The specific arrangement of these cells is critical for their functionality, making the 3D tissue a more accurate model compared to traditional 2D cell cultures used in laboratories. By closely replicating the interactions within a real liver, the 3D tissue provides more reliable results. Organova claims that this advancement not only accelerates the drug discovery process but also reduces its cost. The bioprinting technique employed by Organova resembles that of desktop inkjet printers, using needle-like nozzles to deposit cells precisely in a predetermined pattern. Remarkably, the printed liver tissue remains alive for up to 42 days which enables researchers to subject it to various experimental medication on it [28].

Human Ear

"Professor Alex Selfalian and his research team at University College London" have achieved a significant breakthrough: they've discovered a method to replicate the intricate structure of the human ear using a special biological "ink." This replicated ear is then fixed beneath a flap of skin on the arm, where it undergoes a process called vascularization prior to being affixed to the head's side. Previous studies have shown the feasibility of creating ears on the backs of rats.

In situations where serious facial deformities which are present from birth; surgeons typically resort to invasive and painful procedures, such as extracting cartilage from other parts of the body like the ribs. This cartilage is then manually shaped into the desired ear shape, forming a scaffold that is inserted beneath the patient's skin. However, this innovative technique is changing the game. Scientists now utilize advanced scanning technology to create an exact mirror image of the unaffected ear. This digital replica is then 3D printed using a porous plastic material to form a scaffold. The scaffold is subsequently rooted under the patient's arm, where it gradually becomes integrated into the surrounding arm tissue as blood vessels form around it over a period of four to eight weeks. Once fully integrated, the ear is surgically removed from the arm and attached to the patient's head.

Trials of this groundbreaking procedure are scheduled to begin in India and in the UK in the upcoming year. In Mumbai, where

the need for facial reconstruction is urgent, more than a dozen children are eagerly awaiting the opportunity to take part [28].

Skin

In Canada, a group of engineering students has developed a revolutionary 3D printer named Print Alive, designed specifically to produce skin grafts for burn victims. Arianna McAllister and Lain Leng, engineering students at the University of Toronto, collaborated with Boyang Zhang, Dr. Marc Jeschke and Professor Axel Guenther, who oversees "Sunnybrook Hospital's Ross Tilley Burn Center", to create this innovative machine.

Traditionally, healthy skin from different parts of the body is taken which later grafted onto the afflicted area to treat severe burns. But the PrintAlive machine presents a viable substitute: it prints thick, continuous layers of tissue onto a hydrogel substrate, complete with minute details like sweat glands and hair follicles, among other complicated aspects of human skin. This innovative method has the potential to do away with the unpleasant processes involved in skin harvesting.

The PrintAlive machine prints the patient's skin cells in patterns of spots or stripes rather than a continuous sheet, optimizing their usefulness because it usually takes more than two weeks to cultivate a culture of the patient's skin cells suitable for grafting. This result in a treatment for wounds that is inhabited with cells that closely resemble the key components of human skin. Furthermore, the printed tissue's composition, thickness, and structure may all be precisely adjusted to match the demands of the individual patient. [28].

Cartilage Bioprinting

Cartilage tissue is a form of flexible connective tissue that requires replacement surgery since it cannot heal itself after injury. Nevertheless, cartilage lesions have resulted from typical artificial substitutes' inability to promote spontaneous repair [29–31]. Within the field of 3D printing, chondrocyte-, mesenchymal-, and bone marrow-derived bioink-infused cartilaginous tissue scaffolds have been created that allow for the production of desired tissue architectures. Though significant progress has been made, questions have been expressed about the stiffness and poor mechanical strength of the implanted cartilage tissue.

However, because 3D bioprinting can imitate the precise in vivo environment of cartilage tissue, it has a great deal of promise for cartilage tissue restoration. A researcher's team in Sweden accomplished a significant milestone by successfully implanting 3D printed human cartilage technology into mice. They created structures using a hydrogel made of nanocellulose combined with cartilage

cells taken from humans, which were subsequently surgically transplanted into the mice. Surprisingly, upon implantation, fresh blood vessels developed within the produced cartilage, highlighting the potential of 3D bioprinting to promote cartilage tissue regeneration [29].

Kidney

The kidney is a complex organ, making its construction through 3D printing a very difficult task. The printing of kidneys has been made possible by researchers using bioinks that comprise kidney cells and surrounding elements. Because these bioinks can be extruded at room temperature and have a viscosity similar to toothpaste, complex structures can be developed. The nephron, the kidney's functional unit in charge of filtering and reabsorbing essential substances, has been successfully rejuvenated by one study team.

Furthermore, this technology is being effectively applied in the field of regenerative medicine to create a variety of substitute organs, which are referred to as analogs, prostheses, implants, grafts, and precursors.

Several case studies demonstrate the successful transplantation of 3D printed organs. For instance, a patient suffering from spina bifida, a condition where a portion of the spinal cord protrudes outside the spinal column, experienced bladder dysfunction leading to urine leakage and subsequent kidney complications. In this case, the patient's own healthy cells were utilized to construct the urinary tract and bladder. In another instance, a 3D printing medical model company, in collaboration with surgeons at Belfast City Hospital in Northern Ireland, produced a functional 3D printed replica of the kidney [19][32].

Heart

Creating a printed heart is considered relatively uncomplicated due to its straightforward structure and primary function of pumping blood. The process begins with a CT and MRI scan of the donor's heart to determine its size and structure. The patient's blood cells are then extracted, and growth agents are used to transform them into stem cells. These stem cells are then utilized, along with bioink mixed with hydrogel, in 3D printers to regenerate heart tissue. The bioink is layered onto a scaffold that mimics the precise outline of the patient's heart, providing mechanical support besides facilitating cell growth. As time progresses, the cells integrate and start to beat, resembling a natural heart. Once the scaffold is detached, the heart is ready for implantation. Using the patient's own stem cells for organ regeneration reduces the risk of host versus graft disease, eliminating the necessity for immunosuppressive medications [33–35].

BioLife4D, a biotech startup, achieved success in engineering a miniature heart for experimentation in small animals. Furthermore, researchers at Carnegie Mellon University devised an innovative method for producing 3D bioprinted tissue scaffolds using collagen, enabling the printing of full-sized, adult human hearts. However, in a separate study, a 3D bioprinted heart incorporating human cells failed to beat as anticipated due to its small size, comparable to that of a rabbit's heart, rendering it unsuitable for human applications [36,37].

ADVANTAGE AND DISADVANTAGE OF 3D BIOPRINTING

The potential benefits of 3D bioprinting technology are immense. With this technology, it becomes possible to fabricate most human organs by reprogramming fibroblasts to function as specific organ cells. This advancement holds promise for overcoming the shortage of organs available for transplantation, potentially saving countless lives. Moreover, since the organs are constructed using the patient's own DNA, the risk of organ rejection post-transplantation is significantly reduced.

One of the main benefits of 3D bioprinting is its capacity to customize organs for each patient, guaranteeing their efficacy and compatibility. But even with the recent noteworthy advancements in 3D bioprinting's medical uses, major scientific and legal obstacles still stand in the way. It will take some time for this technology to grow into its most revolutionary uses.

Furthermore, the cost of implementing 3D bioprinting remains prohibitive, limiting its widespread adoption. Additionally, the viability of the printed organ depends on the establishment of blood vessels within the tissue, which presents a significant challenge. While using an individual's stem cells for organ development is a promising approach, it comes with its own cost implications.

In summary, while 3D bioprinting holds tremendous potential for revolutionizing healthcare, it is important to acknowledge and address the scientific, regulatory, and financial challenges that currently impede its widespread use and impact [28].

CONCLUSION

A cutting-edge production method for distributing cell-loaded hydrogels, bioprinting has a promising future particularly in the field of organ donation but is also fraught with difficulties. The emergence of 3D printing has shown great promise and could revolutionize a number of fields, most notably medicine. The technology of 3D bioprinting holds the potential to overhaul and redefine the landscape of healthcare. By translating data from MRI, CT scans or x-rays images into digital formats, this technology

enables the fabrication of implants and prostheses in virtually any conceivable shape or form. This advancement holds promise for saving lives and improving medical outcomes.

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ARTICLE

IN-SILICO PREDICTION OF THE NEURO-INFLAMMATION MECHANISM OF CAPPARIS SEPIARIA

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ABSTRACT

Neuroinflammation or neural dysfunction is a major risk factor that can initiate multiple intracellular signaling cascades to release different proinflammatory cytokines, chemokines and various reactive oxygen species leading to multiple neurodegenerative diseases, such as Alzheimer's disease, Parkinson's disease, and Huntington's disease. The adverse effects associated with the long-term use of conventional non-steroidal anti-inflammatory drugs is attracting herbal medicines as potential therapeutic candidates worldwide. Capparis sepiaria L (C. sepiaria) belonging to Capparaeae is therapeutic medicinal plant used to relieve various ailments including skin diseases, tumours, blood purification, toxaemia, snakebite and disease of the muscles. The objective of this study is to determine the pharmacokinetic and pharmacodynamic properties of C.sepiaria phyto-constituents as therapeutic molecules against neuro inflammation by using in-silico docking analysis and drug disposition. Six phyto- constituents identified from the leaves of C. sepiaria were docked against six pro-inflammatory markers of neuroinflammation followed by the prediction of their safety and bioavailability using GOLD 5.2, admetSAR softwareS respectively. The docking scores obtained were comparable and even better than the five standard marketed drugs. β-amyrin and quercetine present in the leaves of C.sepiaria showed highest fitness score with P38 MAP kinase, NF-kB, mTOR, TACE AChE, BChE

markers which are also the targets of the drugs like Galantamine, Donepazil and Rivastigmine. To our understanding this is the first study investgating the inhibitory effect of C. sepiaria in the neuroinflammation. Thus, Capparis sepiaria may prove to be a potential anti-neuroinflammatory agent and may be further explored as a potential therapeutic candidate for the management of neurodegenerative diseases.

Keywords: *Neuroinflammation, Alzheimer's disease, Capparis sepiaria, GOLD 5.2, admetSAR*

INTRODUCTION

Capparis sepiaria L. an important medicinal plant belonging to family, Capparidaceae is a prickly, evergreen shrub indeginously used as food, medicine and fuel. It is commonly known as "Himsara or Indian caper" and is traditionally used for treatment of asthma, allergy, jaundice, rheumatism and dysentery. It has also been reported to possess anti-inflammatory, analgesic, hepato-protective and anti-oxidant activity [1-4].

The medicinal potency presented by this herb is attributed to terpenoids, phenolic derivatives, alkaloids, glycosides, and steroidal saponins that have several therapeutic effects. The leaves of C.sepiaria contain naturally occurring phytochemicals viz. α- amyrine, β-amyrine, quercetine, β-sitosterol, erythrodiol.

Neuroinflammation play a critical role in neurodegenerative diseases such as Alzheimer disease, Parkinson disease, traumatic brain injury, and stroke leading to their higher prevalence [5-7]. Previously the diseases that were largely believed to be based on dysregulation of neurotransmitter systems such as mood disorders/depression/anxiety, schizophrenia, and chronic pain now seem to have emerged due to inflammation of the nervous system [5].

The uncontrolled over activation of brain immune cells, especially microglial cells, lead to self-perpetuating inflammatory responses, accompanied by secretion of various inflammatory mediators like cytokines, reactive oxygen and nitrogen species, damaged protein products 'etc' [8-9]. Due to its complex multi-factorial mechanisms, therapeutic intervention in neurodegenerative diseases is a major challenge. Conventionally, anti-inflammatory drugs like Galantamine, Donepazil and Rivastigmine are generally prescribed in such condition, but, their long term usage is also associated with several side effects like gastrointestinal problems, dizziness, headache 'etc'[10]. Nowadays natural products like terpenoids, phenolic derivatives, alkaloids, glycosides, and steroidal saponins have emerged promising therapeutics for neuroinflammation and neurodegenerative diseases because of their pleiotropic nature, and least side effects

There are several factors viz. aging, air pollution, oxidative stress, infection,

neurotoxins, tissue damage and mutation which resulted in to the microglial activation. The microglial activation resulted into the abrupt excessive expression of several pro-inflammatory mediators viz. IL-1β, Reactive Oxygen Species (ROS), TACE; COX-2; mTOR; NF-kB, p38 map kinase. Consequently, this abrupt excessive expression of these pro-inflammatory mediators resulted into the neuronal dysfunction and death. In case of chronic neuroinflammation excessive damage of the neuronal cells were take place which resulted into the neurodegenerative disorders like Alzheimer disease. Extensive literature search revealed that many synthetic and natural drugs were reported for the treatment of neuro-inflammation although treatment with these drugs showed some side effects as shown in the Table 1. Additionally, these reported drugs were not found to be so much effective against the neuro-inflammation.

Table 1: Marketed drugs for the treatment of neuro inflammation with their side effects

Marketed Drugs	Targets	Side Effects
Galantamine	Acetylcholinesterase, Neuronal acetylcholine receptor subunit α-7, Muscle nicotinic acetylcholine receptor, Cholinesterase	Nausea, vomiting, loss of appetite and increased frequency of bowel movements.
Donepezil	Acetylcholinesterase, 5-hydroxytryptamine receptor 2_A, Cholinesterase, Tumor necrosis factor-inducible gene 6 protein, Interleukin-1 β, Nuclear factor NF-kB, NMDA receptor	Nausea, vomiting, loss of appetite, muscle cramps and increased frequency of bowel movements.
Rivastigmine	Acetylcholinesterase, Cholinesterase	Nausea, vomiting, loss of appetite and increased frequency of bowel movements.
Memantine	5-hydroxytryptamine receptor 3_A, α-7 nicotinic cholinergic receptor subunit, Dopamine D_2 receptor, Glutamate receptor ionotropic, NMDA 1, Glutamate (NMDA) receptor (Protein Group), GABA(A) Receptor (Protein Group), Glycine receptors (Protein Group)	Headache, constipation, confusion and dizziness.

Hence in the present study, research efforts were made to identify the important chemical classes of the phyto constituents responsible for the attenuation of the neuronal inflammation. Detailed literature search indicated that broadly terpenoids, phenolic derivatives, alkaloids, glycosides, and steroidal saponins were found to be effective in neuroinflammation and neurodegenerative diseases. Additionally, few published scientific reports described the activity of naturally occurring phyto-constituents viz. α- amyrine, β-amyrine, quercetine, β-sitosterol, erythrodiol against the neuro-inflammation. Hence in the present research, the plant *Capparis sepiaria* was selected for the evaluation of its activity against the neuroinflammation as this plant consist of all the above-mentioned chemical constituents which are important for the activity against the neuroinflammation.

Capparis sepiaria L is a profusely branched hedge plant with slender prickly shrubs, zigzag stems. Traditionally, *C. sepiaria* is used as blood purifier, stomachic, tonic and appetizer. It2 s flowers, leaves and roots are used in cough and toxaemia and root powder is also used as a cure for the snakebite. It also possesses febrifuge properties and is used to treat skin diseases, tumours, inflammation and diseases of the muscles.

Phytoconstituents reported from the leaves of *Capparis sepiaria* consisted of the phytoconstituents 6 major are from the class of flavonoids and alkaloids (Figure 1). List of these phytoconstituents along with their structure are shown in Figure 1. There are many studies on the anti-neuroinflammatory activity of these individual phytochemicals reported from different plant sources individually. Thus, C. sepiaria is an ideal candidate for prediction potential anti neuoinflammatory drug source as it consists of all of the important reported phytocontituents. In the present study, an attempt has been made to identify and compare the inhibition of neuroinflammation between C. sepiaria phytoconstituents and the synthetic drugs Galantamine, Donepazil and Rivastigmine by targeting the key receptors in the neuroinflammation pathway to determine anti-neuroinflammatory potential of *Capparis sepiaria.*

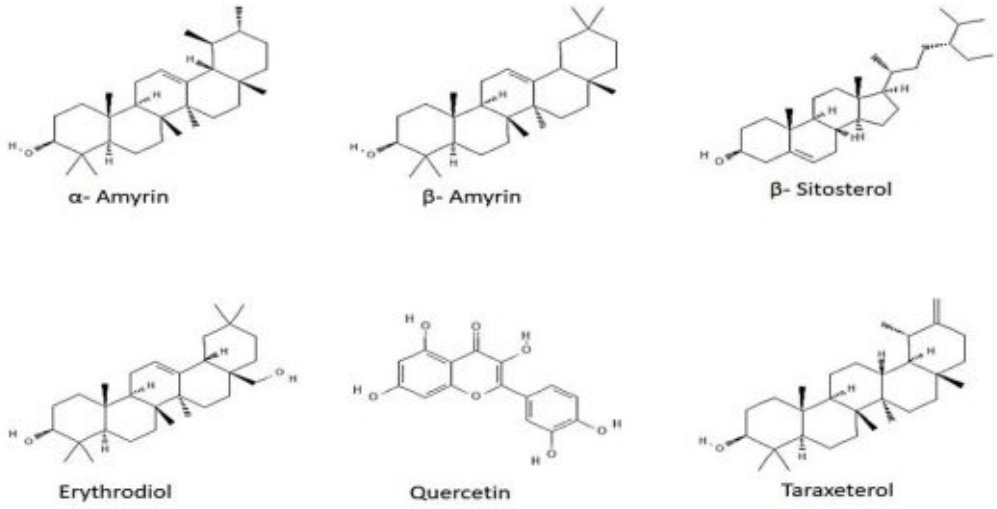

Figure 1. Important phyto-constituents of the leaves of *Capparis Sepiaria*

MATERIAL AND METHODS

In-silico ADMET prediction

The admetSAR is an online reliable freeware utilised widely for the prediction of Adsorption, Distribution, Metabolism and Excretion along with the acute oral toxicity of any compound. This online free tool stores the data based upon the previously reported library of the compounds and predicts the *in-silco* ADMET property of the desired compounds. In the present research SMILE format of the desired phytoconstituents were generated and ADMET properties of these phytoconstituents were predicted. Major advantage of this tool is of *in-silico* toxicity prediction in the form of class-I chemicals (LD50 d" 50mg/kg), class-II chemicals (500mg/kg > LD50 > 50mg/kg), class- III chemicals (5000mg/kg > LD50 > 500mg/kg) and class-IV chemicals (LD50 > 5000mg/kg). It also gives an idea about the carcinogenicity of the chemical.

Target Identification

Through the extensive literature search molecular targets for the neuro-inflammation were identified. Most of the literature revealed the exploration of molecular targets viz. COX-2, TACE, P38 MAP Kinase, NF-kB in the area of the neuroinflammation. Overall based upon literature search; COX-2, TACE, P38 MAP Kinase, NF-kB, AChE, & BChE were explored for the molecular docking study to identify the mechanism of action of the *Capparis Sepiaria*.

Molecular docking study

The co-crystallized structures of different proteins viz. COX-2 (PDB ID: 3LN1), TACE (PDB ID: 1ZXC), P38 MAP Kinase

(PDB ID: 3GCS), NF-kB (PDB ID: 5T8P), acetyl choline esterase (PDB ID: 2X8B) and butyl choline esterase (PDB ID: 2WIL) were downloaded from the protein data bank. Prior to molecular docking study, both, ligand and receptor were prepared. Water molecules and co-crystallized ligands were removed from the protein. The binding position of the co-crystallized ligand was considered to be the binding pocket of the protein. After the extraction of these co-crystalized ligand from the binding pocket, all the desired phytoconstituents were docked on their respective protein.

RESULTS AND DISCUSSION

In-Silico ADMET prediction

In-silico ADMET of the phytoconstituents of Capparis sepiaria were evaluated in terms of partition coefficient (AlogP), molecular weight, hydrogen bond acceptor (HBA), hydrogen bond donor (HBD), carcinogenicity, acute oral toxicity, human intestinal absorption (HIA) and water solubility (Log S) shown in Table 2.

Table 2. In-Silico ADMET prediction of phytoconstituents of leaves of Capparis Sepiaria

ADMET Properties of the chemical constituents	ALogP	Molecular Weight	HBA	HBD	Rotatable Bonds	Carcinogenicity	Acute Oral Toxicity	Human Intestinal Absorption	Water Solubility (LogS)
α-amyrine	8.02	426.73	1	1	0	No	Class-III	More than 30%	-4.251
β-amyrine	8.17	426.73	1	1	0	No	Class-III	More than 30%	-4.251
β-sitosterol	8.02	414.72	1	1	6	No	Class-I	More than 30%	-4.703
Erythrodiol	7.14	442.73	2	2	1	No	Class-III	More than 30%	-4.307
Quercetin	1.99	302.24	7	5	1	No	Class-II	More than 30%	-2.999
Taraxeterol	8.02	426.73	1	1	0	No	Class-III	More than 30%	-4.123

ALogP value usually indicates the hydrophilic capacity of the chemical component. Higher the ALogP lesser is the hydrophilcity of the given chemical component. Most of the *capparis sepiaria* phytoconstituents indicated the AlogP value more than 5 except quercetin which proved that these phytoconstituents are in hydrophobic in nature. This hydrophobic nature of phytoconstituents of *capparis sepiaria* responsible for the activity in neuroinflammation as these phytoconstituents able to cross the blood brain barrier (BBB).

Almost all the phytoconstituents of capparis sepiaria are small molecules and having molecular weight less than 500 which is one of the drug like property. Similarly as per the Lipinski rule of five molecules must possess hydrogen bond acceptor (HBA) less than 5 and hydrogen bond donor (HBD) not more than 10. In lieu of this all the phytoconstituents of capparis sepiaria following this Lipinski rule where all the phytoconstituents does not have the HBA and HBD more than 5 and 10, respectively.

All the phytoconstituents are non-carcinogenic in nature. In case of acute oral toxicity β-sitosterol and quercetin falls in the category of class-I and class-II chemicals, respectively. While on the other hand all the remaining phytoconstituents are falling in the class-III chemical clearly indicated the non-toxic nature of these phytoconstituents. In addition to this, most of the phytoconstituents revealed the human intestinal absorption more than 30% which proved the drug ability of all the phytoconstituents. As it is clearly observed from the hydrophobicity (ALogP) of the phytoconstituents that these components were less soluble in the water.

Molecular docking study

Various molecular targets viz. COX-2, TACE, P38 MAP Kinase, NF-kB, acetyl choline esterase (AChE) and butyl choline esterase (BChE) which are involved in the pathogenesis of the neuroinflammation were taken for the detailed molecular docking study as shown in the Table 3.

Table 3. Molecular docking score of the important phytoconstituents of leaves of *Capparis Sepiaria*

	Docking Score (GOLD FITNESS)						
Phyto Constituents	**P38 MAPK (PDB ID: 3GCS)**	**NFkB (PDB ID: 5T8P)**	**mTOR (PDB ID: 4JT6)**	**COX-2 (PDB ID: 3LN1)**	**TACE (PDB ID: 1ZXC)**	**AChE (PDB ID:2X8B)**	**BChE (PDB ID:2WIL)**
α-amyrine	98.62	118.2	121.58	116.79	110.03	102.08	105.7
β-amyrine	98.88	119.88	123.97	116.66	112.09	109.4	104.5
β-sitosterol	40.97	2.001	35.98	-4.08	42.86	30.34	39.42
erythrodiol	30.06	-106.03	15.27	-152.18	41.66	40.21	30.23
quercetin	52.56	60.11	58.99	59.27	62.22	113.2	114.5
taraxeterol	-93.44	-57.42	28.18	-156.19	26.94	32.21	33.45

β-amyrin was found to be best among all the other phytoconstituents of *capparis sepiaria* in terms of docking score as it shown highest GOLD Fitness against P38 MAP kinase, NF-kB, mTOR, TACE. In addition to this it showed same binding interactions as that of the respective co-crystallized ligand of various proteins shown in the Figure 2.

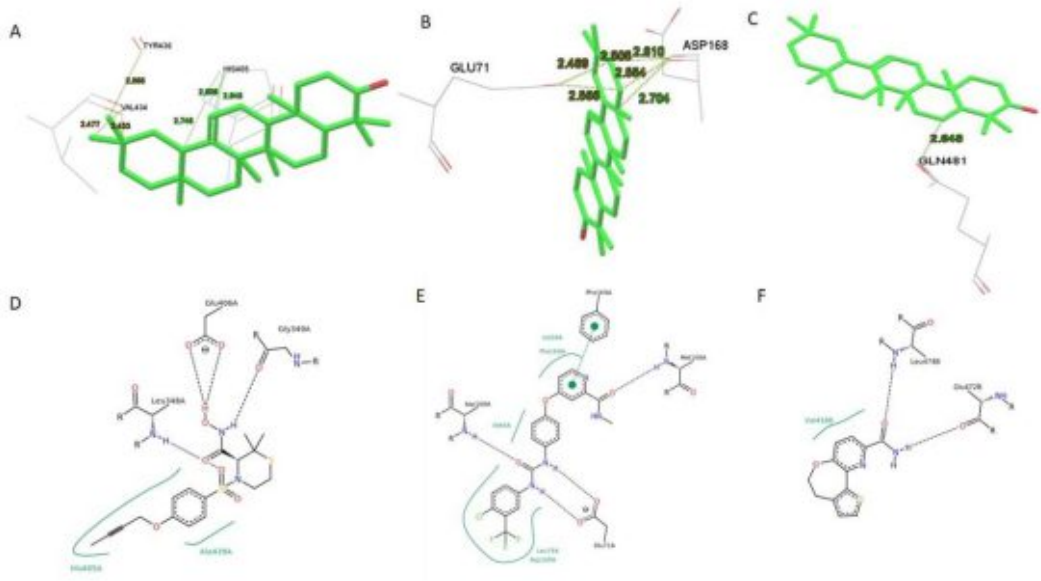

Figure 2: Molecular docking interactions of β-amyrin with (A) TACE (PDB ID: 1ZXC) (B) P38 MAP kinase (PDB ID: 3GCS) (C) NF kB (PDB ID: 5T8P). Docking interactions of standard co-crystallized ligands with the (D) TACE (PDB ID: 1ZXC) (E) P38 MAP kinase (PDB ID: 3GCS) (F) NF kB (PDB ID: 5T8P)

In case of COX-2, α-amyrine showed highest docking score. Overall molecular docking study revealed that α-amyrine and β-amyrine were acting predominantly on molecular targets which are involved in the pathogenesis of the neuro-inflammation. In case of TACE, double bond of β-amyrin at 12th position showed π-π stacking with His 405 which is considered to be the hydrophobic binding interaction which is similar to the binding interaction of the co-crystallized ligand of the TACE shown in the Figure 2(A). In case of P38 MAP kinase; β-amyrin showed binding interactions with the Glu71 and Asp168 which is similar to the binding interactions of the co-crystallized ligand of the P38 MAP Kinase shown in the Figure 2(B). In case of NF-kB, β-amyrin showed binding interactions with the Gln481 as shown in the Figure 2(C) although it didn't show any similar binding interactions as that of the standard co-crystallized ligand of the NF-kB. Molecular docking study revealed that β-amyrin was one of the important phyto constituent of the *capparis sepiaria*

which played an important role in the inhibition of the inflammation markers like NF-kB, TACE and P38 MAP kinase.

For this purpose the standard marketed drugs against the neuroinflammation were taken into the consideration. The marketed drugs Galantamine, Donepazil and Rivastigmine were showed interactions against the acetylcholine esterase and butylcholine esterase which is also evident in the literature. Based upon this phytoconstituents of *capparis sepiaria* were taken for the molecular docking study against the AChE and BChE. In case of AChE, molecular docking study revealed that Donepazil showed good docking score and binding interactions compared to the Galantamine and Rivastigmine. Additionally it also showed similar binding interaction as that of the co-crystallized ligand of the AChE. Interestingly the phytoconstituents of the *capparis sepiaria* also shown good binding interactions with the AChE. But among all the other phytoconstituents, quercetine showed highest docking score and similar binding interactions as that of the standard shown in the Figure 3.

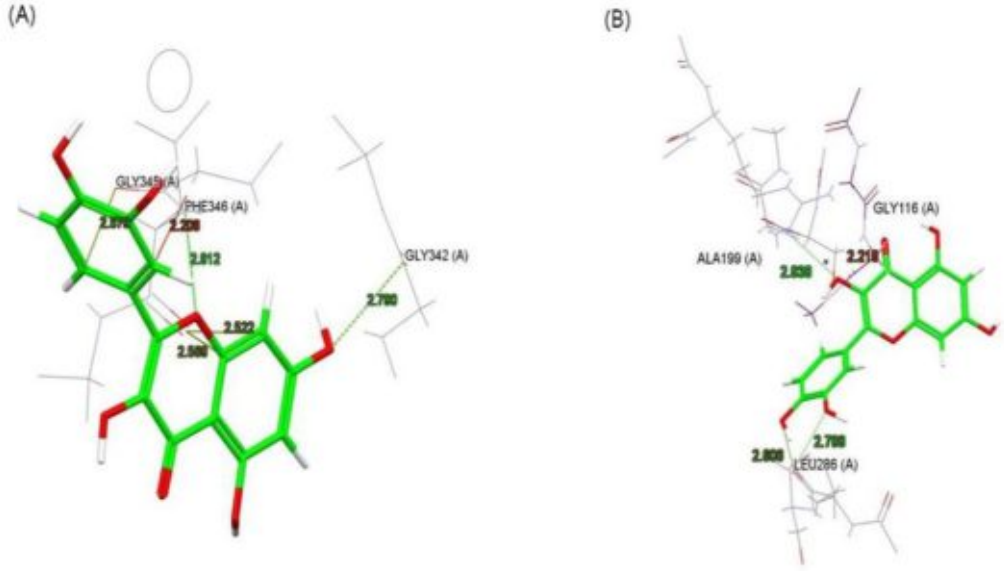

Figure 3: Molecular Docking Interactions of Quercetine with (A) Acetyl Choline Esterase (PDB ID: 2X8B) (B) Butyl Choline Esterase (PDB ID: 2WIL)

This outcome indicated that the leaf extract of *capparis sepiaria* might be active against the neuroinflammation as most of the phytoconstituents showed inhibition of the AChE. Similarly, in case of BChE, donepezil showed highest docking score compared to the other marketed drugs. In this docking study quercetine showed highest docking score compared to the donepezil.

Interestingly docking study against both AChE & BChE revealed that the quercetine is one of the important phytoconstituent of *Capparis Sepiaria* which might be playing an important role against the AChE and BChE and showing the activity of *capparis sepiaria* against the neuroinflammation.

CONCLUSION

Neuroinflammation is the major problem associated with the Alzheimer's disease where older people are most affected. Available drugs for the treatment of neuroinflammation having some side effects & toxic effects on the different organs. In addition to this these drugs were found to be in ineffective for the long-term treatment of the neuroinflmmation. Hence in the present research phytoconstituents of *Capparis sepiaria* leaf were explored against the molecular targets of neuroinflammation where viz. α-amyrine, β-amyrine, and quercetin were found to be most potent and safe at molecular level for the treatment of Neuroinflammation. Hence our study concludes that the leaf extract of *Capparis sepiaria* would be effective against the neuroinflammation as it comprises α-amyrine, β-amyrine, and quercetin. Additionally, our study also suggests that components like α-amyrine, β-amyrine, and quercetin could be used separately for the new drug development as active pharmaceutical ingredient.

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