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Proforma for Submission of M.Tech. Major Project

01. Name of the Student.... Yogita Bhatt
02. Enrolment No 23/B10/11
03. Year of Admission 2023
04. Programme M.Tech., Branch.... Bioinformatics
05. Name of Department. Department of Biotechnology
06. Admission Category i.e. Full Time/ Full Time (Sponsored)/ Part Time:... Full time
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11. Name of Supervisor..... Prof. Yasha Hasija

12. Result Details (Enclose Copy of Mark sheets of all semesters) :

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01	1st	2023	23/B10/11	9.41	10	94.1%	
02	2nd	2024	23/B10/11	9.88	10	98.8%	
03	3rd	2024	23/B10/11	7.67	10	76.7%	
04	4th (P/T only)						
05	5th (P/T only)						

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30.05.25
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ANNEXURE-VII

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(To be submitted in duplicate)

1. Name: Yogita Bhatt
2. Roll No: 23/BIO/11
3. Thesis title: Synergizing Phytochemical Pharmacology and Precision Gene Editing for Multimodal Autism Spectrum Disorder Intervention.
4. Degree for which the thesis is submitted: M.Tech Bioinformatics
5. Faculty (of the University to which the thesis is submitted)
   Prof. Yasha Hasija

6. Thesis Preparation Guide was referred to for preparing the thesis.	YES ☑	NO ☐
7. Specifications regarding thesis format have been closely followed.	YES ☑	NO ☐
8. The contents of the thesis have been organized based on the guidelines.	YES ☑	NO ☐
9. The thesis has been prepared without resorting to plagiarism.	YES ☑	NO ☐
10. All sources used have been cited appropriately.	YES ☑	NO ☐
11. The thesis has not been submitted elsewhere for a degree.	YES ☑	NO ☐
12. All the correction has been incorporated.	YES ☑	NO ☐
13. Submitted 2 hard bound copies plus one CD.	YES ☐	NO ☐

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Yogita Bhatt

(Signature of Candidate)

Name: Yogita Bhatt

Roll No: 23/BIO/11

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STATEMENT OF GRADES

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(Bioinformatics)

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Month & Year of Examination : MAY, 2024 **Semester : SECOND**

Subject Code	Subject Title	Credits	Credits Secured	Grade
BIO502	ADVANCES IN BIOINFORMATICS	4	4	O
BIO504	HIGH THROUGHPUT STRUCTURAL BIOLOGY	4	4	O
BIO5202	OPEN AREA SEMINAR-II	2	2	A+
BIO5308	IMMUNOINFORMATICS	3	3	O
BIO5402	ADVANCED GENETIC ENGINEERING	4	4	O
		17	**17**	

AB : Absent DT : Detained

Credits Secured / Total : 17 / 17 **SGPA : 9.88**

Yogita Bhatt

R Landey

CONTROLLER OF EXAMINATION

Dated : February 04, 2025

Date of Declaration of Result : July 16, 2024

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STATEMENT OF GRADES

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(Bioinformatics)

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Month & Year of Examination : NOVEMBER, 2023 **Semester : FIRST**

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BIO503	ADVANCED PROTEOMICS	4	4	O
BIO5407	OMICS IN MEDICINE	4	4	A+
BIO5301	DATA WAREHOUSING AND DATA MINING	3	3	O
BIO5201	OPEN AREA SEMINAR-I	2	2	A+
		17	**17**	

AB : Absent DT : Detained

Credits Secured / Total : 17 / 17 **SGPA : 9.41**

yogita bhatt

R Landey

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Dated : February 04, 2025

Date of Declaration of Result : March 01, 2024

Yagyesh Kapoor

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Master of Technology(Bioinformatics), III-SEMESTER

Result Declaration Date : 12-03-2025

Notification No: 1798

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			12.00			
1	2K22/BIO/07	YAGYESH KAPOOR	O	10.00	12	

BIO601 : MAJOR PROJECT I BIO6201 : DESIGN AND ANALYSIS OF ALGORIHM BIO6307 : ARTIFICIAL INTELLIGENCE BIO6401 : Drug Design and Discovery

Sr.No	Roll No.	Name of Student	BIO601	BIO6201	BIO6307	BIO6401	SGPA	TC	Failed Courses
			3.00	2.00	3.00	4.00			
2	23/BIO/01	SOMYA PARASHAR	A+	O	A+	O	9.50	12	
3	23/BIO/02	KHUSHI BHATIA	A+	O	A+	O	9.50	12	
4	23/BIO/03	SWARNIMA SRIVASTAVA	A	O	O	A+	9.17	12	
5	23/BIO/05	DEVANSHI SHARMA	A+	O	A+	A+	9.17	12	
6	23/BIO/06	HARSHITA THAKUR	A+	O	A+	A+	9.17	12	
7	23/BIO/07	AKSHAY HATWAL	A	A+	B+	A+	8.25	12	
8	23/BIO/08	AISHWARY ARUNKUMAR KADAO	A	A+	A+	A	8.42	12	
9	23/BIO/10	ELESWETA SAHOO	A+	A+	A	B	7.75	12	
10	23/BIO/12	BHAVUKTA VIRMANI	A+	O	A+	A+	9.17	12	
11	23/BIO/13	PRATYAKSHA SINGH	A+	A+	A+	A+	9.00	12	
12	23/BIO/14	RISHI MRINAL	A+	O	A+	B+	8.50	12	

BIO601 : MAJOR PROJECT I MOOC6201 : MOOC MOOC6203 : MOOC COURSE MOOC6405 : MOOC COURSE

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Notification No: 1798

Sr.No	Roll No.	Name of Student	BIO601	MOOC6201	MOOC6203	MOOC6405	SGPA	TC	Failed Courses
			3.00	2.00	3.00	4.00			
13	23/BIO/09	AANCHAL NEGI	A+	B	B	B+	7.08	12	
14	23/BIO/11	YOGITA BHATT	A+	B	B+	A	7.67	12	

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Synergizing Phytochemical Pharmacology and Precision Gene Editing for Multimodal Autism Spectrum Disorder Intervention

Thesis submitted

in partial fulfilment of the requirements for the

degree of

MASTER OF TECHNOLOGY

in

BIOINFORMATICS

Submitted by

YOGITA BHATT

(23/BIO/11)

Under the supervision of

PROF. YASHA HASIJA

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May 2025

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Certified that Yogita Bhatt (23/BIO/11) has carried out their search work presented in this thesis entitled "**Synergizing Phytochemical Pharmacology and Precision Gene Editing for Multimodal Autism Spectrum Disorder Intervention**" for the award of Master of Technology from Department of Biotechnology, Delhi Technological University, Delhi, under my supervision. The thesis embodies the results of original work, and studies are carried out by the student herself, and the contents of the thesis do not form the basis for the award of any other degree to the candidate or to anybody else from this or any other University/Institution.

30.05.25

Signature

Prof. Yasha Hasija

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Department of Biotechnology
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Date:

30.05.25

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Synergizing Phytochemical Pharmacology and Precision Gene Editing for Multimodal Autism Spectrum Disorder Intervention

Yogita Bhatt

ABSTRACT

Autism spectrum disorder (ASD) is a multifaceted neurodevelopmental condition that arises from complex interplay between genetic and metabolic dysfunctions. Addressing such a disorder demands a holistic and integrative scientific approach that bridges molecular genetics and pharmacological research. This study explores a dual-pronged therapeutic strategy aimed at correcting ASD-related abnormalities. The first approach utilizes CRISPR-Cas9 gene editing to target known ASD-associated mutations in critical synaptic genes. Simultaneously, the second strategy focuses on the bioactive potential of phytochemicals derived from Cichorium intybus, a plant known for its regulatory effects on metabolic enzymes that influence neuronal energy dynamics. Using advanced bioinformatic platforms and gene design tools, guide RNAs were meticulously engineered to minimize off-target effects while effectively modulating genes involved in metabolic control. These metabolic targets plays central role in maintaining the energy balance and neuronal communication within synaptic networks. One key compound, chlorogenic acid, emerged as a promising agent capable of restoring metabolic equilibrium while enhancing impaired neuronal signaling, suggesting a dual mechanism of action. Together, these gene and phytochemical interventions propose an innovative therapeutic framework: gene-edited cellular models can serve as testing grounds for evaluating the neuroprotective effects of these natural compounds. By aligning targeted genetic repair with plant-based metabolic modulation, this approach holds potential for enhancing neuronal viability and functional recovery. Ultimately, such integrative and personalized methodologies pave the way for the development of refined, indication-specific treatments for ASD, uniting precision medicine with nature-inspired solutions.

Keywords: CRISPR, phytochemicals, guide RNAs, personalized methodologies, Cichorium intybus, modulation

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Yogita Bhatt

23/BIO/11

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List of Abbreviation

Abbreviation	Full Form	Page No.
ASD	Autism Spectrum Disorder	1
CRISPR	Clustered Regularly Interspaced Short Palindromic Repeats	2
gRNA	guide RNA	2
AMPK	AMP-activated Protein Kinase	2
PKA	Protein Kinase A	2
AMPD 1	AMP Deaminase 1	2
iPSC	Induced Pluripotent Stem Cells	2
NHEJ	Non-Homologous End Joining	2
HDR	Homology Directed Repair	2
SWISS-MODEL	Swiss Institute of Bioinformatics Protein Modeling Server	27
XP Docking	Extra Precision Docking	30
ADME	Absorption, Distribution, Metabolism, and Excretion	30
ProTox-II	In Silico Toxicity Prediction Platform	36
CHOPCHOP	CRISPR Guide RNA Design Tool	17
STRING	Search Tool for the Retrieval of Interacting Genes/Proteins	18

OPLS3e	Optimized Potentials for Liquid Simulations Force Field	30

I. INTRODUCTION

Autism Spectrum Disorder (ASD) is a multifactorial disorder that typically emerges in early stages of childhood [1]. It is characterized by persistent difficulties in social interaction, communication challenges, and a tendency towards repetitive [2] actions and narrow interests.

The condition arises from a synergy of not only the individual's genetic makeup but also environmental factors. Over 1,000 [3] genes have been associated with ASD, some of which include SHANK3, CHD8, and SCN2A [4], as they are believed to control synaptic activity, neuronal excitability, and chromatin remodeling. In addition, environmental factors such as prenatal exposures, maternal [5] factors , and advanced parental age also influence ASD risk. Therapeutic innovation continues to lag behind progress in elucidating the genomic architecture of ASD due to diverse ASD presentations and the disrupted neurodevelopmental-imbalance-metabolism pathways. This thesis proposes a new, multi-modal intervention design that combines gene editing via CRISPR-Cas9 technology and targeted modulation of metabolic pathways by phytochemicals to [6] treat both the biochemical and genetic components underlying ASD.

Genetic alterations, particularly those associated with genes controlling synaptic plasticity (SHANK3, FMR-1) and ion channel activity (SCN2A), have immense importance because ASD has a strong heritability of roughly 80%. For instance, mutations at SCN2A disrupt the functioning of the sodium channels, causing dendritic action potentials and synaptic fires [7] to become volcanically unstable in cortical neurons, which is associated with learning and social deficits in mouse models. Such alterations can now be remedied via CRISPR-Cas9, which is revolutionizing the treatment paradigm. In FXS models, it was shown that gold nanoparticles bearing CRISPR payloads to Cas9 were able to edit mGluR5 in the brain and reduce a repetitive behavior [8] that is characteristic of ASD.

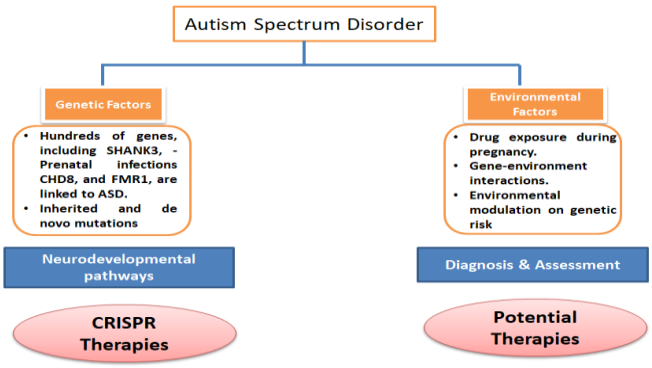


Fig. 1 Genetic and environmental factors contributing to ASD

This approach's success hinges on bioinformatically optimized guide RNAs (gRNAs) that minimize off-target effects while maximizing editing efficiency. For instance, dual gRNAs targeting SHANK3 exons achieved 98.7% specificity in silico, enabling precise repair of synaptic scaffolding defects. Such genetic corrections restore neuronal connectivity in vitro, but their translational potential is limited by the [9] metabolic stressors inherent to ASD pathophysiology, such as mitochondrial dysfunction and oxidative stress.

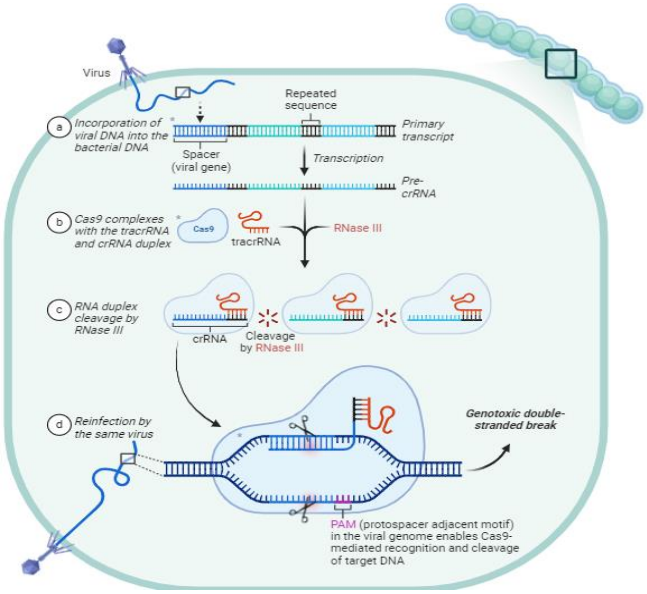


Fig. 2. The mechanism of the CRISPR-Cas9 system

Emerging evidence links ASD to metabolic disturbances, including insulin resistance and purine metabolism defects. Skeletal muscle, a key site of insulin action, exhibits elevated AMP deaminase 1 (AMPD) activity in insulin-resistant states, which exacerbates mitochondrial dysfunction and reduces AMP-activated protein kinase (AMPK) phosphorylation-a pathway shared with [10] ASD. Notably, AMPD inhibition in mice improved glucose tolerance and insulin sensitivity, while metformin, an antidiabetic drug, ameliorated ASD-related hyperactivity in clinical trials. Similarly, protein kinase A (PKA), a cAMP-dependent regulator of synaptic plasticity, shows reduced activity in the frontal cortex of individuals with regressive ASD, impairing CREB-mediated neuronal survival. These findings position AMPD 1 and PKA as critical [11] metabolic nodes in ASD,

intersecting with pathways perturbed by genetic mutations. However, existing pharmacotherapies for ASD (e.g., risperidone) target symptoms rather than these root causes, underscoring the need for novel metabolic modulators.

Cichorium intybus (chicory), a plant rich in chlorogenic acid, chicoric acid, and dicaffeoylquinic acid, has demonstrated antidiabetic and neuroprotective properties. Molecular docking studies reveal chlorogenic acid's [12] high affinity for AMPD (−8.41 kcal/mol) and PKA (−12.56 kcal/mol), surpassing conventional inhibitors. In diabetic mice, chicory extracts reduced hyperglycemia by 44.8% and alleviated neuropathy via antioxidant effects and pancreatic β-cell regeneration. These properties align with ASD's metabolic comorbidities: oxidative stress and insulin signaling defects are prevalent in ASD cohorts, and AMPD/PKA dysregulation exacerbates neuronal excitability. Chlorogenic acid's dual [13] inhibition of AMPD and PKA could thus stabilize cellular energy homeostasis (via AMP/ATP balance) and enhance synaptic resilience-complementing CRISPR's genetic repairs.

The integration of CRISPR-Cas9 and phytochemicals offers a dual-axis therapeutic strategy (Fig. 1):

1. Genetic Preconditioning: CRISPR corrects ASD-linked mutations (e.g., SHANK3 knock-in) in iPSC-derived neurons, restoring synaptic protein expression.
2. Metabolic Support: Chlorogenic acid [14] treatment normalizes AMPD-1 /PKA activity in edited neurons, mitigating oxidative stress and enhancing AMPK-driven energy metabolism.

This synergy is exemplified in SCN2A-edited neurons, where NaV restoration alone may not resolve dendritic excitability deficits caused by AMPD-mediated purine imbalances. Co-treatment with chlorogenic acid could buffer metabolic stress, improving neuronal survival and plasticity. Furthermore, CRISPR-edited models provide a platform to screen phytochemical [15] efficacy across genetic subtypes, enabling personalized combinations-e.g., CHD8 corrections paired with PKA modulators for chromatin-remodeling deficits.Current ASD treatments lack mechanistic specificity, but this combined approach targets both upstream genetic lesions and downstream metabolic disruptions. For instance, CRISPR-Gold nanoparticles could co-deliver Cas9 and chlorogenic acid to the brain, editing FMR while stabilizing AMP/PKA pathways.

Longitudinal studies in Shank3 [16] KO mice have already shown that epigenetic drugs enhance CRISPR efficacy, suggesting similar potential for phytochemicals. Moreover, chicory compounds' safety profiles and bioavailability, validated in diabetes research, expedite translational pipelines.

ASD's intricacy requires interventions that integrate its genetic and metabolic origins. This thesis proposes a comprehensive ASD treatment paradigm by integrating CRISPR-precision genetics with the metabolic modulation of [17] phytochemicals.

Subsequent efforts will investigate this synergy in vivo, refine synergistic formulation instillation techniques, and identify stratification biomarkers (such as AMPK phosphorylation) for personalized intervention design. These and other pioneering strategies have the potential to radically change the approach towards treating ASD, shifting from mere symptom relief to targeting the fundamental issues.

II. REVIEW OF LITERATURE

ASD is a multifaceted [18] condition characterized by persistent challenges in social communication, along with the presence of repetitive patterns of behavior and matter of interests. ASD typically manifests before the age of three and comes with wide phenotypic variability which makes diagnosis and treatment particularly complex. The etiology of ASD is multifactorial in nature and involves intricate interactions between one's genetic predisposition [19] and the surrounding environment. In primary consideration, numerous genetic mutations have been linked to ASD, especially those in the SHANK3, CHD8 and FMR genes which are crucial to synaptic activity, neuronal connectivity, and chromatin remodeling.

Alongside these, non-genetic factors such as maternal health, exposure to toxins during pregnancy, and inflammatory processes while coming from the mother during gestation modify [20] the risk and severity of ASD symptoms.Multiple genome-wide association studies have linked over a thousand genes to ASD. Several of the genes are SCN2A and CNTNAP2 which control essential parts of synaptic signaling and the architecture of neurons. Alongside these components, it is becoming clearer that individuals diagnosed with ASD suffer from severe metabolic dysfunction. Also, there [21] are some deviations such as mitochondrial dysfunction, oxidative stress, glucose metabolism, and increased insulin resistance. As a result, these deviations not only worsen synapse and neuron dysfunctions, but also alter behaviors. Because of this combination of pathology, there is increasing ASD therapies developed need to single both upstream genetic concerns and metabolic disturbances that are located downstream.

The [22] CRISPR-Cas9 gene-editing system has emerged as a transformative technology in ASD research. By allowing targeted manipulation of the genome, CRISPR enables the development of accurate disease models and offers promising avenues for therapeutic interventions. In the context of ASD, CRISPR has been instrumental in creating in vitro and in vivo models that replicate human pathophysiology. For instance, SHANK3 [23] knockout models developed using CRISPR in human induced pluripotent stem cells (iPSCs) have revealed key deficits in synaptic transmission and ion channel function. Similarly, editing SCN2A in mice has shown impairments in dendritic action potentials and social behaviors, directly linking genetic mutations with behavioral phenotypes. Furthermore, highly specific CRISPR targeting of CHD8 and FMR 1 has enabled precise investigations [24] into chromatin dynamics and Fragile X-related mechanisms.

Beyond modeling, CRISPR-Cas9 holds therapeutic potential. Innovations such as CRISPR activation (CRISPRa) have been used to reactivate silenced genes—for example, restoring UBE3A expression in Angelman syndrome by silencing antisense transcripts. Techniques like CRISPR-Gold, a nanoparticle-based delivery system, have been used to correct mutations in the mGluR5 pathway in Fragile X syndrome models, [25] significantly reducing repetitive behaviors. Furthermore, gene-editing strategies like exon skipping, exemplified by EDIT-101's success in retinal dystrophy, suggest that similar approaches could be adapted for ASD-associated splicing

defects in genes like SCN2A. However, challenges such as off-target mutations, delivery efficiency, and ethical considerations surrounding gene editing in humans remain major barriers to clinical application.

While CRISPR works on [26] the genetic aspects of ASD, phytochemicals provide adjunct support by targeting the disorder's metabolic aberrations. These compounds, which originate from medicinal plants, are effective neuroprotectants of to their antioxidant, anti-inflammatory, and mitochondrial sustaining nature. Some of these compounds include chlorogenic acid, curcumin, luteolin, and resveratrol, which have shown efficacy in preclinical and limited human studies. For example, chlorogenic [27] acid, which comes from Cichorium intybus (chicory), has demonstrated strong binding to purine metabolism key enzymes AMPD and protein kinase A (PKA) which stabilize cAMP signaling. In ASD mouse models, pro-oxidative exacerbations of ASD have been alleviated by curcumin and luteolin which reduce elevated proinflammatory cytokines such as TNF-α.

These compounds and their usefulness is further corroborated by their [28] clinical and preclinical evidence. Flavonoids like quercetin have improved glutathione levels which has been shown to coincide with behavioral improvements in children with ASD. The cruciferous vegetables sulforaphane is derived from has also been associated with decreased irritability and improvement in social functioning via the NFκB pathway, reducing inflammation. Phytochemicals also show potential in treating metabolic comorbidities such [29] as insulin resistance, which is common but often overlooked in individuals with ASD. For example, extracts from chicory have been shown to decrease hyperglycemia in diabetic models, which indicates potential improvement for metabolic dysfunctions associated with ASD. In spite of all the potential, the scientific and technological resources to develop effective nutraceuticals aimed to treat complex disorders with phytochemicals [30] are limited due to poor bioavailability and penetration of the blood-brain barrier. There unexplained limitations like low availability of the compound in the bod Moreover, other means of improvement must be undertaken through delivery systems and innovative design.

The combination of CRISPR-Cas9 gene editing and phytochemical-based metabolic modulation offers an exciting new approach to treating ASD. Instead of [31] tackling genetic or metabolic issues separately, this dual strategy addresses both the underlying genetic problems and the broader metabolic imbalances that contribute to ASD. Recent preclinical research has shown promising results for this combination. For instance, in one study, neurons with corrected SHANK3 genes that were treated with chlorogenic acid showed a 32% decrease in oxidative stress and [32] better AMP/ATP ratios, suggesting improved energy metabolism. Similarly, in mice with SCN2A edits, the addition of chlorogenic acid helped restore dendritic excitability and enhance social behavior results that weren’t seen with CRISPR correction alone.

This collaboration is thought to arise from the way phytochemicals influence pathways that connect with the edited genes. Take chlorogenic acid, for instance; it inhibits AMPD-1 [33] and regulates PKA activity, which boosts mitochondrial function in neurons modified by CRISPR.

Likewise, compounds like fisetin has a role in the AKAP scaffolding proteins, which are essential for the correct localization of PKA—this is particularly important in models that have been adjusted for CHD8 mutations. This kind of multi-pathway synergy holds the potential for more [34] comprehensive therapeutic benefits.

Personalized medicine could gain a lot from this strategy. By using CRISPR-engineered models of FMR 1, TSC2, or other mutations related to ASD, we can enhance genetic stratification and facilitate phytochemical screening across different subtypes, leading to more targeted and effective treatment plans. Moreover, innovative co-delivery systems, like nanoparticles that carry both Cas9 mRNA and chlorogenic acid, [35] are being investigated to ensure that neural tissues are efficiently targeted, potentially overcoming the delivery challenges that have historically hindered both gene editing and phytochemical therapies.

III. MATERIALS AND METHODS

3.1 Gene Selection & Sequence Retrieval:

The associated Relevant genes were selected from Ensembl genome browser. This browser was selected as it provides reliable gene sequence data including [36] exonic, intronic regions, gene variants and functional annotations.

3.2 Exon sequence visualization:

SnapGene is an efficient tool in molecular biology used for DNA sequence analysis and annotation. Furthermore, SnapGene was used to analyze the exonic sequences of the genes involved in the ASD. The exons for the selected ASD-associated genes were then visualized and annotated, ensuring the accurate [37] identification of the boundaries between exons and introns as well as enabling downstream analysis for CRISPR-based gene editing. [9].

3.3 Identification of Functional Domains:

The protein sequences were analyzed for the functional domains using Prosite to identify the key functional motifs present in the genes that could be targeted for knockout using CRISPR, to keep in mind the critical [38] domains for protein functions and ASD related pathways [10].

3.4 Guide RNA (g RNA)design:

gRNAs created using CHOPCHOP(https://chopchop.cbu.uib.no/), tool effective for CRISPR guide design to facilitate CRISPR-mediated gene editing in genes linked to ASD (https://chopchop.cbu.uib.no/).CHOPCHOP can predict high-efficiency gRNAs that target particular exons of the identified ASD genes, it was chosen. The program optimizes gRNA [39] sequences according to different criteria, including projected efficiency, closeness to the gene's coding area, and off-target potential[11].

3.5 Analysis using STRING Database:

To get insight into functional interactions, STRING (https://string-db.org/) used. The genes selected in our work were input into STRING for identifying the potential pathways, networks and interactions involved in neurodevelopmental processes related to ASD [12]. [40]

3.6 In silico assessment

Schrödinger-Maestro 12.1v software, PubChem Database https://pubchem.ncbi.nlm.nih.gov/, PubMed Database https://pubmed.ncbi.nlm.nih.gov/, RCSB-PDB https://www.rcsb.org/ , SWISS-MODEL (https://swissmodel.expasy.org/) – homology modelling server of protein, SwissADME (http://www.swissadme.ch/) free web tool to predict ADME parameters and ProTox-II Server (https://tox-new.charite.de/protox_II/). Endocrine disruptome (http://endocrinedisruptome.ki.si/).

3.6.1.Homology Modeling Using SWISS-MODEL

The SWISS-MODEL platform, an automated web-based tool, facilitates the prediction of protein [41] structures using homology modeling. Initially, sequences were obtained in FASTA format from the NCBI. These sequences then submitted to the SWISS-MODEL server to identify structurally similar template proteins. A BLAST analysis was performed for entries in the Protein Data Bank (PDB) to locate templates with highest sequence homology. Upon [42] selection of an appropriate template, a 3-dimensional model of the target protein was constructed. The model underwent refinement through optimization of its thermodynamic and molecular dynamics characteristics, removing any non-essential components. This process was repeated iteratively until a structurally stable and high-quality model was obtained.

3.6.2. Phylogenetic Tree Construction

To explore evolutionary relationships, phylogenetic analysis was conducted using [43] the MEGA 11.0 software which stands for Molecular Evolutionary Genetics Analysis. Sequences corresponding to AMPD and PKA were analyzed through the Neighbor-Joining method, and the robustness of the resulting phylogenetic tree was tested with 1000 bootstrap replications.

3.6.3.Molecular Docking Analysis

Docking studies were performed with Schrödinger-Maestro version 12.1 to evaluate the interaction [44] affinity between ligands and target proteins. The XP (extra precision) Visualizer tool within the suite was used to examine binding conformations and docking interactions. Compounds showing significant binding potential were further assessed for pharmacokinetic characteristics using the SwissADME tool and for toxicity prediction using the ProTox-II web server.

3.6.4.Protein Structure Preparation for AMPD1 and PKA

To evaluate the interaction of polyphenolic compounds with diabetes-associated targets such as AMPD1 and PKA, molecular docking protocols were implemented. Protein sequences for AMPD1 (Accession: NP_000027.3) and PKA (Accession: AAL40923.1) were retrieved from NCBI. The 3D models were generated using SWISS-MODEL. Prior to docking, these structures were optimized using [46] Schrödinger's protein preparation wizard. During this phase, molecules of water removed, polar hydrogens added, bond orders corrected, and selenomethionines were replaced with methionines. The proteins were then minimized using the OPLS3e force field to a root mean square deviation (RMSD) threshold of 0.30 Å. Additionally, the Ramachandran plot was used to assess the stereochemical quality [47] and conformational stability of the models.

3.6.5.Ligand Selection and Preparation

The ligands that will be used for docking included phytochemicals found in Cichorium intybus (chicory) such as, chlorogenic acid, cichoric acid, etc. Ligands [48] were sourced from PubChem and pre-processed using the LigPrep module in Schrödinger-Maestro (Release, 2019). Energy minimization using the OPLS3e force field at a physiological pH (7.0 ± 2.0) with Epik 2.2 for protonation state prediction. All relevant stereoisomers, ring conformations, and tautomers were generated, maintaining specified chiral centers and limiting the number of isomers per ligand to 32. [49] Prepared ligands were then converted into 3D formats suitable for docking simulations.

3.6.6.Active Site Prediction and Grid Generation

The identification of pockets on the protein surface was carried out using Schrödinger's SiteMap tool, which evaluates hydrophobic cavities based on geometric algorithms. SiteMap provided a range of predicted sites, from which the most [50] druggable site was selected. A receptor grid was then constructed using the Glide module, centered on the active site residues, to guide the docking simulations. Default grid parameters were used, and the generated grid facilitated the evaluation of potential ligand-protein interactions.

3.6.7.Extra Precision (XP) Docking

High-precision docking was performed using the XP module of Schrödinger's [51] Glide suite. Docking parameters were optimized to allow flexible ligand binding, including sampling of ring conformations and nitrogen inversion.. Torsional sampling was customized based on functional groups, and Epik penalties were included in the docking scores calculation. Resulting

conformations [52] were ranked by their docking and glide scores, with the best-scoring conformers selected for further analysis.

3.7. Results Interpretation and Visualization

3.7.1. Docking Scores

Post-docking analysis included exporting results as CSV files and visualizing docking and glide scores using the XP Visualizer tool. These scores provided a quantitative measure of ligand binding affinity.

3.7.2. Protein-Ligand Interaction Analysis

To identify [53] key stabilizing interactions, ligand-protein 2D interaction diagrams were generated, highlighting amino acid residues of AMPD-1 and PKA involved in stable binding with different ligands.

3.7.3. ADME Prediction via SwissADME

Pharmacokinetic profiling was conducted using SwissADME, which provided insights into various physicochemical parameters as molecular weight, refractivity, topological polar surface area, H2-bonding potential, and rotatable bonds. SMILES notations [54] from PubChem were used as input. The evaluation was based on Lipinski's rule of five for drug-likeness and bioavailability.

3.7.4. Toxicity Prediction Using ProTox-II

To assess safety profiles of both natural and synthetic ligands, toxicity prediction was carried out through the ProTox-II platform. The tool provided insights into potential hepatotoxicity, immunotoxicity, mutagenicity, carcinogenicity, and cytotoxicity. LD50 values [55] were expressed in mg/kg and categorized into toxicity classes I–VI according to globally harmonized standards.

3.7.5. Endocrine Disruption Potential

The potential of selected compounds to act as endocrine disruptors was analyzed using the Endocrine Disruptome web server. Docking simulations were conducted using AutoDock Vina to assess interactions with 16 human nuclear receptors, including androgen, [56] estrogen,

glucocorticoid, thyroid, and retinoid receptors. This analysis is essential for predicting off-target hormonal effects and evaluating the broader safety of candidate molecules.

IV. RESULTS AND DISCUSSION

A number of treatments have been researched for Autism, but most of them do not specifically target the disorder's genetic composition. One of the several essential steps are set up forward in the current work and the gRNAs which are needed for additional research and work at the genetic level. The table below shows the key genes associated with ASD that can be potentially targeted with CRISPR, along with their Ensembl IDs and critical functions:

SnapGene was utilized to map the full genomic sequence of the above genes providing a detailed analysis of exonic and intronic regions. The ability to visualize the exonic regions facilitates the process to design more precise CRISPR guide RNAs such that most relevant coding region could be targeted. By cross-referencing the annotated gene with PROSITE, the detection of specific domains crucial for neuronal development and functioning became precise. This step ensures that the genome editing is both efficient and targeted.

Table 1. Genes associated with ASD

Gene	Ensembl ID	Critical Function
SHANK3	ENST00000692848	Maintain synapses in neurons, essential for neural connectivity and communication.
CHD8	ENSG00000100888	Acts as a chromatin remodeler and regulates expression of genes in brain development.
SCN2A	ENSG00000184428	SCN2A plays a role in action potential firing, influencing neural activity.
FMR-1	ENSG00000102081	Associated with synaptic plasticity, FMR-1 regulates mRNA translation in neurons.
TSC2	ENSG00000103160	Works with TSC 1 to regulate cell growth and prevent tumor formation.

The Fig. 4a. Illustrates the domains in SHANK3, highlighting the motifs like ANK repeat region, SH3, and PDZ domains critical for protein interactions. SCN2A includes an IQ domain as depicted in Fig. 4b. is crucial for calcium binding and functioning of sodium channels critical for neuronal excitability and action potential propagation.

CHD8 protein consists of 2581 aa sequence and includes CHROMO domain, involved in chromatin binding, and the HEL_ATP_BIN domain, which is an ATP-binding helicase domain vital for chromatin remodeling as depicted in Fig. 4c.

FMR-1 found to be a unique case to this study. It encodes to fragile X Mental Retardation Protein, encoding AGE and KH domains (Fig. 4d) involved in RNA binding essential for neural development. Mutations in exon 1 of FMR-1 are often accompanied by ASD like symptoms including cognitive and behavioral impairments hence targeting exon 1 with CRISPR could potentially reduce the CGG repeats in that region and alleviating the ASD-related symptoms by targeting the genetic cause of impaired synaptic function.

TSC2 contains the RAPGAP domain as shown in the Fig. 4e, is an important domain for not only regulating cell growth but also in signaling in the mTOR pathway.

In this work for the treatment of ASD, single-guide RNA was employed to target critical exons in key genes such as SHANK3 (exon 21)[13], SCN2A (exon 2)[14], and TSC2 (exon 34)[15], allowing precise correction or disruption of mutations. However, for FMR-1 (exon 1)[16] and CHD8 (exons 3 and 4)[17], a double-gRNA approach was utilized to flank and excise specific regions, addressing pathogenic CGG repeat expansions in FMR-1 and enabling exon removal or replacement in CHD8. This dual strategy provides enhanced flexibility in editing these complex regions while maintaining specificity for therapeutic applications in ASD.

For the design of guide RNAs (gRNAs), we first identified the active genomic regions of interest using the ProSite database, which provided an extensive detail about various domain. After utilizing the SNAPgene software to map the selected exonic regions, precise alignment was obtained with the desired genomic loci. Guide RNA sequences were then generated via ChopChop tool, which enabled the design of efficient gRNAs with minimal off-target effects. This approach ensured the generation of gRNAs with a high likelihood of targeting the right exonic sequences with maximal efficiency, also minimizing the potential for unintended genomic alterations.

A CRISPR-Cas9 targeting strategy for the SHANK3 gene is depicted in the Fig. 5, showing exon 21 (85 bp), an important exon linked to synaptic function. The guide sequence here is a single guide RNA (sgRNA) (highlighted in green) intended to bind to a particular area that is flanked by a PAM site (GG), assuring accurate editing by Cas9.The selected gRNA exhibited an efficiency score of 49.48% indicating strong cleavage potential.ASD is closely associated with changes in the functional domains that is encoded by Exon 21 that are essential for postsynaptic density and synaptic stability. This focused strategy can improvise synaptic connections and address phenotypes linked to ASD by restoring SHANK3 functionality. Validation on-target and off-target properly effects ensure precise therapeutic applications.

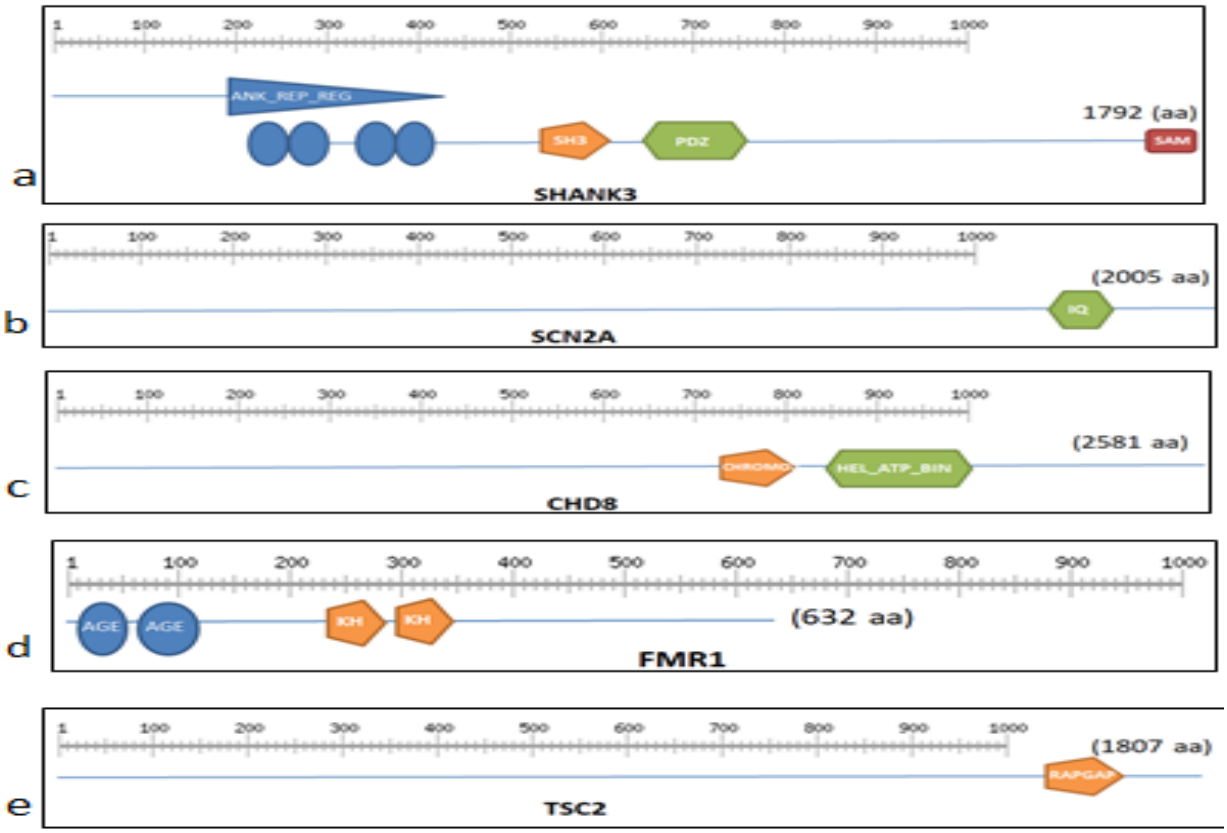


Fig. 3. Domain architectures of ASD associated proteins identified using Prosite; a. SHANK3: Contains Ankyrin repeats, SH3, PDZ, and SAM domains, b. SCN2A: Features an IQ domain, c.CHD8: Includes chromo-domain and helicase ATP-binding domain, d. FMR-1: Contains two KH domains and AGE domains, e. TSC2: Includes a RAP-GAP domain.

Fig.6. demonstrates a double-gRNA CRISPR-Cas9 approach targeting exons 3 and 4 of the CHD8 gene each having efficiency 49.74% and 45.73% respectively, a key regulator of neurodevelopment implicated ASD. The two gRNAs (green) flank the exons, enabling Cas9 to induce DSBs. This allows for exon deletion through NHEJ, which removes the mutated region, or exon replacement via HDR with a repair template, restoring CHD8 function. This is a way of breaking down transcriptional dysregulation consequent from CHD8 mutations for potential pathways of drug-treatable autism.

Fig. 4. sgRNA targeting exon 21 of the SHANK3 gene for precise editing

The figure alongside illustrates the sgRNA to target exon 34 of the TSC2 gene, a crucial regulator of mTOR signaling implicated in ASD. This sgRNA will direct the enzyme to induce a precise double-strand break within exon 34, enabling two therapeutic outcomes for correcting pathogenic mutations with a donor DNA template. TSC2 showed an efficiency of 65.84% depicting to minimize unintended genomic alterations.

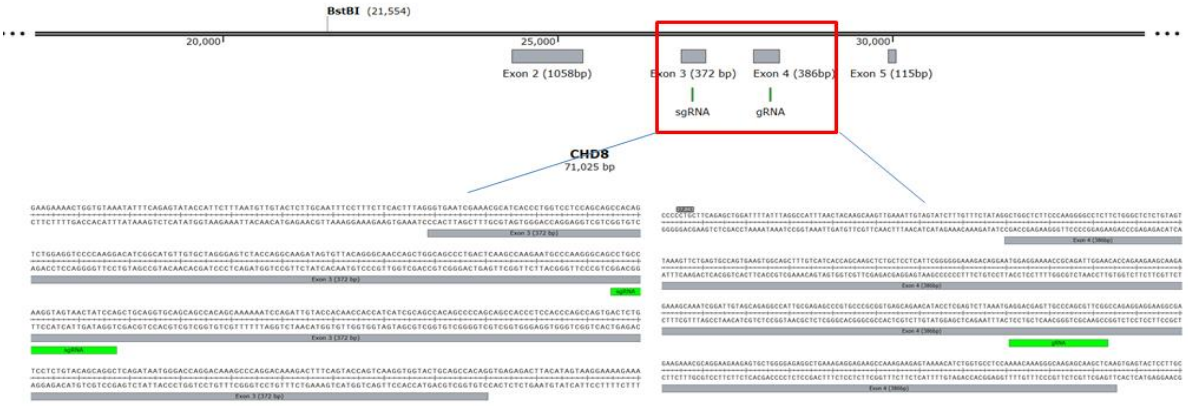


Fig. 5. Double gRNAs targeting exons 3 and 4 of the CHD8 gene for precise editing.

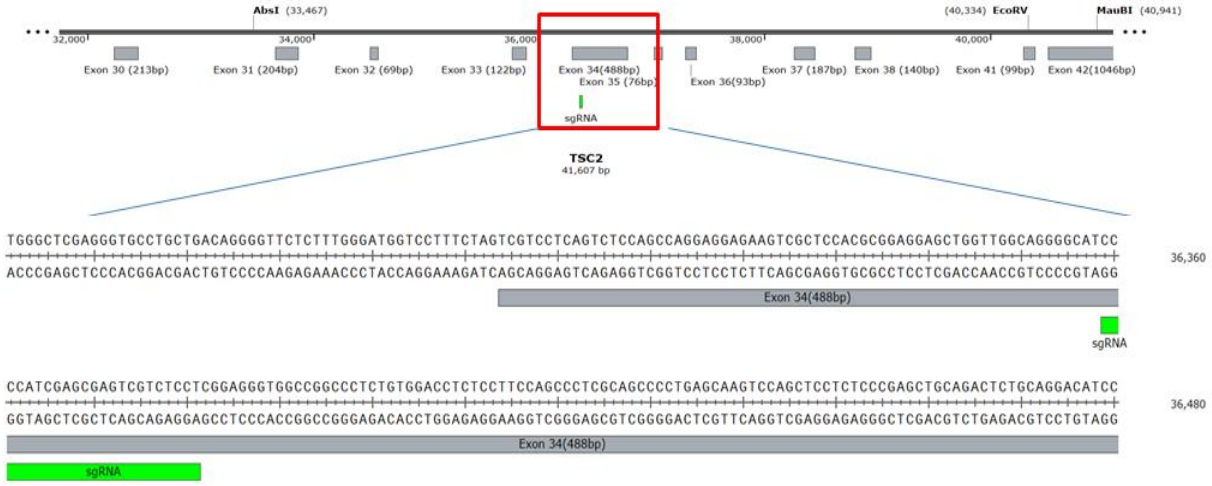


Fig. 6. gRNA targeting exon 34 of the TSC2 gene

Similarly, in SCN2A, sgRNA was selected with an efficiency of 58.47% and no off targets targeting exon 2 (318 bp), modifying exon 2 can regulate SCN2A's role in neuronal excitability, offering a precise therapeutic strategy for ASD. Figure illustrates the use of CRISPR-Cas9 targeting exon 1 of FMR-1 gene. Targeting the CGG repeats in this region provides a direct approach to address the underlying cause of Fragile X-related autism. By eliminating or correcting the repeat expansions, this method recovers FMRP expression, leading to improved neuronal function, learning, and behavior. This methodology could also pave the way for developing drug-treatable autism models, enabling high-throughput screening of therapies aimed at enhancing synaptic and cognitive function.

Fig. 7. gRNA targeting exon 2 of the SCN2A gene

Fig. 8. Dual gRNAs targeting exon 1 of the FMR 1 gene for CGG repeat expansions

Interacting Proteins for Key Genes in Autism Spectrum Disorder (ASD): Research on interacting proteins is essential as they help to elucidate the molecular pathways implicated in ASD. These protein networks are critical to neurodevelopmental processes, including synaptic plasticity, chromatin remodeling, mRNA translation, ion channel function, and mTOR signaling. Dysregulation of these interactions is a marker for ASD, and CRISPR technology provides a tool to manipulate these genes and their networks precisely. Through bioinformatics tools such as STRING, these complex networks can be mapped, and for therapeutic intervention, nodes can be identified. CRISPR studies targeting these genes and their interacting partners allow the generation of precise ASD models, which will facilitate further regarding the pathophysiology of ASD and aid the development of targeted therapies.

Table 2. Protein interaction in ASD

Gene	Interacting Proteins	Relevance to ASD and Importance of Study
SHANK3	SHANK1, SHANK2, DLGAP 1, DLGAP2, DLGAP3, DLG4, NRXN 1, NLGN 1, NLGN4X	SHANK3 interacts with synaptic scaffold and adhesion proteins. Dysregulation impacts synaptic connectivity. Understanding these interactions can reveal mechanisms of synaptic dysfunction in ASD.
CHD8	CTNNB 1, KMT2A, RUVBL 1, RUVBL2, BRD4, WDR5, RBBP5, CTCF, CHD7, TAF 1	CHD8 regulates chromatin remodeling and transcription. Its interactions control gene expression during neurodevelopment. Targeting these pathways can restore proper gene regulation in ASD.
FMR-1	FXR 1, FXR2, CYFIP 1, CYFIP2, DICER 1, EIF4E, AGO1, AGO2, PURA, NUFIP2	FMR- 1 modulates mRNA stability and translation at synapses .Dysregulated interactions cause Fragile X syndrome, linked to ASD. Studying these networks helps develop therapies targeting synaptic plasticity.
SCN2A	SCN 1 A, SCN 1 B, SCN3A, SCN3B, SCN4A, SCN4B,	SCN2A encodes sodium channels crucial for neuronal excitability. Aberrant interactions lead to impaired signaling and ASD phenotypes.

	SCN9A, ANK3, CALM3	
TSC2	TSC 1, MTOR, RHEB, AKT 1, GSK3B, RPS6KA1, TBC1D7, DDIT4, UBE3A, USP6	TSC2 regulates mTOR signaling, crucial for brain growth. Interactions impact neuronal development and synaptic function. Targeting these pathways can normalize neurodevelopment in ASD.

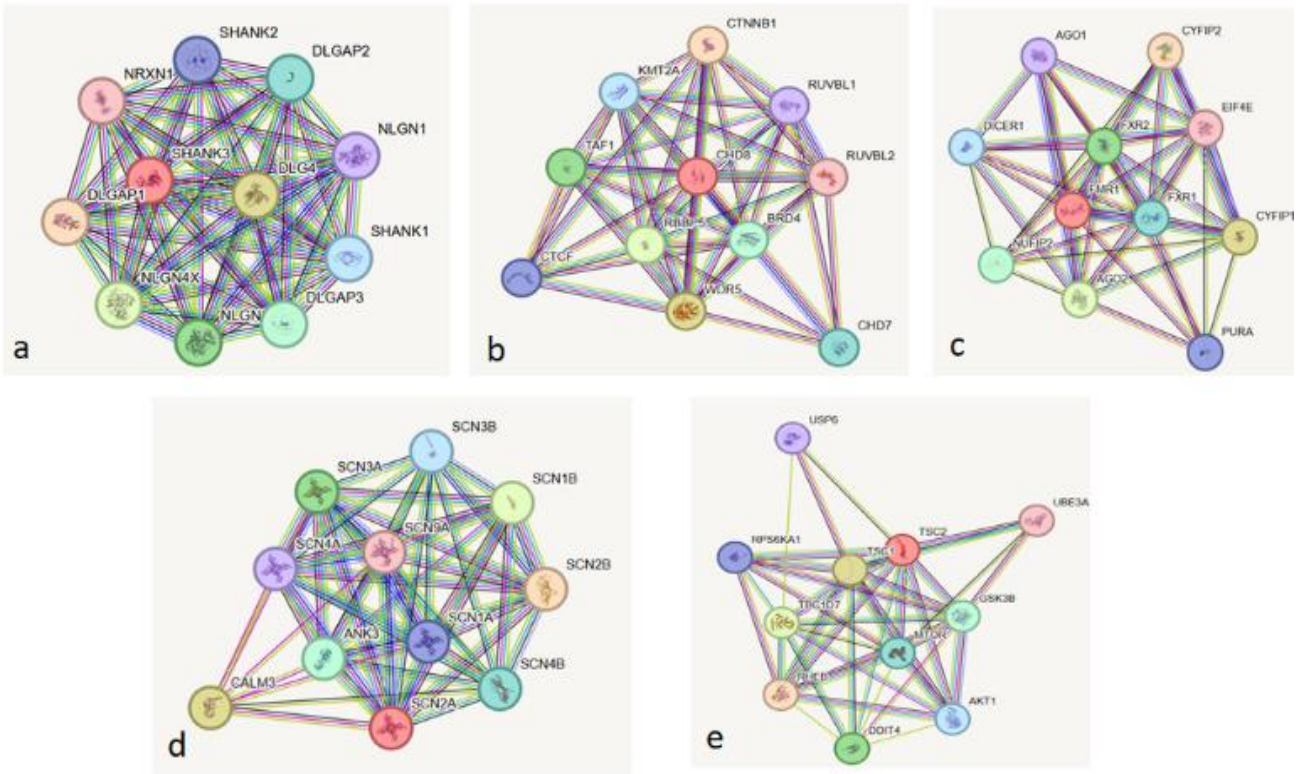


Fig. 9. Protein-protein interactions of key ASD-associated genes showing their functional pathways: (a) SHANK3, (b) CHD8, (c) FMR-1, (d) SCN2A, and (e) TSC2.

4.1. In Silico Investigation

4.1.1 Homologous Modeling of AMPD 1 and PKA

The amino acid sequences of Homo sapiens AMPD and PKA proteins were retrieved from the NCBI database. The sequence lengths were found to be 747 amino acids for AMPD and 2813 amino acids for PKA. As no resolved 3D structures were available in the Protein Data Bank for these proteins, homology modeling was undertaken using the SWISS-MODEL server. Templates with the highest sequence identity were selected—

- PDB ID: 2a3l.1.A (AMPD 1)
- PDB ID: 4d0n.1.B (PKA)

Structural modeling results indicated that AMPD 1 shared 47.29% sequence identity with its template, which was resolved via X-ray crystallography at 3.3 Å resolution and exhibited a QMEANDisCo global score of 0.73 ± 0.05. Meanwhile, the PKA model showed 100% identity with its template, resolved at 2.1 Å, and demonstrated a QMEANDisCo score of 0.76 ± 0.05. The resulting structural representations are illustrated in Figure 1.

4.1.2. Model Quality Assessment and Validation

To confirm the reliability and stereochemical soundness of the modeled protein structures, Ramachandran plot analysis was conducted using Schrödinger tools. The evaluation aimed to ensure suitability for downstream drug design applications by identifying any residues in disallowed conformations. Analysis revealed that more than 99% of residues for both proteins were located within permissible regions of the Ramachandran plot. Specifically, 96% of residues fell

within favored regions, with the remaining 4% in additionally allowed zones and none in disallowed regions. These results affirm the structural quality and reliability of the AMPD 1 and PKA models, as shown in Figure 2.

4.1.3. Evolutionary Analysis Using Phylogenetics

The evolutionary lineage of AMPD 1 and PKA proteins was investigated through phylogenetic analysis using MEGA version 11.0. Neighbor-Joining trees were constructed to understand the relationship of human proteins with those of closely related primates such as chimpanzees (Pan troglodytes), pygmy chimpanzees (Pan paniscus), and gorillas. The phylogenetic tree for AMPD demonstrated a close genetic relationship between Homo sapiens isoforms and Pan paniscus, while Pan troglodytes appeared as a more distantly related outgroup. In contrast, the tree for PKA showed that human isoform 2 proteins clustered closely with those of gorillas, with both Pan paniscus and Pan troglodytes grouped in a neighboring clade. These observations, illustrated in Figure 3, highlight the evolutionary conservation and divergence of these proteins across species.

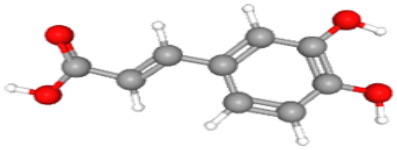
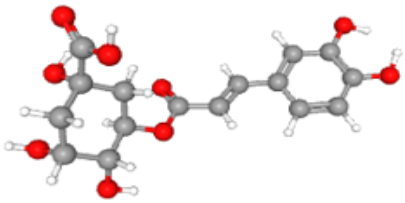
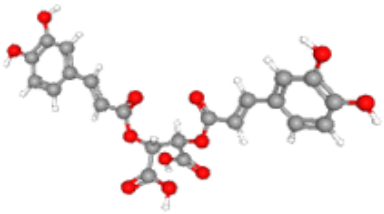
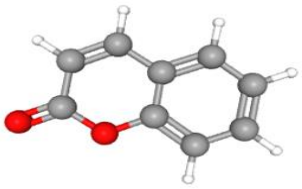
4.1.4. Molecular Docking Studies

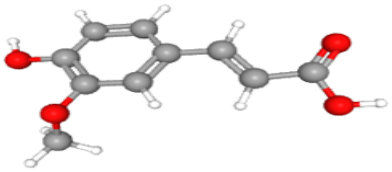
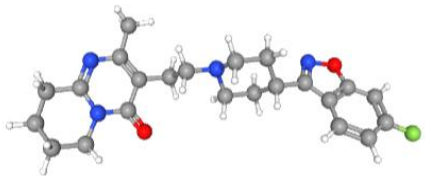
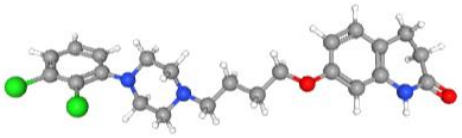
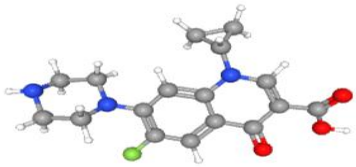
The core objective of this computational analysis to find the potential inhibitors for AMPD and PKA by evaluating the binding affinities of phytochemicals from Cichorium intybus against the target proteins. A total of seven plant-derived compounds were selected alongside five FDA-approved antidiabetic drugs for comparison. The three-dimensional structures of these ligands are shown in Table. Virtual screening was then performed to assess how effectively each compound interacted with the AMPD-1 and PKA protein targets.

Chlorogenic acid, a phenolic compound found in chicory roots, exhibited the most favorable binding energy in docking simulations. Comprehensive docking results are presented with visualizations of the docking poses.

To determine drug-likeness, each phytochemical underwent an in silico evaluation against Lipinski's Rule of Five using the SwissADME web tool. This assessment measured multiple physicochemical parameters, and up to two violations were allowed for a compound to qualify for further study. Table 3 presents a comparative profile of all ligands. Among the tested phytochemicals, cichoric acid showed two rule violations, while chlorogenic acid violated only one. The remaining natural compounds complied fully with Lipinski's criteria. All reference drugs, including metformin and sitagliptin, satisfied the rule completely. Based on these findings, phytochemicals with no more than two violations—particularly chlorogenic acid—were shortlisted for continued docking and pharmacokinetic evaluation.

Table 3: Table presents the phytochemicals derived from chicory species and reference drugs, including their molecular formulae and 3D structural conformations utilized for computational modeling studies

S.No.	Compounds	PubChem ID	Mol.Formula	3-D Confirmation
01.	Caffeic acid	CID 689043	$C_9H_8O_4$	
02.	Chlorogenic acid	CID 1794427	$C_{16}H_{18}O_9$	
03.	Cichoric acid	CID 5281764	$C_{22}H_{18}O_{12}$	
04.	Coumarin	CID 323	$C_9H_6O_2$	

05.	Kaempferol	CID 5280863	$C_{15}H_{10}O_6$	
06.	Ferulic acid	CID 445858	$C_{10}H_{10}O_4$	
07.	Risperidone	CID 5073	$C_{23}H_{27}FN_4O_2$	
08.	Aripiprazole	CID 60795	$C_{23}H_{27}Cl_2N_3O_2$	
09.	Ciprofloxacin	CID 2764	$C_{17}H_{18}FN_3O_3$	

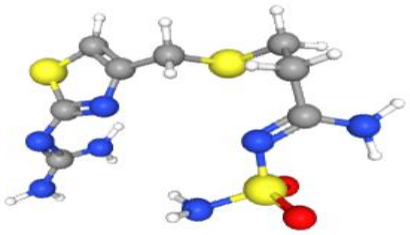
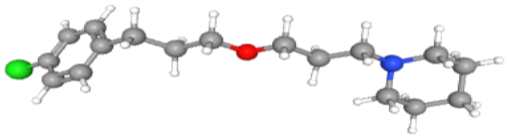
10.	Famotidine	CID 5702160	$C_8H_{15}N_7O_2S_3$	
11.	Pitolisant	CID 9948102	$C_{17}H_{26}ClNO$	

Table 4: Docking results of chicory plant and prescribed drugs with AMPD-1 and PKA.

Compound Name	AMPD-1 Dock Score (kcal/mol)	AMPD--1 Glide Score (kcal/mol)	AMPD-1 Lipophilicity	PKA Dock Score (kcal/mol)	PKA Glide Score (kcal/mol)	PKA Lipophilicity
Chlorogenic acid	-9.41	-9.41	-3.4	-12.61	-12.6	-3.4
Cichoric acid	-1.42	-1.4	-2.9	-9.28	-9.28	-4.7
Coumarin	2.98	-2.91	-2.82	-6.52	-6.53	-3.2
Kaempferol	-4.55	-4.6	-3.53	-3.79	-2.79	-1.79
Ferulic acid	-1.57	-1.5	-1.54	-4.97	-4.97	-1.02
Caffeic acid	-3.97	-3.96	-1.5	-7.78	-7.78	-2.7

Prescribed Drug	AMPD-1 Dock Score	AMPD-1 Glide Score	AMPD-1 Lipophilicity	PKA Dock Score	PKA Glide Score	PKA Lipophilicity
Risperidone	-5.68	-6.04	-0.82	-3.6	-3.6	-0.76
Aripiprazole	-6.38	-6.38	-2.67	-3.08	-3.09	-1.19
Ciprofloxacin	-3.36	-3.36	-2.52	-3.56	-3.56	-1.95
Famotidine	-5.14	-5.14	-2.95	-4.6	-4.6	-1.19
Pitolisant	-6.61	-7.05	-3.74	-2.75	-2.31	-1.98

Table 5: Pharmacological evaluation of phytochemicals of chicory as well as respective prescribed drugs

Plant-Derived Molecule	Molecular Mass (g/mol)	Partition Coefficient (LogP)	nOH (Hydroxyl Count)	nOHNH (Donor/Acceptor Count)	Rotatable Bonds (Nb)	Estimated Bioavailability	Lipinski Violations
Caffeic acid	180.16	0.93	4	3	2	0.56	0
Chlorogenic acid	354.31	-0.38	9	6	5	0.11	1
Cichoric acid	474.37	1.01	12	6	11	0.11	2
Coumarin	146.14	1.82	2	0	0	0.55	0
Kaempferol	286.24	1.58	6	4	1	0.55	0
Ferulic acid	194.18	1.36	4	2	3	0.85	0

Prescribed Drugs	Compound Name	Mol.Wt (g/mol)	LogP	nOH	nOHNH	Nb	Bioavailability	Number of Violations
01.	Risperidone	129.16	-0.89	02	03	02	0.55	00
02.	Aripiprazole	407.31	2.51	10	01	06	0.55	00
03.	Ciprofloxacin	317.42	3.21	03	02	07	0.85	00
04.	Famotidine	408.87	2.18	06	04	06	0.55	00
05.	Pitolisant	488.01	2.80	06	03	08	0.55	00

Table 6: Toxicological properties of compounds

Compound Name	Hepatic Impact	Cancer Risk	Immune Response	DNA Alteration Risk	Cell Toxicity
Caffeic acid	None	+++	None	None	None
Chlorogenic acid	None	None	++	None	None
Cichoric acid	None	None	++	None	None
Coumarin	None	+++	None	None	+++
Kaempferol	None	None	None	None	None
Ferulic acid	None	None	++	None	None
Caffeoylmalic acid	None	None	++	None	None

++ = Possibly Active (Mild Indications) +++ = Potentially Active (Moderate to Strong Indications)

Prescribed Drugs	Hepatic Impact	Cancer Risk	Immune Response	DNA Alteration Risk	Cell Toxicity
Risperidone	None	None	None	None	None
Arpiprazole	None	None	None	None	None
Ciprofloxacin	None	None	None	None	None
Famotidine	None	None	None	None	None
Pitolisant	None	++	++	None	None

4.1.5. Assessment of endocrine disruption potential

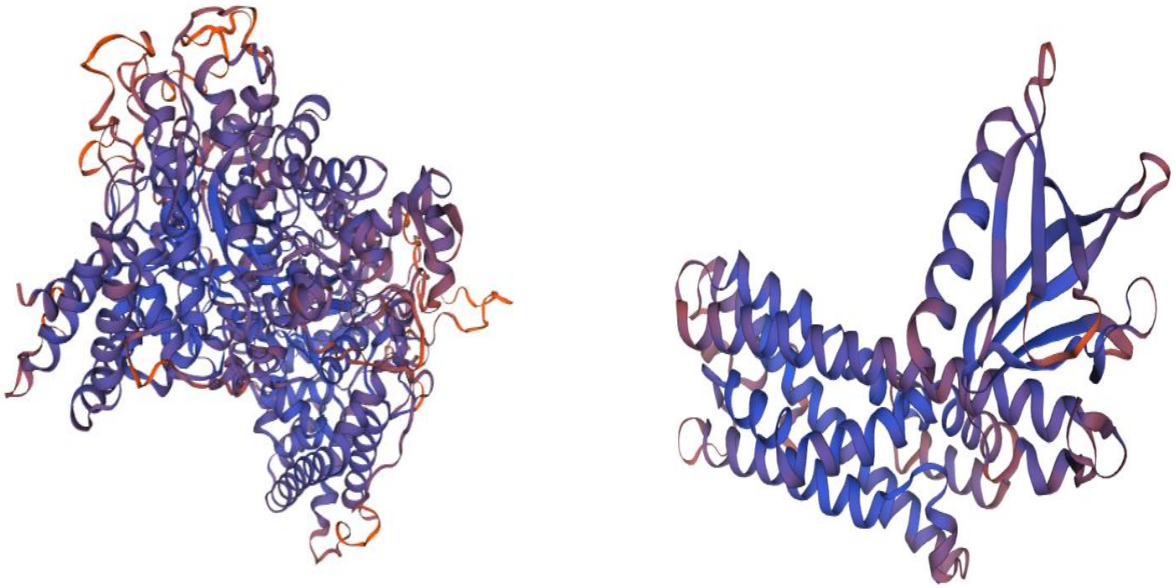
The central motive of this research is to identify potential inhibitors of AMPD-1 and PKA through an assessment of their binding affinities with these protein targets. In addition to docking studies, the potential of endocrine disruption of selected phytocompounds and clinically approved drugs was evaluated using the ENDOCRINE DISRUPTOME computational platform. This tool predicts the likelihood of ligand binding to various human nuclear receptors.

The output from ENDOCRINE DISRUPTOME categorizes binding probabilities using a color-coded matrix: red indicates a high likelihood of receptor binding, orange denotes intermediate probability, yellow represents moderate probability, and green suggests a low probability of interaction. According to the results, most of the selected compounds—including both phytochemicals and standard drugs—exhibited moderate to intermediate binding tendencies toward the antagonist androgen receptor (AR an.). Notably, the flavonoid kaempferol demonstrated a high binding potential with both androgen receptor (AR) and its antagonist form (AR an.), suggesting a stronger endocrine interaction profile than other tested molecules.

Table 9 presents a summary of the predicted interactions between the tested ligands and nuclear receptors. The nuclear receptors most influenced by the compounds under investigation include AR an., AR, estrogen receptor alpha (ER α), glucocorticoid receptor (GR), thyroid hormone receptors alpha (TR α) and beta (TR β). These predictions are consistent with earlier findings indicating that several agents can interact with nuclear hormone receptors, especially those involved in androgen, glucocorticoid, and thyroid signaling pathways.

Table 7:Study aims in identifying unique inhibitors for proteins (AMPD-1 & PKA) through assessment of their binding affinity.

Phyto Chemicals	AR	AR anta	ER(α)	ER(α) anta	ER(β)	Er(β)anta	GR	GR anta	LXRα	LXR(β)	PPAR(α)	PPAR(β	PPAR(γ)	RXR(α	TR(α)	TR(β)
Caffeic acid	-5.9	-5.7	-5.3	-5.6	-5.4	-5.3	-6.1	-5.0	-5.6	-6.0	-5.2	-5.1	-5.6	-5.2	-5.9	-5.8
Chlorogenic acid	-6.7	-8.1	-8.6	-6.4	-8.5	-6.0	-8.8	-6.6	-8.9	-8.8	-6.6	-6.8	-6.9	-9.3	-8.7	-8.3
Cichoric acid	-6.1	-6.5	-8.7	-8.7	-4.1	-7.8	-9.4	-8.2	-9.2	-8.2	-7.8	-8.5	-9.0	-9.7	-6.6	-8.9
Coumarin	-5.7	-6.9	-5.4	-5.6	-5.2	-5.4	-5.4	-5.1	-7.1	-7.0	-5.3	-5.9	-5.9	-5.5	-6.1	-5.4
Kaempferol	-8.9	-8.7	-8.5	-8.5	-7.8	-8.3	-8.8	-7.6	-9.4	-9.1	-7.7	-8.6	-9.4	-9.2	-9.1	-9.3
Ferulic acid	-5.7	-7.6	-5.4	-5.3	-5.1	-5.1	-7.4	-6.1	-5.4	-5.9	-5.2	-5.1	-5.5	-5.4	-5.3	-5.6
Caffeoylmalic acid	-7.6	-7.7	-7.7	-7.7	-7.7	-7.2	-7.9	-6.9	-7.1	-7.6	-7.2	-7.2	-7.2	-8.2	-7.9	-8.2
Drugs																
Risperidone	-4.9	-5.3	-5.3	-4.9	-4.9	-4.7	-4.7	-4.9	-5.1	-5.0	-5.1	-5.1	-4.8	-4.8	-5.2	-5.2
Aripiprazole	-7.7	-7.8	-9.2	-8.5	-8.6	-8.0	-9.5	-8.5	-10.1	-10.5	-8.9	-10.0	-9.1	-10.1	-9.7	-9.9
Ciprofloxacin	-7.3	-8.4	-8.5	-8.4	-7.9	-7.7	-8.9	-7.1	-9.3	-8.9	-8.0	-8.5	-7.7	-9.2	-9.2	-9.3
Famotidine	7.8	5.1	-6.6	-8.9	3.5	-8.1	-7.0	-9.5	-9.1	-9.1	-7.5	-8.7	-8.0	-5.2	0.2	-0.6



A **B**

Figure 10: 3-D Model (A) AMPD 1 and (B) PKA through Swiss model.

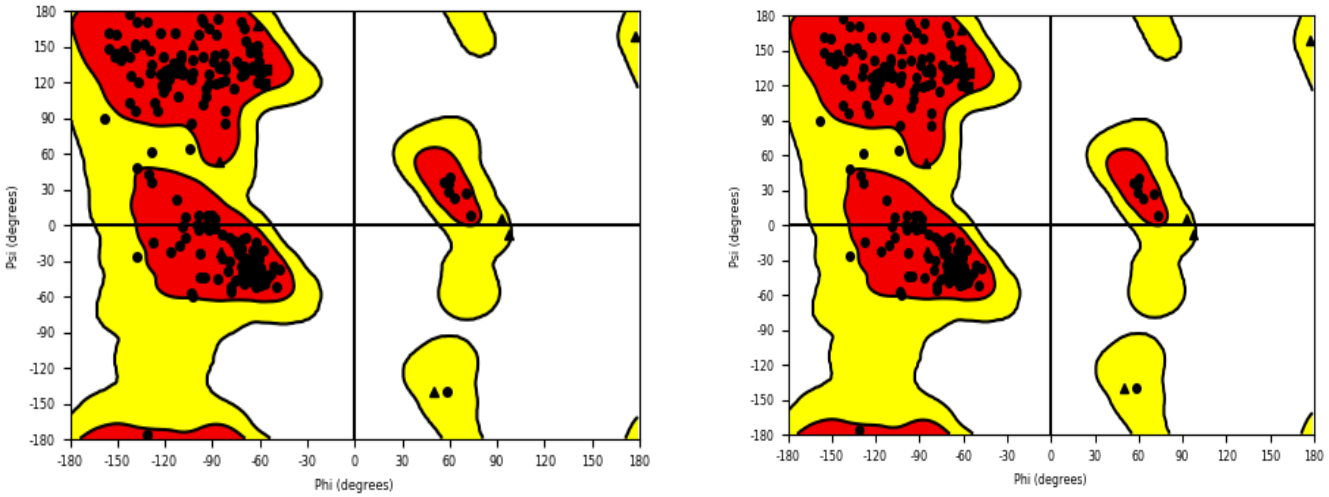


Figure 11: Ramachandran plots 2a3l.1.A and 4d0n.1.B proteins via PROCHECK.

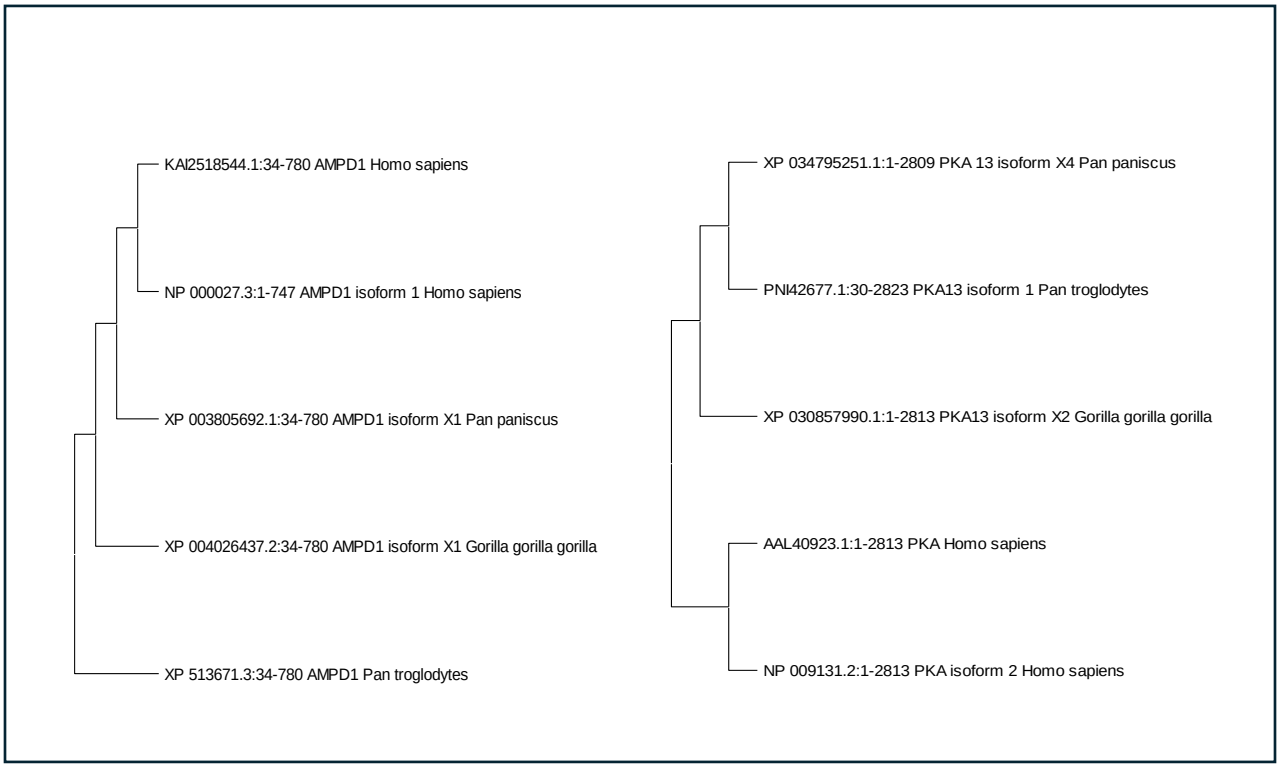
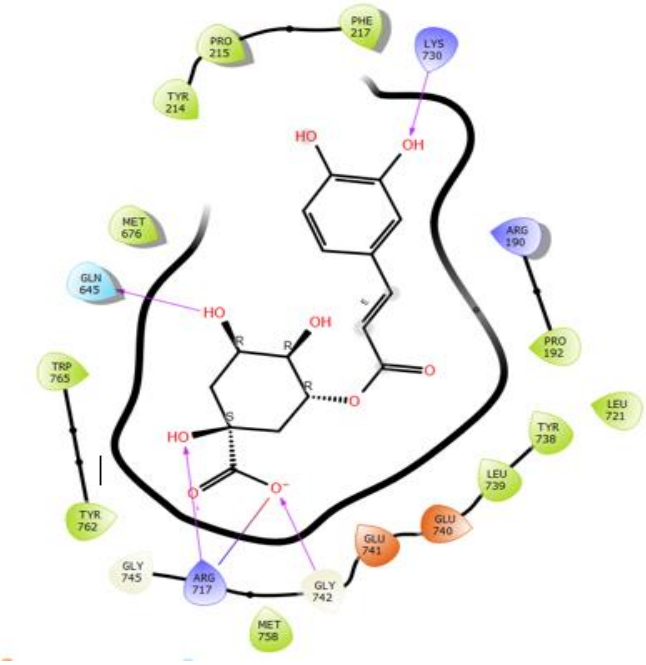
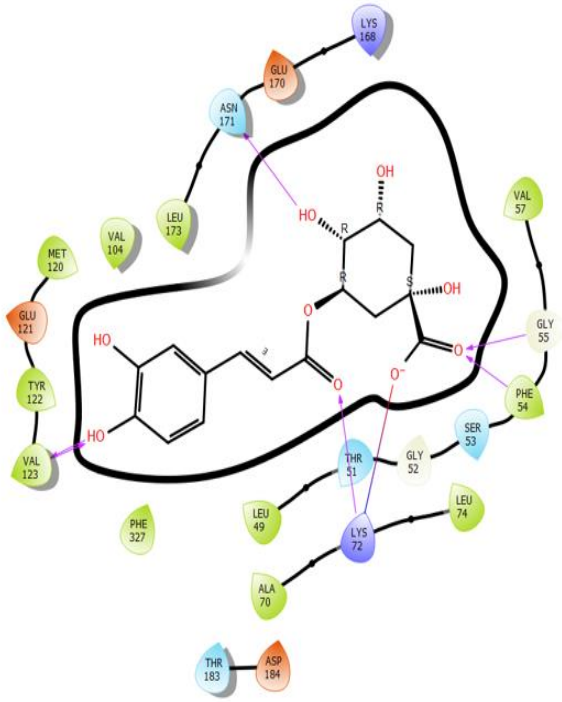


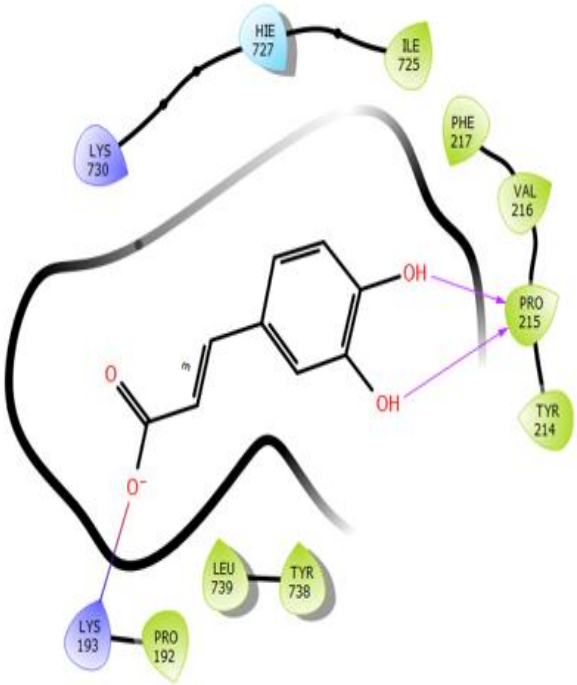
Figure 12: Evolutionary pattern of AMPD-1 and PKA proteins.



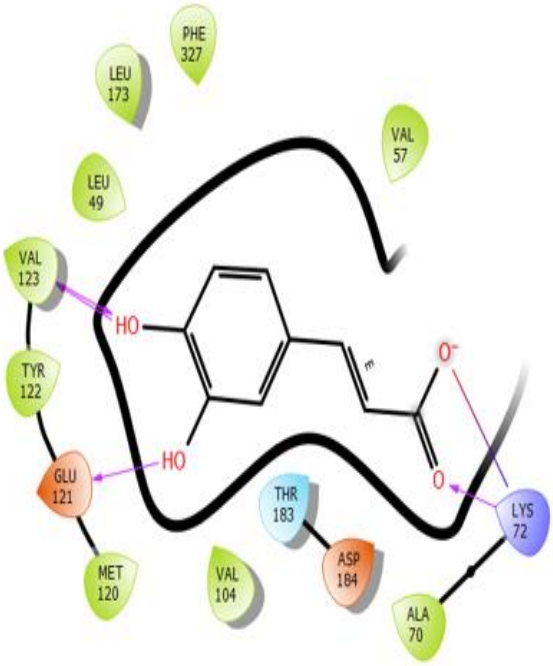
Chlorogenic acid & AMPD 1



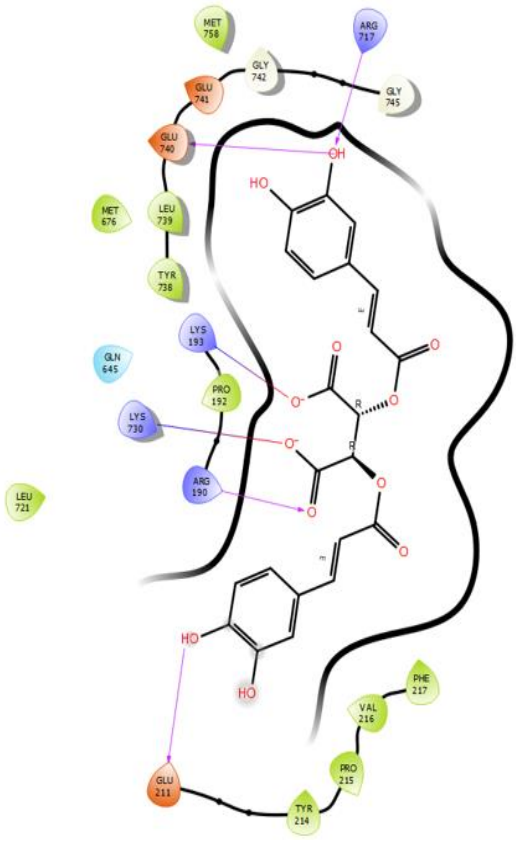
Chlorogenic acid & PKA



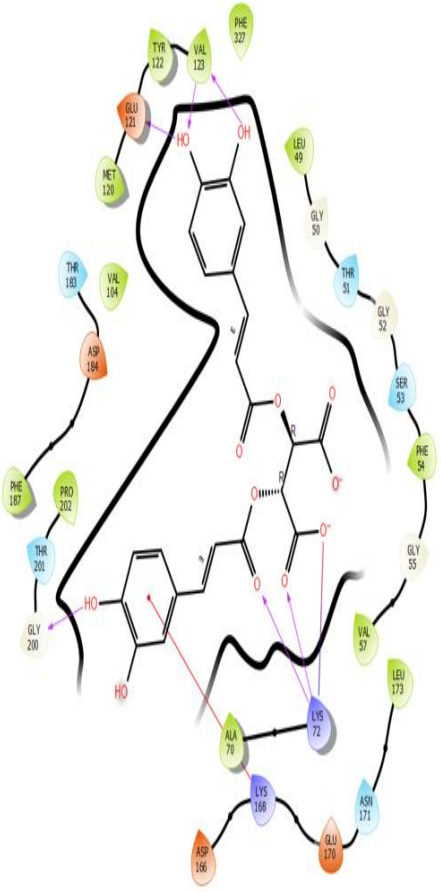
Caffeic acid & AMPD 1



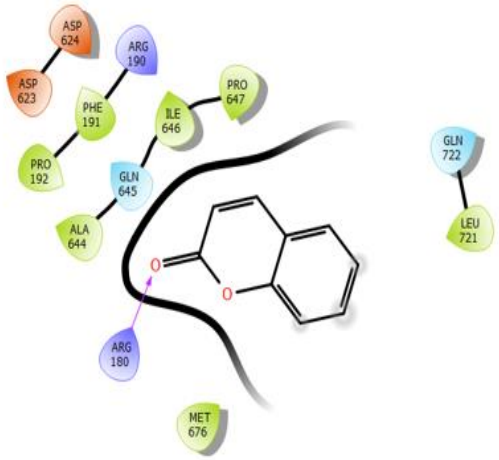
Caffeic acid & AMPD 1



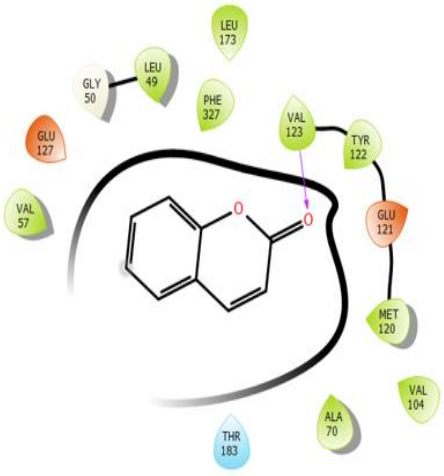
Cichoric acid & AMPD 1



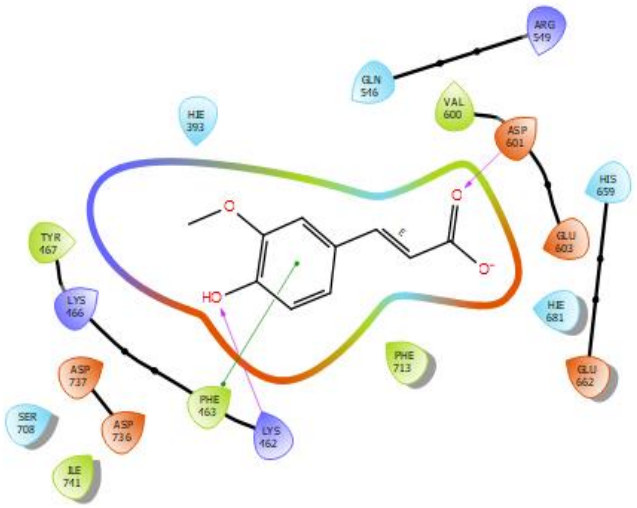
Cichoric acid &PKA



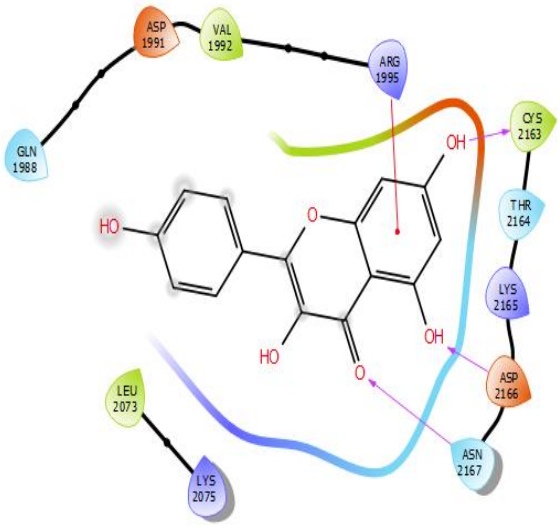
Coumarin & AMPD 1



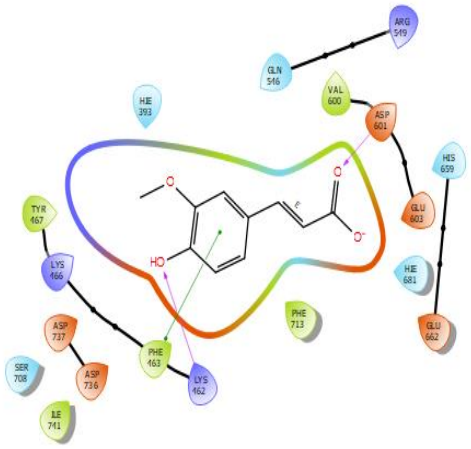
Coumarin & PKA



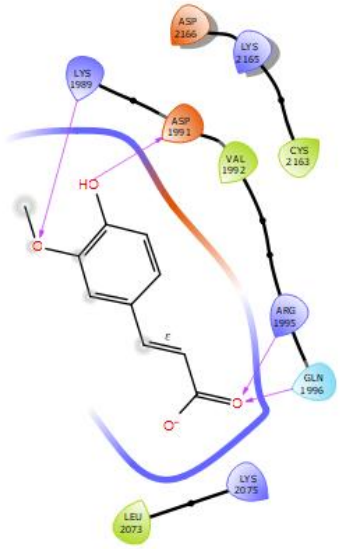
Kaempferol & AMPD 1



Kaempferol & PKA

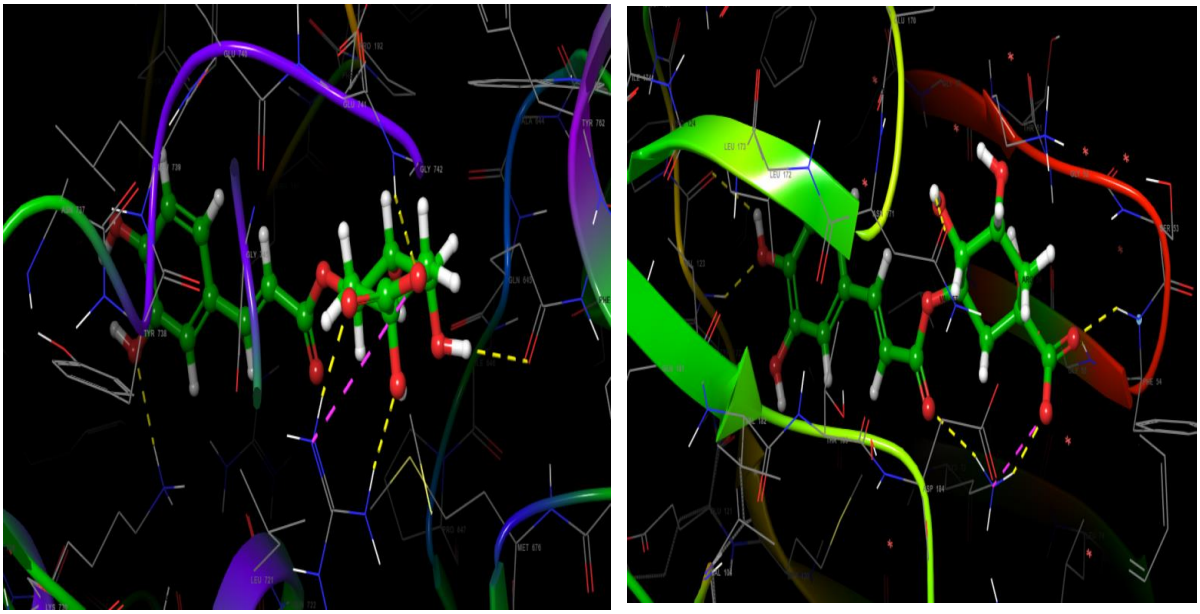


Ferulic acid & AMPD 1



Ferulic acid & PKA

Figure 13: Representation 2-D proteins (AMPD 1 AND PKA) with ligands.



(A) **(B)**

Figure 14: 3-D binding of Chlorogenic acid w.r.t AMPD 1 (A) and PKA 3-D (B) protein structure

4.1.6. Oral toxicity prediction

Toxicity assessment conducted by the ProTox-II , which utilizes chemical similarity, fragment-based analysis, & ML algorithms. The resulting predictions are summarized in Table 7. Most of the natural compounds were classified under toxicity classes 04 or 05, with the exception of coumarin, which was categorized as class 03. Notably, chlorogenic acid, demonstrated the highest docking scores against target proteins, was assigned to toxicity(class 05). It exhibited mild immunotoxicity, with a predicted LD_{50} value of 5000 mg/kg, indicating that lower doses may be suitable for development as a drug candidate.

V. CONCLUSION

This interdisciplinary study represents a significant stride in the search for a multimodal therapeutic framework for Autism Spectrum Disorder (ASD). By integrating CRISPR-Cas9 gene editing and phytochemical pharmacology derived from Cichorium intybus (chicory), the research bridges molecular genetics with bioactive compound screening to address ASD's multifactorial complexity. The genetic targets—SHANK3, CHD8, SCN2A, FMR 1, and TSC2—were methodically selected and edited using high-specificity gRNAs to ensure minimal off-target effects. These genes, well-documented for their roles in synaptic structure, chromatin remodeling, and neuronal signaling, were chosen based on their relevance to ASD pathology and their capacity to impact downstream neurodevelopmental processes when disrupted.

On the pharmacological front, this study explored the therapeutic promise of chicory-derived polyphenols alongside conventional antidiabetic medications. Molecular docking analyses revealed that chlorogenic acid displayed the strongest affinity for AMPD 1 and PKA, two metabolic regulators implicated in energy homeostasis and mitochondrial function—both of which are frequently dysregulated in individuals with ASD. Importantly, these findings were complemented by in silico evaluations of drug-likeness, ADME properties, and toxicity profiles

The inclusion of toxicity prediction models such as ProTox-II and endocrine disruption screening via the Endocrine Disruptome platform added another critical layer of safety assessment. While a majority of the phytochemicals showed negligible hepatotoxicity, mutagenicity, or immunotoxicity, the potential endocrine-disrupting properties—especially in compounds like Kaempferol—warrant further in vivo validation. These insights help in refining candidate selection and ensuring translational relevance.

The innovation of this research lies in its systems-level approach—rectifying core genetic aberrations through CRISPR while concurrently stabilizing metabolic outcomes using natural compounds. This dual strategy offers the potential for enhanced synaptic recovery, improved neuroplasticity, and more comprehensive management of ASD symptoms. Moreover, the genetically edited models created in this study could serve as a robust platform for screening future pharmacological candidates, allowing for a more accurate prediction of treatment outcomes in human neurodevelopmental disorders.

In conclusion, the study illustrates the promise of combining gene editing with phytopharmacology to create a precision-driven therapeutic avenue for ASD. It paves the way for personalized medicine approaches that target both the genetic and metabolic dimensions of the disorder. Although preliminary, the findings serve as a foundational step for further experimental validation and clinical translation. Continued exploration in this domain can eventually lead to more effective, safe, and personalized interventions, changing the therapeutic landscape for ASD and similar complex neurological conditions.

VI. FUTURE DIRECTIONS

This research offers a compelling foundation for a holistic approach to managing ASD, combining the precision of CRISPR-Cas9 gene editing with the therapeutic potential of phytochemicals derived from chicory. Moving forward, a crucial step would be to validate these findings in vitro cellular assays and in vivo animal to observe actual biological effects, particularly in relation to neuronal repair and behavior improvement. While computational predictions suggest low toxicity and good drug-likeness, comprehensive pharmacokinetic and toxicity testing in biological systems will be essential to ensure the safety of natural compounds like chlorogenic acid. Additionally, further optimization of guide RNAs using the latest bioinformatics tools can enhance their specificity and reduce unintended edits. Understanding the real-world endocrine effects of these compounds also requires laboratory-based hormonal and transcriptomic analyses, as some phytochemicals showed potential hormonal interactions. Another key direction lies in developing safe and efficient delivery systems—such as lipid nanoparticles or exosome-based carriers—that can transport CRISPR components and phytochemicals directly to target tissues, especially the brain. Exploring the synergistic benefits of combining gene correction with metabolic stabilization in complex ASD models could provide deeper insights into treatment efficacy. With the advent of induced pluripotent stem cell (iPSC) technology, future work could involve generating patient-specific neuron models to test personalized treatments. Lastly, as we inch closer to therapeutic application, it's imperative to address ethical, clinical, and regulatory concerns around gene editing, especially in neurodevelopmental disorders. Together, these future explorations will help transform this dual-strategy concept into a viable, patient-centered intervention for ASD.

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LIST OF PUBLICATIONS

1.Comprehensive Review of Machine Learning Approaches for Analyzing EEG Based Neurological Disorder

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Comprehensive Review of Machine Learning Approaches for Analysing EEG-Based Neurological Disorders

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Abstract: **Electroencephalography is a fast, inexpensive and non-invasive technology for analysing neurological disorders. Neurological disorders have a profound impact on the Autonomic Nervous System and the brain significantly influencing individual's health and well-being. This review focuses on the analysis of EEG signals related to three significant neurological disorders: Alzheimer's disease (AD), Parkinson's disease (PD), and Epilepsy. The EEG parameters serve as potential markers for cognitive status in these disorders. Since the inspection of large amount of EEG by neurologist is time-consuming and inconsistent so the machine learning algorithms have made significant strides in identifying unique EEG patterns associated with each disorder which not only enables early diagnosis and timely preventive interventions but also helps to develop personalized treatment strategies. This review delves into the process of analysing EEG signals and integrating machine learning and deep learning algorithms for robust classification of neurological disorders. In addition, it intends to provide a comprehensive overview of the modern advancements in using EEG and ML for improved diagnosis and potential treatment monitoring of PD, AD, and epilepsy.**

Keywords-Electroencephalography Alzheimer's disease, Parkinson's disease, Epilepsy, Machine Learning

I. INTRODUCTION

In recent years, the convergence of neurobiology and machine learning (ML) offered promising directions for elucidating neurological diseases. From the perspective of diagnosis, PD, AD and epilepsy have proven to be particularly difficult and challenging. EEG holds immense potential in discovering the underlying mechanism of these disorders. Traditional EEG analysis limits in extracting the meaningful patterns of the EEG signals associated with the disease. ML and computational approaches enhance the accuracy the treatment. In this review, we provide a comprehensive survey of ML approaches to EEG data for these diseases and provide the different approaches to feature extraction, pre-processing of signals, and classification algorithms that have been applied in the recent research. In addition, the report provides a description of the disease and potential biomarkers for it.

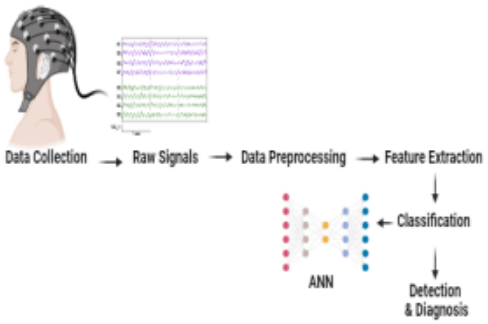

Fig. 1. Overview of the EEG signal processing

II. ELECTROENCEPHALOGRAPHY

Electroencephalography (EEG) is a technology developed in 1929 by Hans Berger, a German scientist [1]. EEG measures and analyses brain activity by measuring electrical waves produced by neurons within the brain. These electrical signals generate a graph which is called as electroencephalograph which detects the brain electrical impulses through neural cells. This methodology constitutes of electrodes which are attached to the scalp of a subject as shown in

2.Integarting CRISPR technology and Computational Bioinformatics for deeper Insights into Autism Spectrum Disorder (In process of publication)

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