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RESEARCH PAPER

INVESTIGATION OF THE PHARMACOLOGIC MECHANISMS INVOLVED IN THE ACTION OF BERBERINE AS A POTENTIAL TREATMENT FOR ALZHEIMER'S DISEASE: NETWORK PHARMACOLOGY-BASED APPROACH

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Alzheimer's disease (AD) is a neurodegenerative disorder characterized by significant memory loss and mental health decline. Berberine, a key active component of the ancient Chinese herb Coptis chinensis, has potential therapeutic effects against AD. To explore this, we utilized the HERB and SwissTargets prediction databases to identify AD-related targets associated with berberine. These targets were used to construct a protein interaction network and were analyzed through gene ontology (GO) and the Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analyses. Additionally, berberine's targets against tau and Aβ pathology in AD were identified using the AlzData database and further validated through molecular docking studies. Following screening and removal of duplicates, 27 berberine-related targets for AD were identified. GO biological process analysis revealed a significant upregulation in the cellular response to chemical stress (GO:0062197) among these targets. KEGG pathway enrichment analysis showed the highest enrichment in the AD pathway (hsa05010), with APP, BID, CASP3, CASP8, CYCS, GSK, and TNF emerging as key protein targets. Molecular docking analysis confirmed that these genes exhibited strong binding interactions and favorable binding energies with berberine. In conclusion, network pharmacology methods demonstrated that berberine may exert therapeutic effects in AD by modulating multiple targets and pathways, suggesting its potential as a multi-target drug in treating this complex disorder. This study highlights the importance of further research into berberine's clinical applications for AD.

Key words: Alzheimer's disease, Berberine, Network Pharmacology, Molecular docking, Alkaloid.

INTRODUCTION

Alzheimer's disease (AD) is the most common type of dementia seen in senior people. It affects more than 47 million individuals around the globe, and Alzheimer's Disease International expects that this figure will rise to 81.1 million by the year 2040. It is anticipated that by the year 2050, India will have one of the oldest populations in the world, with the percentage of individuals aged 60 or older growing from 15% now to 36.5% then [1-3]. In the meanwhile, it has been estimated that there are now more than 9 million people living with AD in India.

However, there is now neither a preventative nor a curative therapy available for AD, which places an immense burden on both public health and society. Aggregation of the microtubule-associated protein tau and the amyloid precursor protein (APP) derivative and Aβ are the hallmarks of AD [4]. In addition, AD is typically accompanied by the death of neurons. There are currently very few therapy options available for AD, and most of the novel approaches, such as optogenetics, are still in the research and development stage. There are now just five drugs that are commercially approved by the Food and Drug Administration (FDA) to treat AD. Nevertheless, it should be noted that the therapeutic interventions for AD may just provide temporary relief from the associated symptoms, without the ability to impede, delay or reverse the progression of the ailment. Furthermore, therapeutic drugs that are used in the treatment of AD are usually accompanied by harmful side effects or lack of effectiveness. Therefore, it is very necessary to investigate therapeutic medications that are based on the suppression of AD pathogenic characteristic.

In recent times, there has been a lot of focus placed on the natural ingredients and active components that herbal therapy offers as a cure for AD and other diseases [5-11]. The essential protoberberine alkaloid known as berberine has been put to extensive use in traditional medicine for hundreds of years. Berberine has been shown to have several beneficial therapeutic effects against a variety of diseases and conditions, including cancer, obesity, congestive heart failure, inflammation, atherosclerosis, neuro-degenerative diseases, rheumatoid arthritis, cardiovascular diseases, and metabolic disorders, such as dyslipidemia, impaired fasting glucose, metabolic syndrome, and diabetes [4]. These benefits have been found in clinical trials. Research conducted both *in vitro* and *in vivo* has shown that berberine has neuroprotective benefits when applied to models of stroke. Berberine was shown to alleviate behavioral functional impairment following brain ischemia generated by permanent middle cerebral artery blockage, according to a recent study. This improvement came about as a result of Berberine's ability to enhance cortical neurogenesis.

Significantly, the use of network pharmacology analysis plays a crucial role in unraveling the underlying mechanisms of medications and offers a highly efficient approach for the development of traditional Chinese medicine by examining the nodes and edges within the biological network [12]. Network pharmacology is a discipline that encompasses the identification of active components and possible therapeutic targets, the elucidation of intricate processes behind the treatment of illnesses by medications and prescriptions, and the exploration of medicinal characteristics. The present research used network pharmacology to identify the anti-AD targets of Berberine. The study included conducting PPI, gene ontology, and KEGG pathway analyses. Additionally, the association between possible target genes and disease-related genes was examined. The core target was subjected to molecular docking verification, and an assessment of the clinical importance of the core target was conducted using the GEO database in order to clarify the underlying processes of the therapeutic impact of berberine on AD.

Therefore, network pharmacology approaches may be suitable for finding the complicated mechanism of berberine. In this investigation, network pharmacology was employed to investigate the distinct anti-AD pathways possessed by berberine. Additionally, we can rule out seven pathologies involving Aβ and tau by using the AD database. The anti-AD targets associated with berberine were verified using the aid of molecular docking and high throughput technologies.

METHOD

Prediction of pharmacokinetics and toxicity

The chemical structure of berberine were sketched using ChemDraw Professional as shown in **Figure 1**. The drug-likeness properties, pharmacokinetics, and toxicity studies of the berberine was determined using in-silico pharmacokinetics web server named as SwissADME [13] and ProTox-II [12].

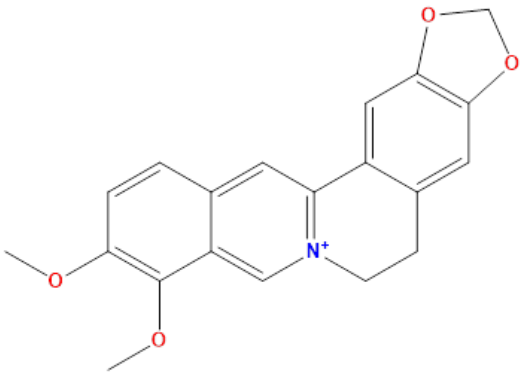

Fig. 1. Structure of Berberine

Prediction of targets related to berberine

The Swiss Target Prediction tool is a web-based application used for predicting targets of small bioactive compounds [14]. In this study, the technology was used to gather information on targets associated with berberine. The SMILES of berberine were imported into Swiss Target Prediction in order to forecast the probable targets associated with this compound. In this study, the HERB database [12] was used to get the relevant targets of berberine, including a wide assortment of pharmacological targets. Subsequently, the gene symbols corresponding to the possible target were acquired via a search conducted on the Uniprot databases [12].

Prediction targets related to berberine against AD

The electronic databases were searched and filtered using the term "AD" to identify and get information related to the AD gene. The GeneCard database [15], OMIM database [16], TTD database [12], PharmaGKB database [12], and DrugBank data [17] are all sources of information often used in academic research. Subsequently, the protein names were rectified as official names and attributed to the respective species, with the human source being specified, use the UniProt database [12].

Screening the intersection targets

We performed an intersection analysis of the targets related to the AD and berberine to clarify the correlation between the targets associated with AD and possible targets affected by berberine. Subsequently, Venn diagrams were constructed using an online application dedicated to Venn diagram visualization [12]. Subsequently, the Panther Classification System [12] was used to classify proteins associated with berberine-related anti-AD targets.

Construction and analysis of the network

The target-disease-compound network was constructed using the STRING database [18], with the species restricted to "Homo sapiens". Subsequently, the STRING results were imported into Cytoscape software 3.8.0 [19], for the purpose of visualization. The key targets were identified by using the Network Analyzer plugin to compute the degree. The top 10 targets were selected according to the degree of core targets.

Gene ontology enrichment analysis

To understand the biological meaning behind large lists of genes, gene ontology (GO) and Kyoto Encyclopedia of genes and genomes (KEGG) pathway enrichment analysis was conducted using DAVID Bioinformatic Resources [20]. The level of statistical significance was established at a threshold of $P < 0.05$ for all conducted tests.

Analysis of berberine targets related to AD pathology

Gene lists for the berberine targets associated with AD pathology were imported into the AlzData database [21], in order to determine the correlation analysis between berberine target and AD pathogenesis. Utilizing the extensive AlzData database, the gene expression analysis was carried out in order to examine the level of expression of genes in AD patients as opposed to healthy control subjects.

Molecular docking

Discovery Studio 2.0 (Accelrys, USA,) was employed for the in-silico and post docking visualization [22]. The molecular docking study was completed by a LibDock program of Discovery Studio which revealed the bioactive binding sites of receptors. In LibDock two protein site features polar and apolar are available called hotspots [23]. Further, berberine interacted with the polar and apolar sites of receptors. Additionally, to identify specific interacting residues of the receptor with a bound ligand, a 2D diagram of the docking study was carried out [24].

RESULTS AND DISCUSSION

Prediction of pharmacokinetic characteristics and toxicity related to berberine

The structure of berberine in the form of SMILES was employed in the SwissADME for the retrievation of the absorption, distribution, metabolism, and excretion (ADME) information. According to the findings, berberine is in accordance with the Lipinski rule of 5, and it is anticipated that it would have a high degree of drug-likeness. In order to conduct an analysis of in-silico toxicological parameters, the Protox-II program was used. According to the findings, berberine did not exhibit any apparent signs of toxicity, except for hepatotoxicity.

Exploring the targets related to berberine against AD

In order to investigate the potential association between berberine and AD targets, a dataset of

183 targets was acquired from SwissTargets and HERB databases. In addition, the GeneCard, DrugBank, and PharmGBK databases were searched in order to get a total of 323 disease-related targets.

On the basis of the data presented above, we were able to identify 27 targets of berberine that are connected to AD via the intersection of 183 targets associated with berberine and 323 targets related to the disease (**Figure 2a**). The information necessary to understand these intersecting targets in further depth may be found in **Table 1**. In addition, using the Panther Classification System, these 27 genes were separated into 11 unique groups (**Figure 2b**). Protein modifying enzymes made up 27.60% of all proteins, metabolite interconversion enzymes 22.5%, intercellular signal molecules 11.80%, transmembrane signal receptors 7.4%, and gene-specific transcriptional regulators 6.9% of all proteins. CASP8, CASP3, CASP8, and CASP9 are proteases; PTGS1 and NOS2 are oxidoreductases; and APP, IKBKB, GSK3B, MAPK1, SRC, and MAPK10 are nonreceptor serine/threonine-protein kinases. These enzymes belong to the categories of protein-modifying enzymes and metabolite interconversion enzymes, respectively.

Table 1. Targets related to berberine against AD

Gene	Gene name	Gene ID	Gene	Gene name	Gene ID
APP	Amyloid beta precursor protein	351	RELA	RELA proto-oncogene, NF-kB subunit	5970
BID	BH3 interacting domain death agonist	12122	TNF	Tumor necrosis factor	7124
CALM 3	Calmodulin 3	808	XBP1	X-box binding protein 1	7494
CASP3	Caspase 3	836	EGFR	Epidermal growth factor receptor	1956
CASP8	Caspase 8	841	JUN	Jun proto-oncogene	3725
CASP9	Caspase 9	842	NTRK1	Neurotrophic tyrosine kinase receptor	4914
CYCS	Cytochrome C, somatic	54205	PTGS1	Prostaglandin-endoperoxide synthase 1	5742
DDIT3	DNA damage inducible transcript 3	1649	SRC	SRC proto-oncogene	6714
GSK3B	Glycogen synthase kinase 3 beta	2932	MAPK1	Mitogen-activated protein kinase 1	5594
IKBKB	Inhibitor of nuclear factor kappa B kinase subunit beta	3551	MAPK10	Mitogen-activated protein kinase 10	5602
IL1B	Interleukin 1 beta	3553	NOS2	Nitric oxide synthase 2	4843
IL6	Interleukin 6	3569	PIK3CB	Phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit beta	74769
INS	Insulin	3630	PIK3CD	Phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit delta	18707
PTGS2	Prostaglandin-endoperoxide synthase 2	5743			

Construction and analysis of the network

Cytoscape was used to build the PPI network based on the PPI associations that were discovered and gathered (**Figure 2c**). The Network Analyzer determined the degree, and the best 8 targets were selected to serve as main targets. The proteins APP, BID, CASP3, CASP8, CASP9, CYCS, GSK3B, and TNF were prioritized as the top 8 targets. When taken together, the findings suggest that primary targets might play an important part in the therapy of AD.

The GO biological process enrichment analysis

The technique of GO enrichment analysis primarily encompasses three key factors, namely biological process (BP), molecular function (MF), and cellular component. The Gene Ontology (GO) enrichment items pertaining to biological processes (BP) are shown (**Figure 3a**), showcasing the top 20 results. The prominent keywords identified within the BP categories were a cellular response to chemical stress (GO:0062197), reaction to lipopolysaccharide

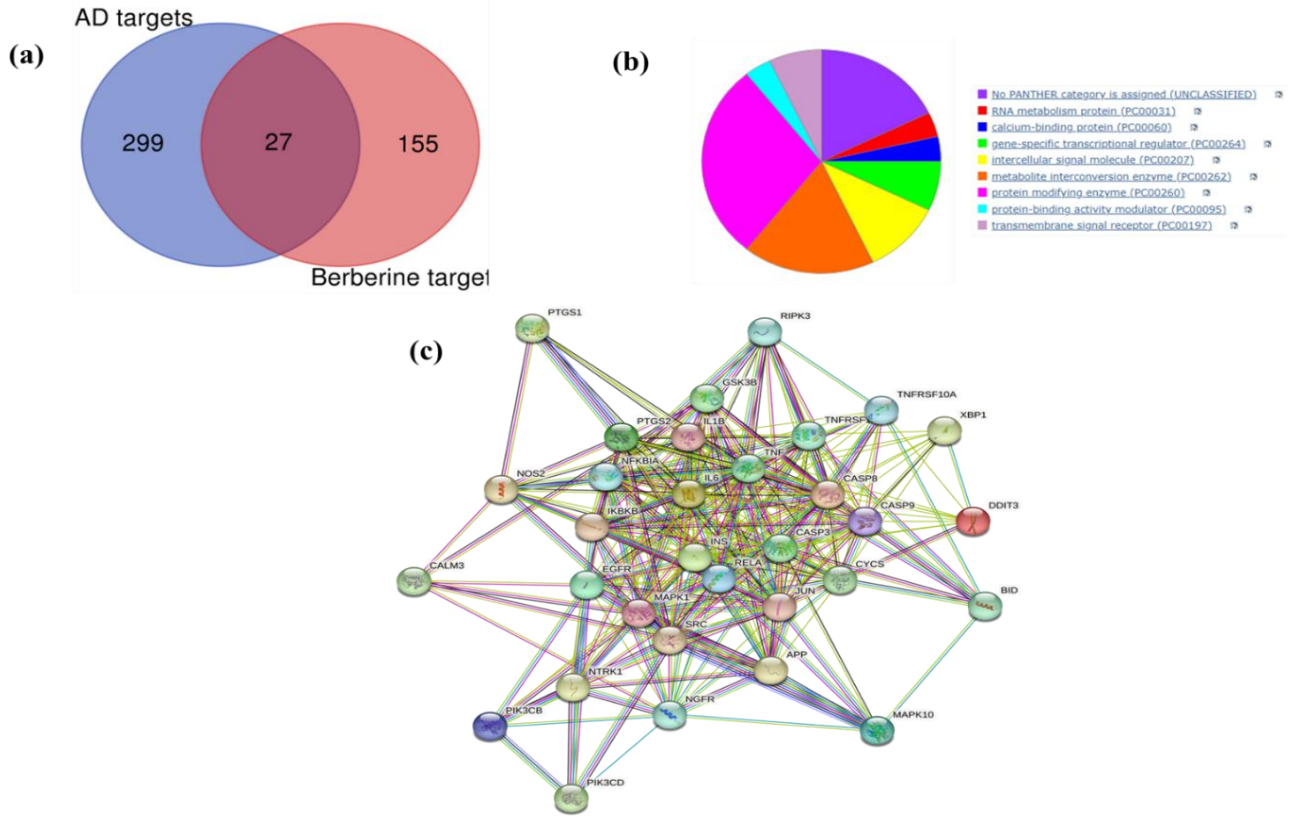

Fig. 2(a). The Venn diagram represents the overlapping region between the main targets related to berberine and AD **(b).** The targets of berberine in the context of AD were classified using the PANTHER categorization method **(c).** PPI networks of berberine in relation to targets associated with AD.

(GO:0032496), response to oxidative stress (GO:0006979), and positive regulation of protein transport (GO:0051222). The aforementioned findings demonstrate that the utilization of berberine in combating AD encompasses a diverse range of targets and is implicated in several BPs.

Analysis of KEGG pathways to investigate the possible therapeutic mechanisms of berberine in the treatment of AD

Following the completion of the KEGG analysis, a total of 163 KEGG pathways were predicted. The figure shown displays the top 10 enriched KEGG pathways (**Figure 3b**). The bulk of the sub-pathways that have been chosen pertains to Alzheimer's disease (hsa05010), lipid and atherosclerosis (hsa05417), non-alcoholic fatty liver disease (hsa04932), human cytomegalovirus infection (hsa05167), Pathways of neurodegeneration - many illnesses (hsa0502 2), and Apoptosis (hsa04210). Comprehensive results of KEGG pathway enrichment analysis were shown in **Table 2**. The mechanistic diagram of AD pathology illustrates targets within Aβ deposition process (**Figure 3c**).

Targets of berberine for treating AD: A bioinformatics study

To examine the possible targets of berberine-associated Aβ and tau pathologies, the Alzdata database was used. Out of the total of 27 targets, it was observed that 8 of them had a significant correlation with Aβ plaques and tau. Among the set of eight targets, a total of seven targets were used in the construction of the protein-protein interaction (PPI) network. Subsequently, the aforementioned targets were assessed and prioritized based on their degree score. As a result, APP, BID, CASP3, CASP8, CYCS, GSK3B, and TNF were identified and projected to be the primary targets. Significant enrichment was seen in many biological processes associated to AD pathology-related targets, including neuron death (GO:0070997), positive regulation of proteolysis (GO:0045862), positive regulation of peptidase activity (GO:0010952), and extrinsic apoptotic signaling pathway (GO:0097191), among others. The KEGG pathway enrichment analysis revealed that the AD (hsa05010) pathway had the highest level of enrichment among the target genes. In the present study, the enrichment genes are summarized in **Figure 4**.

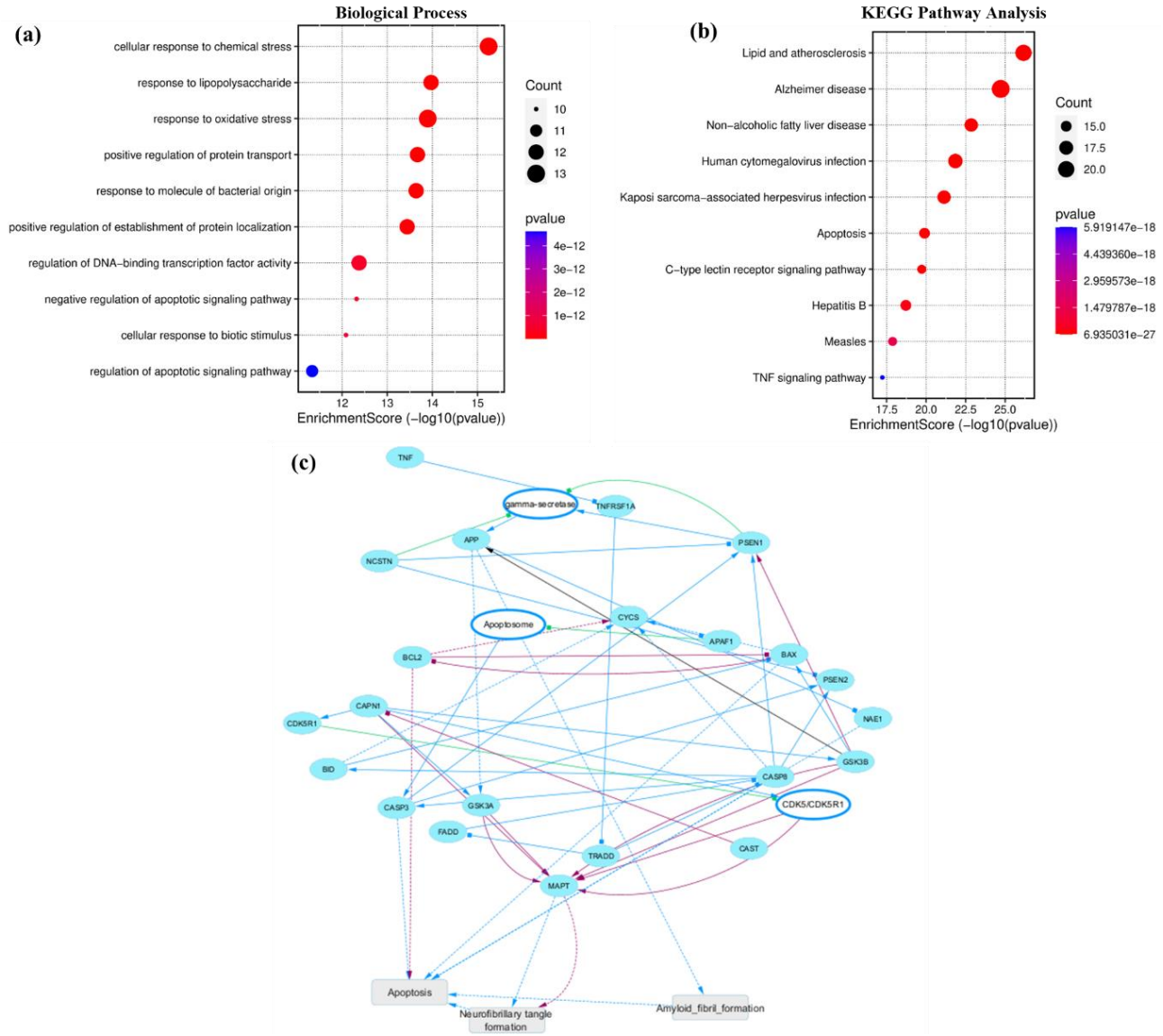

Fig. 3(a). The GO framework manifested the top 20 BPs. **(b).** The top 20 pathways exhibit in the KEGG database. **(c).** Genes implicated in AD are shown in a mechanistic diagram illustrating the pathological processes of AD.

Berberine targets for AD-related Aβ plaques and tau pathologies identified by gene expression omnibus (GEO)

In light of the potential impact of altered expression levels of target genes on the risk of AD, we conducted an additional analysis to examine whether genes associated with AD pathology exhibit differential expression patterns between AD patients and individuals without the disease. This investigation was carried out utilizing the "Differential expression" algorithm of the Alzdata database. In individuals diagnosed with AD, there was a notable upregulation of CASP3, CASP8, and TNF in the hippocampus, whereas a considerable downregulation of APP and GSK3B was seen when compared to the control group, as shown in the provided figure. In the study, it was observed that in patients with AD, there was a considerable upregulation of CASP3, CASP8, and TNF in the temporal cortex. Conversely, there was a significant downregulation of APP and GSK3B in AD patients compared to the control group, as seen in the accompanying **Figure 5**. The findings of this study suggest that the molecular targets of berberine may have a substantial impact on the development and progression of AD.

Molecular docking

In order to get a better understanding of the manner in which berberine binds to its targets involved in AD-related Aβ plaques and tau pathology, molecular docking experiments were

Table 2. The top 20 pathways of targets of berberine against AD

ID	Description	p-value	Gene ID	Count
hsa05417	Lipid and atherosclerosis	6.94E-27	CALM3/BID/CYCS/TNF/CASP9/MAPK1/IKBKB/IL1B/DDIT3/PIK3CB/PIK3CD/JUN/CASP8/MAPK10/GSK3B/RELA/IL6/SRC/XBP1/CASP3	20
hsa05010	Alzheimer disease	1.92E-25	CALM3/BID/CYCS/NOS2/INS/TNF/CASP9/MAPK1/PTGS2/IKBKB/IL1B/DDIT3/PIK3CB/PIK3CD/CASP8/MAPK10/GSK3B/RELA/IL6/APP/XBP1/CASP3	22
hsa04932	Non-alcoholic fatty liver disease	1.41E-23	BID/CYCS/INS/TNF/IKBKB/IL1B/DDIT3PIK3CB/PIK3CD/JUN/CASP8/MAPK10/GSK3B/RELA/IL6/XBP1/CASP3	17
hsa05163	Human cytomegalovirus infection	1.41E-22	CALM3/BID/CYCS/TNF/CASP9/MAPK1/PTGS2/EGFR/IKBKB/IL1B/PIK3CB/PIK3CD/CASP8/GSK3B/RELA/IL6/SRC/CASP3	18
hsa05167	Kaposi sarcoma-associated herpesvirus infection	7.37E-22	CALM3/BID/CYCS/CASP9/MAPK1/PTGS2/IKBKB/PIK3CB/PIK3CD/JUN/CASP8/MAPK10/GSK3B/RELA/IL6/SRC/CASP3	17
hsa04210	Apoptosis	1.27E-20	BID/NTRK1/CYCS/TNF/CASP9/MAPK1/IKBKB/DDIT3/PIK3CB/PIK3CD/JUN/CASP8/MAPK10/RELA/CASP3	15
hsa04625	C-type lectin receptor signaling pathway	1.9E-20	CALM3/TNF/MAPK1/PTGS2/IKBKB/IL1B/PIK3CB/PIK3CD/JUN/CASP8/MAPK10/RELA/IL6/SRC	14
hsa05161	Hepatitis B	1.92E-19	BID/CYCS/TNF/CASP9/MAPK1/IKBKB/PIK3CB/PIK3CD/JUN/CASP8/MAPK10/RELA/IL6/SRC/CASP3	15
hsa05162	Measles	1.33E-18	BID/CYCS/CASP9/IKBKB/IL1B/PIK3CB/PIK3CD/JUN/CASP8/MAPK10/GSK3B/RELA/IL6/CASP3	14
hsa04668	TNF signaling pathway	5.92E-18	TNF/MAPK1/PTGS2/IKBKB/IL1B/PIK3CB/PIK3CD/JUN/CASP8/MAPK10/RELA/IL6/CASP3	13
hsa04657	IL-17 signaling pathway	3.65E-17	TNF/MAPK1/PTGS2/IKBKB/IL1B/JUN/CASP8/MAPK10/GSK3B/RELA/IL6/CASP3	12
hsa05152	Tuberculosis	5.47E-17	CALM3/BID/CYCS/NOS2/TNF/CASP9/MAPK1/IL1B/CASP8/MAPK10/RELA/IL6/SRC/CASP3	14
hsa05142	Chagas disease	1.02E-16	NOS2/TNF/MAPK1/IKBKB/IL1B/PIK3CB/PIK3CD/JUN/CASP8/MAPK10/RELA/IL6	12
hsa05022	Pathways of neurodegeneration - multiple diseases	1.13E-16	CALM3/BID/CYCS/NOS2/TNF/CASP9/MAPK1/PTGS2/IL1B/DDIT3/CASP8/MAPK10/GSK3B/RELA/IL6/APP/XBP1/CASP3	18
hsa05160	Hepatitis C	4.33E-16	BID/CYCS/TNF/CASP9/MAPK1/EGFR/IKBKB/PIK3CB/PIK3CD/CASP8/GSK3B/RELA/CASP3	13
hsa05170	Human immunodeficiency virus 1 infection	5.58E-16	CALM3/BID/CYCS/TNF/CASP9/MAPK1/IKBKB/PIK3CB/PIK3CD/JUN/CASP8/MAPK10/RELA/CASP3	14
hsa05164	Influenza A	1.34E-15	BID/CYCS/TNF/CASP9/MAPK1/IKBKB/IL1B/PIK3CB/PIK3CD/CASP8/RELA/IL6/CASP3	13

hsa05135	Yersinia infection	3.95E-15	TNF/MAPK1/IKBKB/IL1B/PIK3CB/PIK3CD/JUN/MAPK10/GSK3B/RELA/IL6/SRC	12
hsa04620	Toll-like receptor signaling pathway	8.51E-15	TNF/MAPK1/IKBKB/IL1B/PIK3CB/PIK3CD/JUN/CASP8/MAPK10/RELA/IL6	11

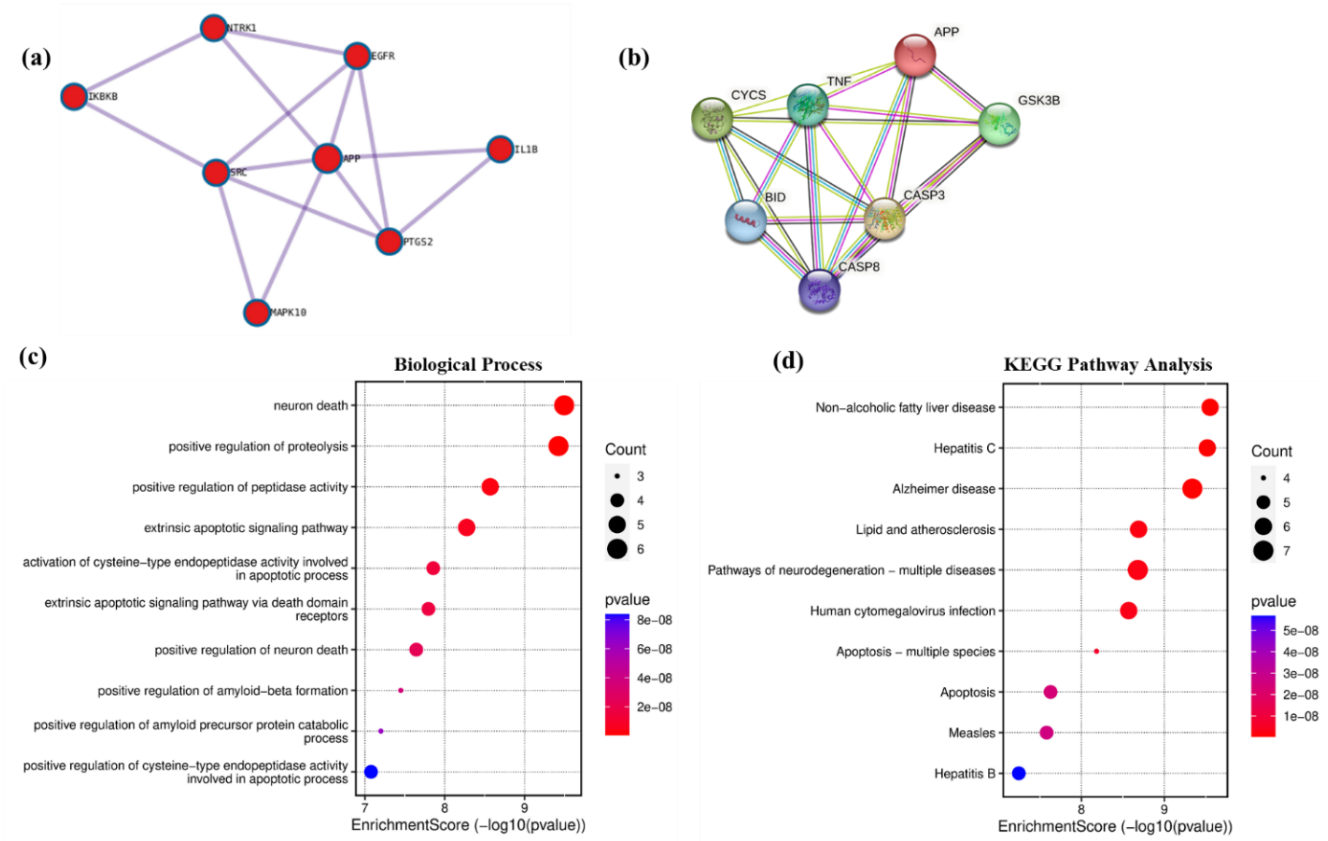

Fig. 4(a). Berberine-related targets significantly associated with Aβ plaques and tau **(b).** PPI networks of berberine-related targets linked with Aβ plaques and tau **(c).** GO enrichment analysis of berberine-related targets linked with Aβ plaques and tau **(d).** KEGG pathway enrichment analysis of berberine-related targets linked with Aβ plaques and tau.

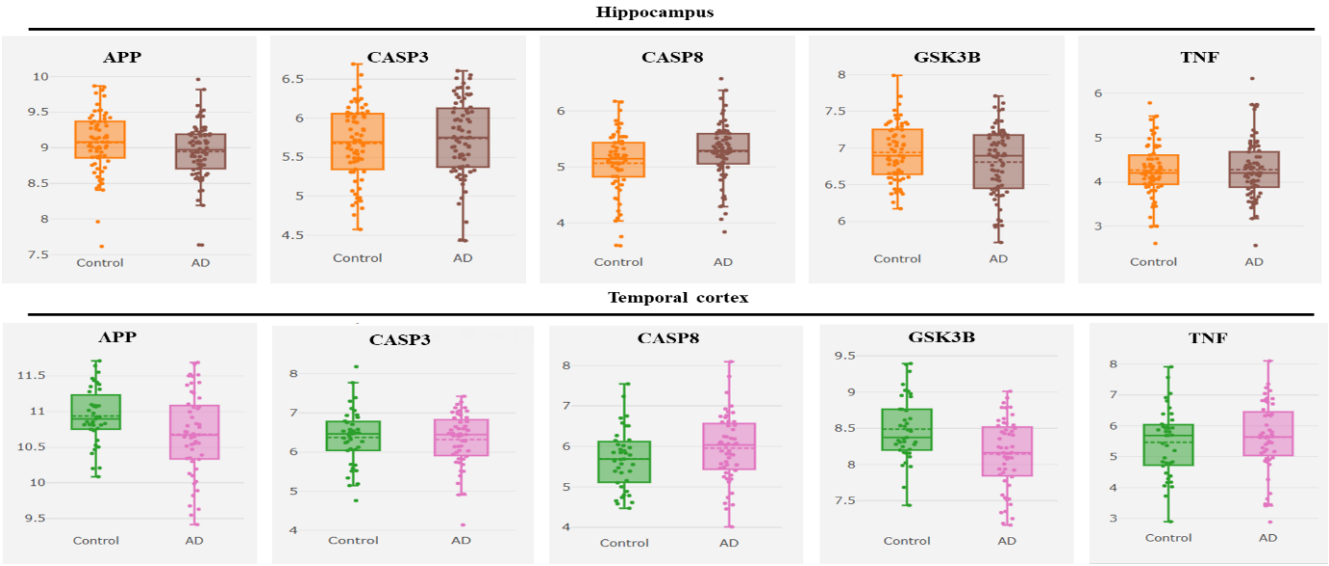

Fig. 5(a-e). Differential gene expression compared to the control group in the hippocampus **(f-j).** Differential gene expression compared to the control group in the temporal cortex

conducted. The molecular docking analysis suggested that the berberine showed the highest docking score 98.96 Kcal/mol and potential binding interactions with CASP3 protein as shown in **Table 3**. Similarly, other macromolecules including CASP8, GSK, TNF, and APP showed good docking scores 84.36, 88.90, 95.61, and 91.23 Kcal/mol respectively.

Table 3. Molecular docking

Ligand	Target	Docking score	Amino acid residues	H-bond distance
Berberine	CASP3	98.96	C-Ile160 O-Glu167 C-Glu167	3.48788 3.09238 3.54727
Berberine	CASP8	84.36	O-Gln286 C-Thr262	2.83983 3.39618
Berberine	GSK	88.90	C-Gly202 C-Asn95 C-Gln89	3.58008 3.53704 4.12414
Berberine	TNF	95.61	C-Tyr151 C-Tyr151 C-Gly121	3.66956 3.57538 3.19987
Berberine	APP	91.23		

Results of molecular docking analysis also manifested that berberine was bound to CASP3 via Ile160, Glu167, and Glu167 with hydrogen bond distances of 3.48788 3.09238, and 3.54727 Å, respectively as shown in **Figure 6a**. Berberine was bound to CASP8, forming two hydrogen bonds with Gln286 and Thr262 via 2.83983 and 3.39618 Å respectively (**Figure 6b**). Berberine also showed binding interactions with the GSK enzyme through three hydrogen bonds interactions Gly202, Asn95, and Gln89 (**Figure 6c**). Berberine bound with TNF by Tyr151, and Gly121 with 3.66956, 3.57538 and 3.19987 Å (**Figure 6d**).

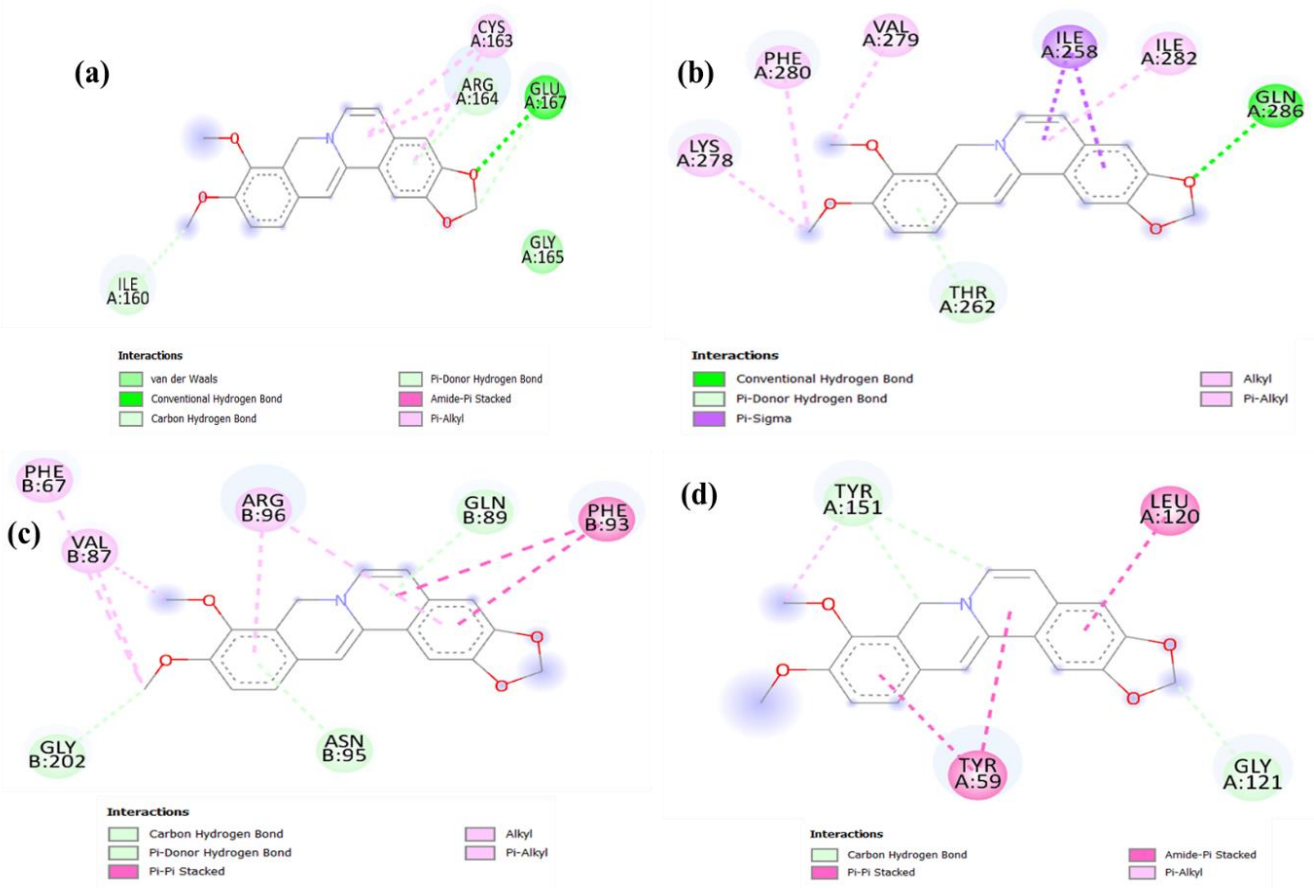

Figure 6(a). Binding interactions of berberine with CASP3 **(b).** Binding interaction of berberine with CASP8 **(c).** Binding interactions of berberine with GSK **(d).** Binding interactions of berberine with TNF

DISCUSSION

In this research, we used network pharmacology and database mining to investigate the processes and molecular targets of berberine in AD mechanisms. Network pharmacology is a discipline that relies on the examination of network models and systems biology [12]. It encompasses a complete approach that integrates classical pharmacology, bio-informatics, chemoinformatics, and network biology [25]. Berberine, a member of the protoberberine group of benzylisoquinoline alkaloids, is a quaternary ammonium salt that has been used for several therapeutic purposes over an extended period. Over the last 10 years, a significant body of scientific study has shown the compound's capacity to regulate several cellular targets, hence exhibiting promising preventative and therapeutic effects in the treatment of diverse illnesses. Berberine exerts its effects on a wide array of molecular targets, including transcription factors, growth factors and their corresponding receptors, cytokines, enzymes, and genes that govern cellular proliferation and death, as well as inflammatory factors [26].

Numerous preclinical investigations have provided compelling evidence demonstrating the therapeutic potential of berberine in various central nervous system disorders, including AD, cerebral ischemia, depression, schizophrenia, epilepsy, and anxiety [27]. It is important to note, however, that these experimental findings have solely been derived from animal models [28]. As a result, berberine was chosen for all following tests.

In the current investigation, the first objective was to ascertain the pharmacokinetic characteristics and toxicity profile of berberine by using computerized databases. The compliance of berberine with the Lipinski rule of 5, as well as its possession of a higher LD_{50} value, was noteworthy. Previous studies in the literature have shown that the intraperitoneal injection of berberine in rats has resulted in an LD_{50} value of 250 mg/kg, a finding that aligns well with the anticipated outcome as indicated by computerized databases. Subsequently, a comprehensive set of 27 targets associated with AD were identified as being affected by berberine. The berberine-AD protein-protein interaction (PPI) network was analyzed to identify the top 10 targets based on its degree. The resulting targets are APP, BID, CASP3, CASP8, CASP9, CYCS, GSK3B, TNF, IKBKB, and IL1B. The highest-ranking position was held by APP. According to genetic and behavioral studies, it is indicated that the physiological process of generating neurotoxic Aβ peptide via successive APP proteolysis plays a critical role in the progression of AD. The APP is a transmembrane protein that is abundantly expressed in the brain. It undergoes a rapid and intricate metabolic process involving a series of sequential proteases. One of these proteases is the intramembranous γ-secretase complex, which is also responsible for processing another important regulatory molecule. Caspases play a crucial role in the process of programmed cell death known as apoptosis. They may be categorized into two types: initiator caspases (such as caspase-3, caspase-8, and caspase-9) that are responsible for both transmitting early apoptotic signals and carrying out the last steps of cell death.

Following the KEGG enrichment analysis, it was shown that the majority of the enriched KEGG pathways were mostly focused on the AD signaling pathway. The proteins BID, CASP3, CASP8, CASP9, CYCS, GSK3B, TNF, IKBKB, and IL1B are implicated in the cleavage of amyloid precursor protein (APP), along with the involvement of the β-secretase component BACE1. Glycogen synthase kinase 3 alpha (GSK3A) and glycogen synthase kinase 3 beta (GSK3B) were chosen because to their role in regulating the generation of amyloid-beta (Aβ) *via* the phosphorylation of amyloid precursor protein (APP) and proteins within the γ-secretase complex. Significant enrichment was seen in the BP and GO categories for cellular response to chemical stress (GO:0062197), reaction to lipopolysaccharide (GO:0032496), response to oxidative stress (GO:0006979), and positive regulation of protein transport (GO:0051222). This observation suggests that berberine may potentially exhibit anti-AD effects *via* many routes and diverse biological processes.

Subsequently, a total of eight targets of berberine against tau and Aβ pathology associated with AD were acquired *via* the use of the AlzDatabase. In our analysis, we specifically examined targets associated with the AD signaling cascade, including CASP3, CASP8, GSK, TNF, and APP. Caspase, namely CASP3 and CASP8, plays a crucial role in the regulation of many cellular death and inflammatory signaling pathways. The first characterization of CASP8 was its identification as a crucial element in the

extrinsic apoptotic pathway, which operates by means of interactions between death receptors and their corresponding ligands, such as TNF. Following the activation of death receptors, adaptor proteins and procaspase are recruited, facilitating the dimerization and auto-processing of procaspases into their active form, CASP8. Subsequently, CASP8 initiates apoptosis by activating CASP3, which ultimately leads to the execution of programmed cell death. The phenomenon of increased expression of death receptors has been documented in individuals diagnosed with AD. Previous research has shown the existence of active variants of CASP3 and CASP8 inside the cerebral regions of individuals diagnosed with AD. Multiple investigations have shown that the atypical generation of Aβ plaque and neurofibrillary tangles induces neuronal harm and inflammation, ultimately leading to the activation of PI3K. Akt phosphorylation is activated, leading to the inhibition of GSK-3β phosphorylation by Aβ1-42, hence protecting neuronal cells inside the brain [29]. On study indicated that the administration of berberine at a concentration of 0.5 μM effectively inhibited the production of TNF-α and MCP-1 caused by Aβ [30]. This inhibition was achieved by suppressing the activation of NF-κB and blocking the PI3K/Akt and MAPK pathways. The accumulation of Aβ may arise due to aberrant processing of APP in AD [31]. The administration of berberine at a dosage of 100 mg/kg by oral gavage for 4 months resulted in a reduction in levels of APP C-terminal fragment, as well as a decrease in APP and tau hyperphosphorylation [32]. These effects were mediated through the Akt/GSK-3 pathway in rats with AD. Further, it was shown that berberine can regulate the production of Aβ via activating the AMPK pathway in N2a mouse neuroblastoma cells. In addition, increased levels of Aβ may facilitate the occurrence of synaptic loss and dysfunction, which are recognized as further pathological indicators of AD. This is achieved by the specific targeting of mitochondria. Moreover, the administration of berberine at a concentration of 1 μM before treatment showed a mitigating effect on axonal mitochondrial abnormalities. This was achieved by maintaining the mitochondrial membrane potential and preventing a decrease in ATP levels. Additionally, berberine treatment increased both the density and length of axonal mitochondria, as well as an improvement in mitochondrial motility and trafficking. These findings were observed in cultured hippocampal mouse neurons treated with 0.5 μM Aβ. Moreover, the impairment of memory and reward systems in AD may be attributed to both neuronal death and cerebral blood flow.

Presently, our examination of GEO datasets has shown a reduction in the expression of CASP3, CASP8, GSK, and TNF in the hippocampus of the AD cohort. The findings suggest that the CASP/GSK/TNF pathway may serve as a potential therapeutic target for berberine in the context of AD. The molecular docking analysis revealed that berberine had a favorable docking affinity with CASP3, GSK, and TNF, resulting in the formation of three hydrogen bonds. Subsequently, berberine displayed a docking interaction with CASP8. The results of molecular docking analysis indicate that berberine exhibits a significant affinity for the expected target protein. Furthermore, it is shown that the therapeutic benefits of berberine against AD are strongly associated with the interaction between berberine and these specific target proteins. This finding provides further evidence that berberine exhibits stable binding affinity to the protein receptor expressed by the core target gene, hence facilitating its therapeutic efficacy in the treatment of AD. The obtained findings have successfully confirmed network pharmacology analysis conducted. These identified components may serve as a foundation for comprehending the mechanisms behind the pathogenesis of AD in future investigations, as well as being potential targets for the development of therapeutic interventions.

CONCLUSION

In this work, an analysis was conducted to investigate the mechanisms by which berberine acts on AD. Utilizing a network pharmacology approach, the primary targets and pathways associated with the therapeutic effects of berberine against AD were found. The findings of our study indicate that GSK3B and NFKB1 may be considered promising targets for the therapy of AD. Additionally, our results demonstrate that berberine has the potential to effectively alleviate the pathological characteristics of stroke via a multifactorial, multitarget, and multi-pathway approach. The findings of this study will provide more robust data to inform future clinical decision-making.

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