REVIEW ARTICLE

Exploring the Potential Targets and Protective Agents in Mercury-Induced Anxiety Disorders Using Network Toxicology, Network Pharmacology, and Molecular Docking

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Received: 9 Aug 2025
Accepted: 19 Aug 2025
Published: 15 Sep 2025

Keywords:

Anxiety disorders
Mercury
Toxicogenomics
Network toxicology
Network pharmacology
Molecular docking

ABSTRACT

Studies show that anxiety is one of the most common symptoms of mercury poisoning. The mechanism of mercury toxicity is not known in detail. This study aimed to broaden our understanding of mercury-induced anxiety and suggest potential protective agents. A list of genes associated with anxiety was extracted from the overlap between GeneCards, DisGeNET, and Diseases to decipher protein-protein interaction (PPI) and core sub-network. The comparative toxicogenomic database (CTD) helped to identify core mercury targets in anxiety disorders. Detailed interactions and relevant functions were obtained through GeneMANIA. Network pharmacology and molecular docking approaches identified potential protective agents-mercury target interactions. Among the eight proteins in the anxiety-related core sub-network (IL1B, IL6, TNF, IFNG, STAT3, TP53, EP300, and ESR1), the top-ranked proteins IL1B, IL6, TNF, and IFNG were revealed as core mercury targets with key interactions in disrupting the inflammatory responses and interfering with cellular processes. GeneMANIA highlighted the functions of CASP1, TNFAIP3, and SQSTM1 as first neighbors of the core mercury targets. Quercetin, selenium, curcumin, and glutathione were specified as factors that target the most mercury-responsive genes. Molecular docking revealed strong binding affinities between protective agents (quercetin and curcumin) and core target proteins. This study presented a network biology approach in toxicology and pharmacology to further understand the mechanism of mercury toxicity and its therapeutic solution for anxiety disorders.

How to cite this article: Farahani M, Bagheri F, Rezaei-Tavirani M, Fateminasab F. Exploring the Potential Targets and Protective Agents in Mercury-Induced Anxiety Disorders Using Network Toxicology, Network Pharmacology, and Molecular Docking. Iran J Child Neurol. 2025;19(4): 1-14. https://doi.org/10.22037/ijcn.v19i4.49672.

Introduction

Anxiety is a psychiatric disorder that affects approximately 15% of the world's population (1). This response relates to anticipated future threats (2). The common symptoms of anxiety are sadness and excessive worrying, which negatively disrupt daily activities and quality of life (1). Physical changes, such as blood pressure, are associated with. Heavy metals, as well-known environmental pollutants, are one of the most critical risk factors for human health (3, 4, 5). According to the Agency for Toxic Substances and Disease Registry (ATSDR), mercury (Hg) ranks as the third heaviest metal in terms of toxicity frequency and the potential risk for human exposure (6). Mercury is released from natural phenomena such as volcanic eruptions, industrial activities, exposed water, and soil. Moreover, marine food and dental amalgam fillings

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can also cause mercury toxicity (7). Many studies have shown that mercury is a nonessential metal for human biological and physiological functions (8), even though the metals Iron (Fe), Magnesium (Mg), and Copper (Cu) are essential nutrients (2). Mercury exposure disturbs the digestive, renal, and nervous system functions (9). Depending on the level of exposure, it may cross the blood-brain barrier (BBB), reach the central nervous system (CNS), and accumulate in tissues, culminating in oxidative stress and changes in brain structure (10, 11). These damages are divided into two groups: Acute exposure-related and chronic-related damages. Acute exposure-related damages cause general physiological modifications; however, chronic exposure-related damages result in anxiety and memory problems (11).

A complex relationship exists between human exposure to heavy metals and nervous system disorders (12). Studies indicate that network toxicology can help understand the toxicological mechanisms of harmful chemical and biological agents from a network perspective and decipher the gene-environment interactions. The toxicological network can be considered as the crucial basis for further toxicological studies (13, 14). Therefore, the present study aimed to explore the underlying mechanisms and core targets in mercury-induced anxiety disorder through a network toxicology approach. In addition, by combining network toxicology with network pharmacology, this study reveals potential protective agents against mercury-gene interactions in anxiety disorders. Finally, molecular docking analysis was used to evaluate the binding stability of protective agents against core mercury targets. Using a network biology approach provided a systematic understanding of the relevant mechanisms.

Materials & Methods

Collecting data related to anxiety disorder and mercury-responsive genes

Anxiety disorder candidate genes were collected from three public databases, including GeneCards (15), DisGeNET (16), and DISEASES (17). Then, a list of common genes between the three databases was obtained using a Venn diagram (https://www.bioinformatics.org/gvenn) and considered as genes associated with anxiety disorder for further analysis. Mercury-gene interactions were extracted from the comparative toxicogenomic database (CTD) (18) and considered mercury-responsive genes. Finally, common genes between the list of anxiety candidate genes and mercury-responsive genes were detected and introduced as mercury target genes in anxiety disorder induction to discern new insights into its detailed toxicity mechanisms.

Functional analysis of mercury-responsive genes in anxiety disorder

Gene ontology (GO) analysis, as a bioinformatics approach, provides a framework of concepts to unify gene representation and describe the function of gene products across organisms (19). The Kyoto Encyclopedia of Genes and Genomes (KEGG) is a bioinformatics resource for systematically investigating high-level links between gene functions (20). DAVID (21) was used as a functional annotation tool to identify and interpret gene ontology biological processes (BPs) and KEGG pathways of the mercury-responsive genes in anxiety disorder (p-value <.05 and enrichment gene count >2).

Constructing the protein interaction network of genes involved in anxiety disorders

The protein-protein interaction information of anxiety disorder-related genes was extracted from the STRING database with a confidence score of ≥ 0.9 (22). The resulting data was imported into the Cytoscape software to build a network and analyze its scaling and topological properties (23).

Discovering the core sub-network in the anxiety network and identifying mercury targets

To find the critical protein sub-network in the whole PPI network, the CytoNca and CytoHubba plugins were used in the Cytoscape software (24, 25). First, to build the initial sub-network by CytoNca, six network topological parameters, including Degree, Betweenness, Closeness, Eigenvector, LAC, and network, were considered. Scores above the median value of each parameter were used to filter the initial critical gene list. This screening method was re-run on the initial results, and the final sub-network of the CytoNca plugin was introduced. The second method to find the critical sub-network in the PPI network was to use the CytoHubba plugin. In this way, the MCC parameter was chosen to analyze the top 17 genes (based on the result of method 1) in the PPI network. Finally, common genes between the results of the two methods were selected to determine the critical PPI sub-network (3, 26). Subsequently, shared genes between the critical sub-network associated with anxiety disorder and the list of mercury targets were identified.

Functional analysis of core sub-network and mercury targets

The extracted sub-network was functionally analyzed using the DAVID resource tool, including

gene ontology and KEGG pathway analysis with a p-value <.05. The GeneMania web server (27) was used to predict more details about the interactions and find related genes to mercury targets in the anxiety core sub-network. The gene list of the critical sub-network is given to this server as a query, and the server can add new genes to the sub-network and evaluate protein interactions in terms of physical, functional, co-expression, genetic, shared protein domains, and co-localization.

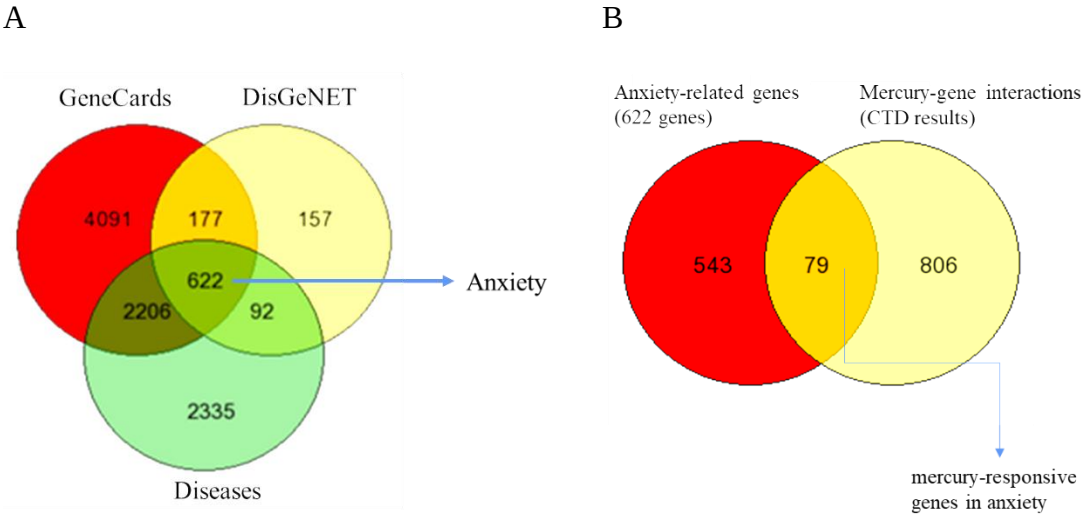


Fig.1. A. Data related to anxiety disorder. B. mercury-responsive genes in anxiety.

Identifying protective factors against mercury-induced anxiety

First, potential protective factors for mercury toxicity were collected using a review of existing resources. Then, information related to protective factors-gene/protein interactions was obtained using the CTD database. CTD is known as a robust and public database that aims to enhance understanding of the mechanisms of environmental effects on human health (18). Protective agent-mercury target interactions were screened based on CTD results to identify potential protective agents against mercury-induced anxiety. In the case of protein-based protective factors, the first relevant interactions were extracted from the STRING (v.12.0) database (22). This study used the option "add more nodes to the current network' and expanded the network. Then, the common proteins between the list of mercury targets and interacting proteins with protein protective factors were extracted.

Construction of protective factor-protein interaction network

A protective factors-mercury targets interaction network was constructed for anxiety disorder using Cytoscape. Factors with more than ten targets were considered to build a protective factor-protein interaction network. Subsequently, protective agent-mercury target interactions in the core sub-network and GeneMANIA network were screened to find potential candidate genes for therapeutic targeting and to investigate treatment effects. In addition, using network pharmacology analysis, high-ranking protective factors were selected for functional annotation and enrichment.

Molecular docking technology

This study employed the molecular docking technique to confirm the interaction between drug candidates and core targets. The 3D molecular structures of compounds were obtained from the PubChem chemical compound library (https://pubchem.ncbi.nlm.nih.gov) (28). The obtained structures were optimized and minimized via the Gaussian09 software package at B3LYP/6-311+G* level of theory (29). The 3D structures of target proteins were obtained from the RCSB database (https://www.rcsb.org/) (30). The rigid (receptor): flexible (ligand) docking was performed by the AutoDock 4.2 molecular docking program, which utilizes an empirical free energy function and the Lamarckian Genetic Algorithm (LGA). The Gastieger charges were computed and added to the macromolecule input files, and AutoGrid was applied to calculate grids (31).

A

B

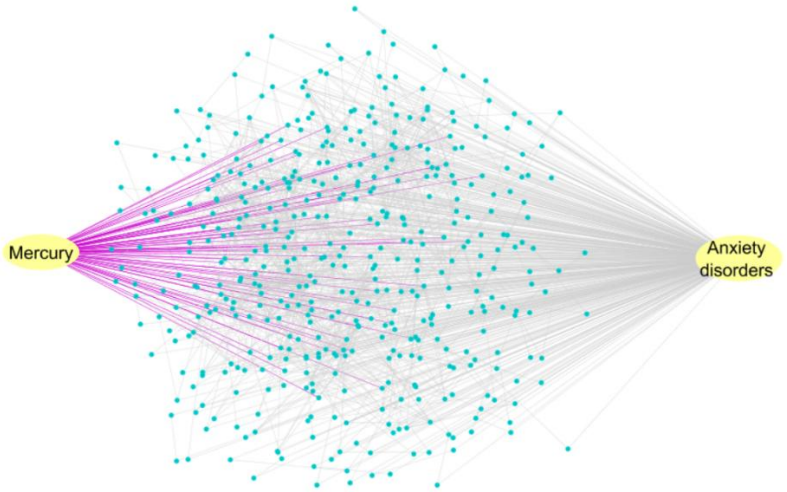
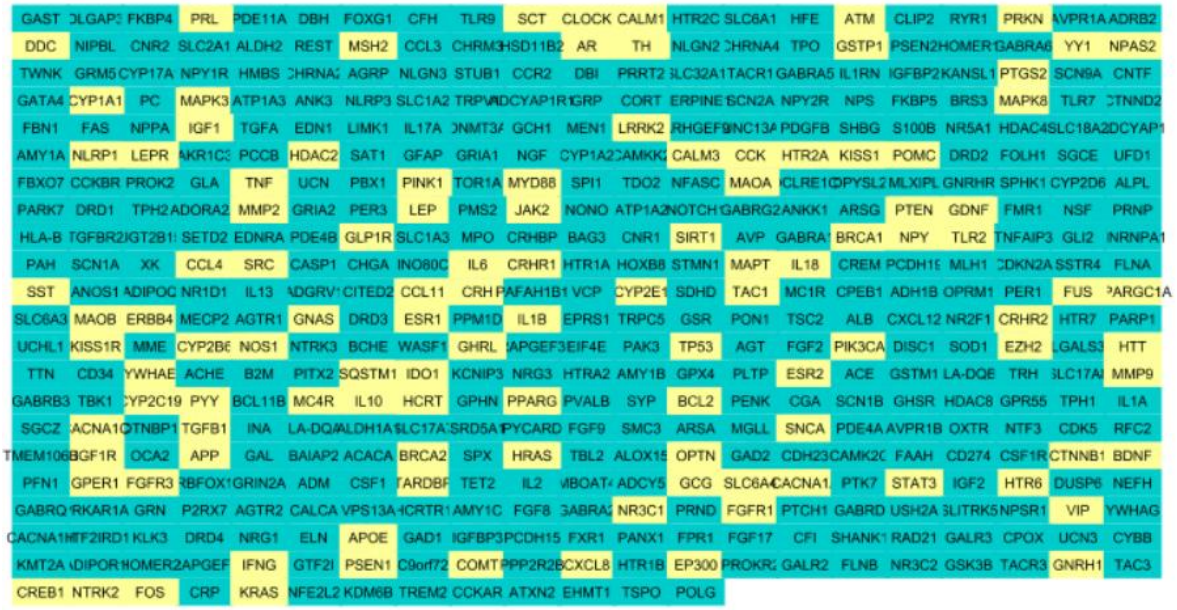
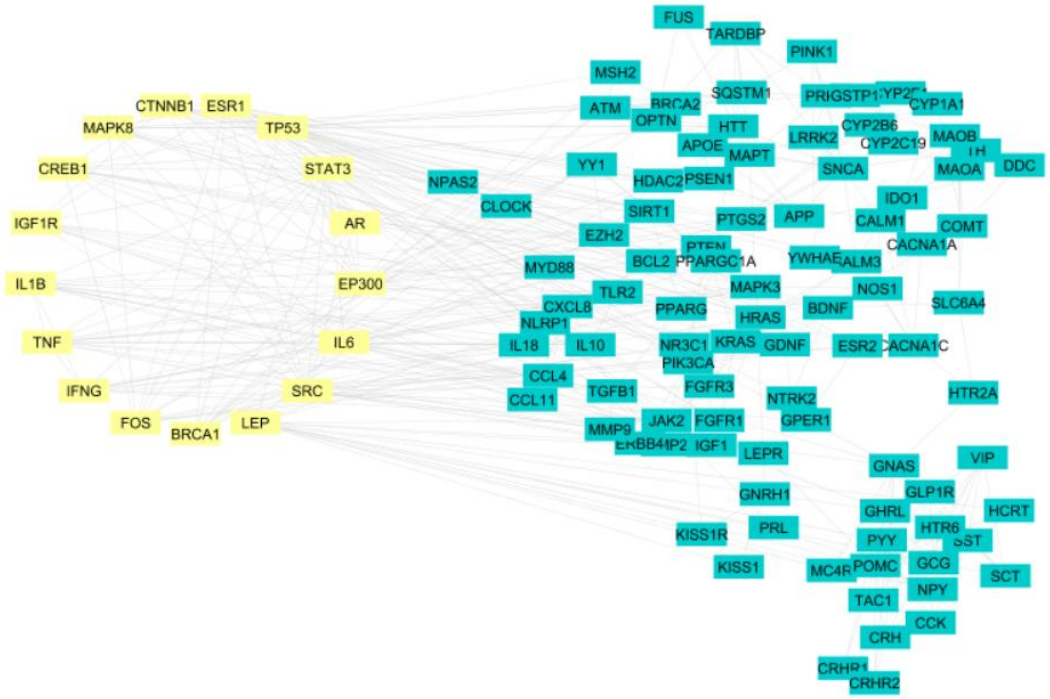


Fig. 2. A. Anxiety protein-protein interaction network; nodes: 454, edges: 1188, with a confidence score ≥ 0.9. B. Network toxicology: Mercury-anxiety interactions in purple edges.

A



B


FUS
TARDBP
PINK1
MSH2
SQSTM1
PRIGSTP1
CYP1A1
CTNNB1
ESR1
ATM
BRCA2
OPTN
HTT
CYP2B6
LRRK2
CYP2C19
MAOB
MAPK8
TP53
APOE
MAPT
TH
CREB1
STAT3
YY1
HDAC2
PSEN1
SNCA
MAOA
DDC
NPAS2
CLOCK
IDO1
SIRT1
PTGS2
APP
CALM1
COMT
IGF1R
AR
EZH2
PTEN
CACNA1A
BCL2
PPARGC1A
YWHAE
ALM3
MYD88
IL1B
EP300
TLR2
MAPK3
NOS1
CXCL8
PPARG
BDNF
SLC6A4
NLRP1
HRAS
TNF
IL6
IL18
IL10
NR3C1
KRAS
GDNF
ESR2
CACNA1C
PIK3CA
CCL4
IFNG
SRC
FGFR3
HTR2A
CCL11
TGFB1
NTRK2
FOS
LEP
JAK2
FGFR1
GPER1
BRCA1
MMP9
IGF1
LEPR
GNAS
VIP
GNRH1
GLP1R
GHRL
HCRT
KISS1R
PRL
HTR6
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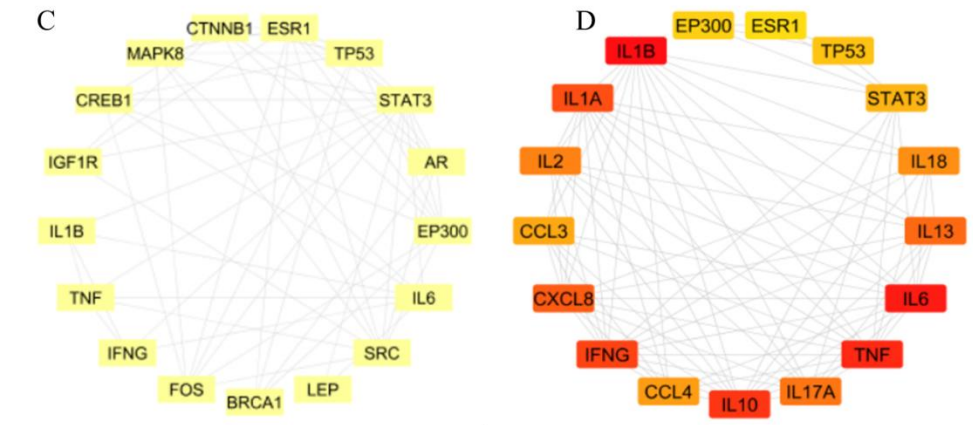
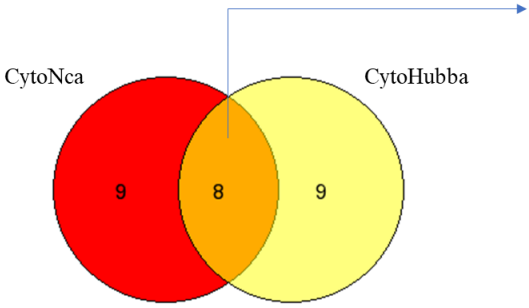


Fig. 3. A. Screening 1 of the anxiety-PPI network (yellow nodes) through CytoNca. B. Screening 2 of the Screening 1 results through CytoNca (yellow nodes). C. Critical Sub-network constructed after two-step screening via CytoNca, 17 nodes and 57 edges. D. Critical sub-network of top 17 nodes by CytoHubba, 17 nodes and 80 edges.



Rank	Name	Mercury target
1	IL1B	✓
2	IL6	✓
3	TNF	✓
4	IFNG	✓
5	STAT3	×
6	TP53	×
7	EP300	×
8	ESR1	×

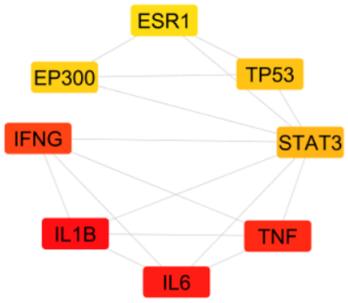


Fig. 4. Shared genes between two key sub-networks and final critical protein sub-network, ٨ proteins and ١٦ interactions. Four top-ranked nodes (IL1B, IL6, TNF, and IFNG) are mercury-responsive targets.

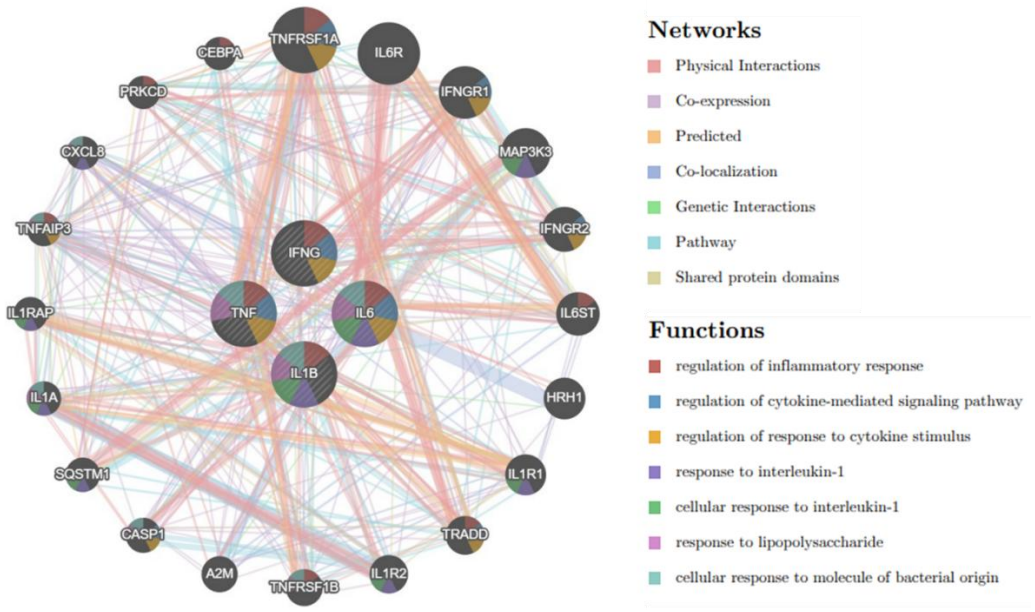
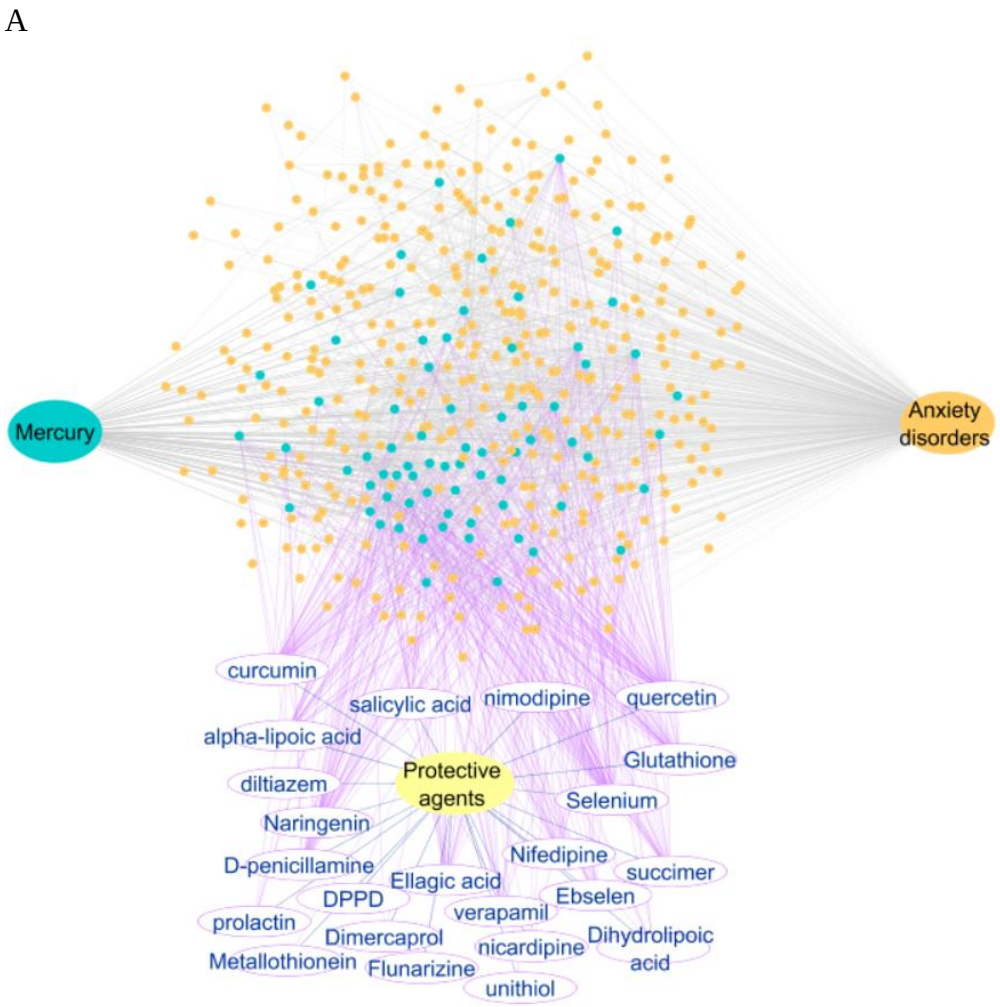


Fig. 5. Key mercury-responsive targets in the core sub-network (IL1B, IL6, TNF, and IFNG) and the GeneMANIA predicted related proteins (20 proteins) and their functional analysis.



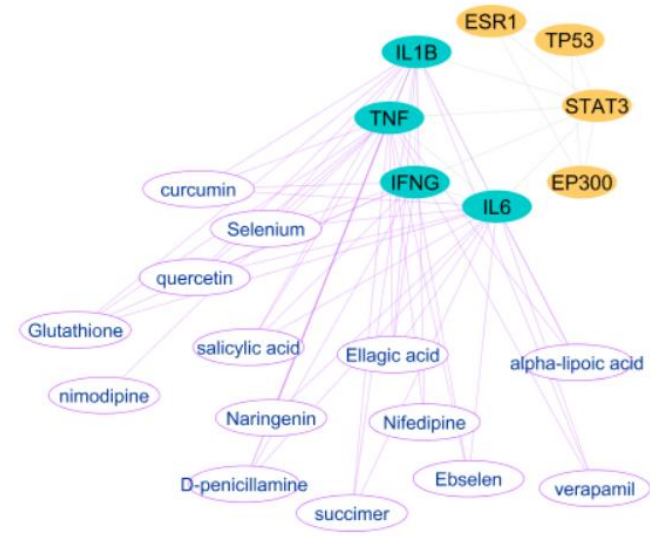
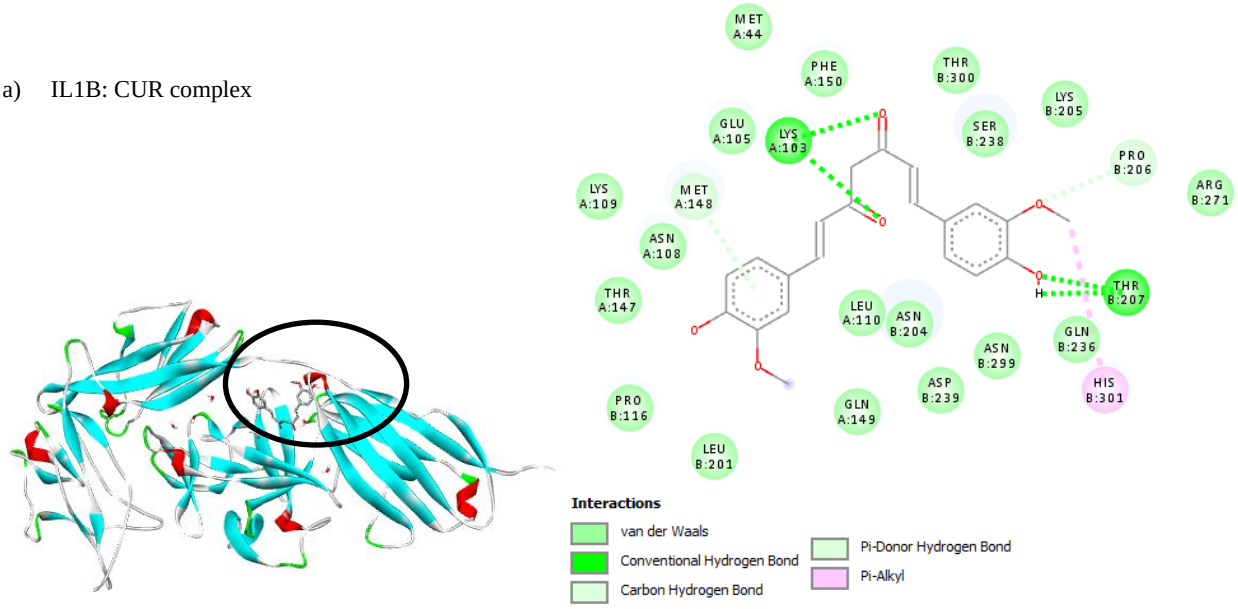


Fig. 6. A. Protective agent-target interactions against mercury-induced toxicity in anxiety. quercetin, selenium, curcumin, and glutathione target the maximum number of mercury-responsive genes (58, 45, 42, and 38 nodes, respectively). B. Protective agent-core target interactions.

a) IL1B: CUR complex



b) IL1B: QUR complex

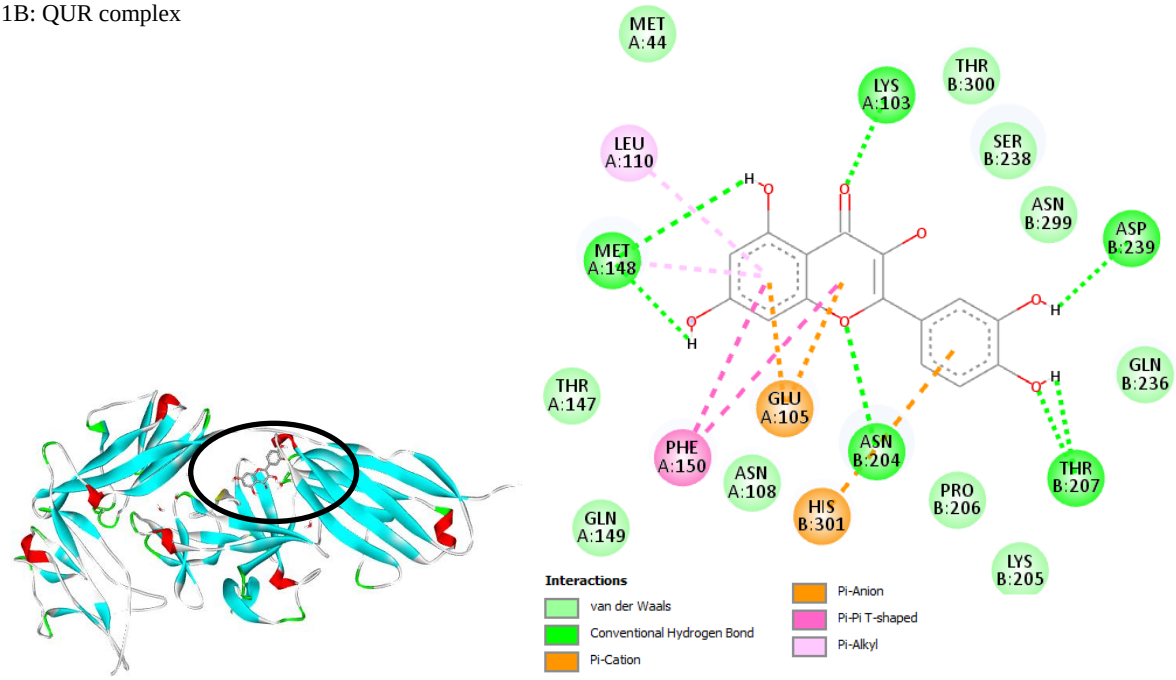


Fig. 7. The complex of IL1B with A) Curcumin, and B) Quercetin. The H-bond interaction is depicted with a green dotted line, and the hydrophobic interaction is depicted with a semicircle**.**

Results

Mercury targets screening and functional annotation in anxiety disorder

At first, 622 common genes for anxiety disorders were identified between the GeneCards, DisGeNET, and Diseases databases, as depicted in Fig.1.A. The overlap of genes between the three databases was presented in a Venn diagram. In addition, 885 mercury-target interactions were retrieved from the CTD database. Fig.1.B. illustrates the overlap of 79 genes between the anxiety-related genes and CTD results. The 79 mercury-responsive genes in anxiety were subjected to the DAVID resource for annotation and functional enrichment. Tables 1 and 2 show the top functional terms. Gene ontology analysis revealed that BP terms of the 79 genes were enriched in inflammatory response and positive regulation of gene expression as the top GO terms (Table 1). KEGG pathway enrichment analysis showed lipid and atherosclerosis, Chagas disease, and pathways in cancer as the top KEGG terms (Table 2).

PPI network structure and core targets

To further decipher the mechanisms of mercury-induced anxiety disorder, the present study built a PPI network for anxiety-related genes and specified mercury-target interactions. Protein interactions were obtained by mapping 622 genes to the STRING database with a confidence score ≥0.9. The interactions were imported into the Cytoscape software for network construction, visualization, and analysis. The resulting network consisted of 454 nodes (protein) and 1188 edges (interaction) and revealed a scale-free pattern with a network degree distribution R-squared value of 0.878 (Fig. 2. A). Figure 2. B revealed that 71 of the 79 mercury targets have protein interactions. ABCB1, ALDH5A1, CADM2, CPZ, ITIH1, ITIH3, LIPA, and SERPING1 did not show any interactions and were absent in the network. Two core sub-networks, consisting of 17 proteins in the total protein network, were screened using CytoNca and CytoHubba (Fig. 3. A-D). The Venn diagram tool was used to find overlaps between the two core sub-networks (Fig. 4). IL1B, IL6, TNF, IFNG, STAT3, TP53, EP300, and ESR1 were identified as the intersection of the two key sub-networks and presented a final core sub-network associated with anxiety disorder. These eight genes were ranked based on their importance in the sub-network, and the first four were identified as mercury targets, including IL1B, IL6, TNF, and IFNG (Fig. 4). Table 1 shows that these four genes are simultaneously enriched in the positive regulation of gene expression GO term. Furthermore, Table 2 indicates the simultaneous presence of these four genes in the KEGG pathways of Chagas disease, Inflammatory bowel disease, IL-17 signaling pathway, and Rheumatoid arthritis.

Table 1. Gene Ontology (BP, biological process) analysis of mercury-responsive genes in anxiety disorder. The top 10 terms are displayed.

GOTERM_BP_DIRECT	Genes	*P*-Value
GO:0006954; inflammatory response	CRP, TGFB1, CXCL8, IL13, TNFAIP3, ADM, FOS, PARK7, TNF, IL6, IL1B, GPER1, CCL3, NLRP3, NLRP1, TAC1, CCR2, NFE2L2	7.90E-13
GO:0010628; positive regulation of gene expression	CRP, APP, TGFB1, CXCL8, NTRK3, IL13, PARK7, IGF1, NGF, FGF2, TNF, SLC6A4, IL6, IFNG, IL1B, GPER1, CCL3, NFE2L2	1.30E-11
GO:0050729; positive regulation of inflammatory response	APP, TGFB1, IFNG, IL1B, CASP1, CCL3, NLRP3, NLRP1, TNF, IL2, CCR2	2.20E-11
GO:0032731; positive regulation of interleukin-1 beta production	APP, IL6, IFNG, CASP1, CCL3, NLRP3, NLRP1, JAK2, TNF	2.20E-10
GO:0033138; positive regulation of peptidyl-serine phosphorylation	APP, IL6, BDNF, NTRK3, BCL2, PARK7, NGF, PTGS2, TNF	3.60E-10
GO:0071222; cellular response to lipopolysaccharide	IL10, IL6, CXCL8, IL1B, GSTP1, CASP1, NLRP3, TNFAIP3, JAK2, PTGS2, TNF, MMP9	4.00E-10
GO:0009410; response to xenobiotic stimulus	IL10, TGFB1, ABCB1, MMP2, FOS, COMT, PTGS2, LIPA, TNF, SLC6A4, SOD1, BCL2, DRD3	4.50E-10
GO:0045429; positive regulation of nitric oxide biosynthetic process	APP, IFNG, IL1B, APOE, OPRM1, JAK2, PTGS2, TNF	6.10E-10
GO:0010629; negative regulation of gene expression	APP, TGFB1, CXCL8, PARK7, IGF1, FGF2, TNF, IFNG, GPER1, CCL3, APOE, TARDBP, HRAS	4.30E-09
GO:0043410; positive regulation of MAPK cascade	APP, IL6, TGFB1, GPER1, IGF1, JAK2, HRAS, TNF, DRD3, FGF2, SOD1	5.20E-09

Table 2. Pathway analysis of mercury-responsive genes in anxiety disorder. The top 10 terms are displayed.

KEGG_PATHWAY	Genes	*P*-Value
hsa05417: Lipid and atherosclerosis	CXCL8, FOS, TNF, MMP9, IL6, IL1B, CASP1, CYP1A1, CCL3, BCL2, FAS, NLRP3, CALM3, JAK2, HRAS, NFE2L2, MAPK3	6.90E-12
hsa05142: Chagas disease	IL10, IL6, TGFB1, CXCL8, IFNG, IL1B, CCL3, FAS, FOS, TNF, IL2, MAPK3	3.70E-10
hsa05200: Pathways in cancer	TGFB1, GSTM1, CXCL8, GSTP1, MMP2, IL13, FOS, IGF1, PTGS2, FGF2, MMP9, IL2, ESR2, IL6, IFNG, BCL2, FAS, CALM3, JAK2, HRAS, NFE2L2, MAPK3	3.70E-10
hsa05133: Pertussis	IL10, IL6, CXCL8, IL1B, CASP1, NLRP3, SERPING1, CALM3, FOS, TNF, MAPK3	4.00E-10
hsa05140: Leishmaniasis	IL10, TGFB1, IFNG, IL1B, FOS, JAK2, PTGS2, TNF, HLA-DQA1, MAPK3, HLA-DQB1	4.50E-10
hsa05321: Inflammatory bowel disease	IL10, IL6, TGFB1, IFNG, IL1B, IL13, TNF, IL2, HLA-DQA1, HLA-DQB1	1.80E-09
hsa04657: IL-17 signaling pathway	IL6, CXCL8, IFNG, IL1B, IL13, TNFAIP3, FOS, PTGS2, TNF, MMP9, MAPK3	2.90E-09
hsa04625: C-type lectin receptor signaling pathway	IL10, IL6, IL1B, CASP1, NLRP3, CALM3, PTGS2, HRAS, TNF, IL2, MAPK3	7.70E-09

hsa05418: Fluid shear stress and atherosclerosis	GSTM1, IFNG, IL1B, GSTP1, MMP2, BCL2, CALM3, FOS, TNF, SQSTM1, MMP9, NFE2L2	1.10E-08
hsa05323: Rheumatoid arthritis	IL6, TGFB1, CXCL8, IFNG, IL1B, CCL3, FOS, TNF, HLA-DQA1, HLA-DQB1	4.80E-08

Functional analysis of key sub-network and core mercury targets

The functional analysis of the key sub-network is displayed in Tables 3 and 4, indicating targeting cellular processes. The GeneMANIA network (Fig. 5) shows 24 genes (4 query genes and 20 related genes) with 507 links based on physical interactions (77.64%), co-expression (8.01%), predicted (5.37%), co-localization (3.63%), genetic interactions (2.87%), pathway (1.88%), and shared protein domains (0.60%). The GeneMANIA analysis-based functions of core mercury targets (IL1B, IL6, TNF, and IFNG) highlight regulation of inflammatory response, regulation of cytokine-mediated signaling pathway, regulation of response to cytokine stimulus, response to interleukin-1, cellular response to interleukin-1, response to lipopolysaccharide, and cellular response to molecule of bacterial origin as the significant terms. GeneMANIA analysis revealed CASP1 (interacts with IL1B, IL6, TNF, and IFNG), TNFAIP3 (interacts with IL1B, IL6, TNF), and SQSTM1 (interacts with IL1B and TNF) among 20 GeneMANIA-predicted proteins as first neighbors of core mercury targets, belonging to the list of 79 mercury-responsive genes in anxiety. The GeneMANIA network contains seven mercury targets: IL1B, IL6, TNF, IFNG, CASP1, TNFAIP3, and SQSTM1.

Table 3. Gene ontology analysis of the key sub-network.

Term	Genes	*P*-Value
positive regulation of transcription by RNA polymerase II	IL6, IFNG, IL1B, STAT3, EP300, ESR1, TNF, TP53	4.41E-09
positive regulation of DNA-binding transcription factor activity	IL6, IL1B, EP300, ESR1, TNF	1.43E-08
positive regulation of DNA-templated transcription	IL6, IL1B, STAT3, EP300, ESR1, TNF, TP53	1.69E-08
positive regulation of interleukin-6 production	IL6, IFNG, IL1B, STAT3, TNF	2.58E-08
positive regulation of gene expression	IL6, IFNG, IL1B, STAT3, TNF, TP53	2.35E-07
positive regulation of calcidiol 1-monooxygenase activity	IFNG, IL1B, TNF	3.34E-07
positive regulation of nitric oxide biosynthetic process	IFNG, IL1B, ESR1, TNF	4.33E-07
regulation of insulin secretion	IL6, IFNG, IL1B, TNF	4.33E-07
cell surface receptor signaling pathway via JAK-STAT	IL6, IFNG, STAT3, TNF	8.78E-07
positive regulation of miRNA transcription	IL6, STAT3, TNF, TP53	1.08E-06

Table 4. KEGG pathway analysis of the key sub-network.

Term	Genes	*P*-Value
Inflammatory bowel disease	IL6, IFNG, IL1B, STAT3, TNF	9.65E-08
African trypanosomiasis	IL6, IFNG, IL1B, TNF	2.32E-06
Hepatitis B	IL6, STAT3, EP300, TNF, TP53	3.69E-06
Graft-versus-host disease	IL6, IFNG, IL1B, TNF	4.22E-06
Influenza A	IL6, IFNG, IL1B, EP300, TNF	4.68E-06
Tuberculosis	IL6, IFNG, IL1B, EP300, TNF	5.73E-06
Malaria	IL6, IFNG, IL1B, TNF	5.82E-06
Lipid and atherosclerosis	IL6, IL1B, STAT3, TNF, TP53	1.13E-05
Human cytomegalovirus infection	IL6, IL1B, STAT3, TNF, TP53	1.36E-05
Pathways in cancer	IL6, IFNG, STAT3, EP300, ESR1, TP53	1.46E-05

Protective agent-target interactions against mercury-induced anxiety

A protective agent-mercury target network was constructed, which included 408 links between 23 agents and 71 targets. As shown in Fig. 6. A, quercetin, selenium, curcumin, and glutathione target the most mercury-responsive genes (58, 45, 42, and 38 nodes, respectively). Fig. 6. B presents agent-mercury target interactions in the core sub-network. Quercetin, curcumin, selenium, succimer, salicylic acid, glutathione, D-penicillamine, and ellagic acid interact with all four-core targets of mercury. It has been previously mentioned that four-core targets (IL1B, IL6, TNF, and IFNG) for mercury were identified in the key sub-network, and three related proteins (CASP1, TNFAIP3, and SQSTM) were also revealed in the

GeneMANIA network. Quercetin is the only one that targets all seven mercury-responsive genes (IL1B, IL6, TNF, IFNG, CASP1, SQSTM1, and TNFAIP3) in the GeneMANIA network (Table 5). Curcumin (IL1B, IL6, TNF, IFNG, CASP1, SQSTM1), selenium (IL1B, IL6, TNF, IFNG, CASP1, SQSTM1), and succimer (IL1B, IL6, TNF, CASP1, SQSTM1, and TNFAIP3) target six mercury-responsive genes. Salicylic acid (IL1B, IL6, TNF, IFNG, CASP1), Glutathione (IL1B, IL6, TNF, IFNG, SQSTM1), and D-penicillamine (IL1B, IL6, TNF, IFNG, CASP1) had five targets among the seven genes. Flunarizine, Prolactin, Dihydrolipoic acid, DPPD, Dimercaprol, Unithiol, Metallothionein, Nicardipine, Diltiazem did not have any target among the seven genes.

Molecular docking of screened protective agents and potential targets

Using molecular docking, as a valuable computational technique, helped to identify potential protective candidates by predicting the binding affinities between drug candidates and key targets.

Based on the protective agent-mercury target network, the positions of drug molecules (curcumin and quercetin) in the binding sites of proteins (IL1B, IFNG, IL6, CASP1, SQSTM1, TNF, and TNFAIP3) were calculated using molecular docking methodology. The free binding energy (ΔGbinding) and involved amino acids in the binding sites of targets are presented in Table 6. As indicated in Table 6, curcumin interacts with six proteins, including IL1B, IFNG, IL6, CASP1, SQSTM1, and TNF, with binding energies of -8.45, -8.38, -5.59, -6.41, -6.78, and -7.34 kcal/mol, respectively. Moreover, quercetin was found to bind with seven proteins: IL1B, IFNG, IL6, CASP1, SQSTM1, TNF, and TNFAIP3, with binding energies of -7.95, -7.24, -5.72, -6.84, -6.24, -5.92, and -5.72 kcal/mol, respectively. Fig. 7, Fig. S1, and Fig. S2 show molecular docking complexes.

Table 5. Protective agents that target mercury-responsive genes in the core sub-network and the GeneMANIA network.

Protective agent	Summary	Targets
Quercetin	It is a plant flavonoid with antioxidant and anti-inflammatory effects	IL1B, IL6, TNF, IFNG, CASP1, SQSTM1, TNFAIP3
Curcumin	Curcumin is a polyphenol that acts as an antioxidant, antibacterial, and anti-inflammatory agent	IL1B, IL6, TNF, IFNG, CASP1, SQSTM1
Selenium	An essential mineral with antioxidant properties	IL1B, IL6, TNF, IFNG, CASP1, SQSTM1
Succimer	Heavy metal chelator	IL1B, IL6, TNF, CASP1, SQSTM1, and TNFAIP3
Salicylic acid	A phenolic compound with anxiolytic and hypnotic effects, functions as an antioxidant	IL1B, IL6, TNF, IFNG, CASP1
Glutathione	A tripeptide with antioxidant properties	IL1B, IL6, TNF, IFNG, SQSTM1
D-penicillamine	A chelating agent	IL1B, IL6, TNF, IFNG, CASP1
Ellagic acid	A polyphenol with antioxidant and anti-proliferative properties	IL1B, IL6, TNF, IFNG
Verapamil	A non-dihydropyridine calcium channel blocker with antihypertensive, anti-inflammatory, and antioxidative properties	IL1B, IL6, TNF, CASP1
alpha-lipoic acid	It is an antioxidant made by the body, with neuroprotective effects	IL1B, IL6, TNF
Ebselen	cytoprotective, anti-inflammatory and antioxidant effects	IL6, TNF, IFNG
Naringenin	A flavonoid with antioxidant, anti-inflammatory, nephroprotective effects	IL1B, IL6, TNF
Nifedipine	antioxidant and anti-apoptotic properties	IL1B, TNF, TNFAIP3
Nimodipine	neuroprotective properties	TNF

Discussion

According to the literature, the interaction between heavy metals, as well-known environmental pollutants, and mental disorders has attracted widespread attention. Mercury is recognized as one of the most hazardous heavy metals, and exposure to this substance can adversely impact multiple systems within the human body. Studies show the occurrence of neurological and behavioral disorders often after exposure to various mercury compounds, posing a serious challenge to the public health of society (32). Mercury penetrates the central nervous system through

the BBB and causes neurological disorders such as anxiety (33, 34, 35). Researchers have found that anxiety is linked to an inflammatory response, which is caused by neuroinflammation, increasing the levels of inflammatory cytokines. Inflammatory pathways are activated by oxidative stress and lead to neuronal damage. Inflammatory cytokines extend central inflammatory responses through increasing BBB permeability and expedite the damage to the CNS by oxidative stress (36). An urgent need exists to constantly control and monitor mercury as a significant public health challenge and provide new insights into the neurobehavioral mechanisms (32). The present study used a network toxicology approach to investigate the effects of environmental mercury exposure on the molecular mechanisms involved in the induction of anxiety disorder. A critical sub-network with eight proteins (IL1B, IL6, TNF, IFNG, STAT3, TP53, EP300, and ESR1) was screened to identify core mercury-responsive targets. IL1B (interleukin-1β), IL6 (interleukin-6), TNF (tumor necrosis factor), and IFNG (interferon-gamma) were detected as core targets of mercury. These results were considered for further detail and functional analysis. Mercury exposure can be linked to the inflammatory reactions (37). Targeting inflammatory pathways and restraining them is an impressive strategy for reducing anxiety and depressive-like behaviors (38). TNF-α and IL1B are major inflammation cytokines in brain tissue, which go up in anxiety and depression (39). The first essential gene is IL1B, which is present in the nervous system during neurogenesis and synapse formation (40). Furthermore, it plays a role in neurological processes and expresses immune responses, implicated in developing mental disorders such as autism, schizophrenia, and anxiety (41). Afsordeh et al. found that exposure to mercury results in abnormal neurogenic inflammatory responses, such as up-regulation of IL1B (42). IL1B is increased in mental disorders through chronic activation of microglia, the brain's endogenous immune cells. It causes neurotoxicity by releasing reactive oxygen species (ROS) and increases depressive symptoms (43, 44). IL6 is an inflammatory cytokine produced by many cells, such as stromal cells, hematopoietic cells, epithelial cells, and muscle cells, and it is involved in various biological processes. It is extended after contact with mercury (45, 46). M. Foley et al. showed an association between IL6 and depression; the activity/bioavailability of IL6 contributes to providing further insight into depression. Its biological effects are exerted through three signaling pathways: Classical signaling, trans-signaling, and trans-presentation, having some interactions between IL6, interleukin 6 receptor (IL6R), and glycoprotein 130. These interactions help to find and measure the trans-signaling strength and reflection of IL6 activity/bioavailability, relating to inflammatory proteins, including CRP, causing depression (47). A study by Gardner et al. concluded that even low mercury levels could dysregulate cytokine signaling pathways (48). A lack of corticotropin-releasing hormone receptor 2 (CRHR2), a receptor that is mainly expressed in the brain, causes anxiety-like behavior and increases neuroinflammation and apoptosis (49). IFNG is a pro-inflammatory cytokine that is increased in response to neurodevelopmental disorders (50). All this research shows mercury affects the regulation of cytokine signaling pathways, the amount of secretion, and the response. The toxicogenomic data mining technique was also used to identify potential protective agents to overcome mercury poisoning and target mercury-gene interactions in anxiety. Molecular docking technique revealed strong binding affinities for curcumin and quercetin with the screened targets. This study's results need to be further verified in future studies.

Table 6. Binding affinities of ligand-receptor complexes.

Receptor	H-bonding	Binding energy
	a) Curcumin	
IL1B	6 H-bond: LysA103 (2), MetA148 (C-H-bond), ProB206 (C-H-bond), ThrB207 (2)	-8.45
IFNG	7 H-bond: AsnA16 (2), LysA12, GlyA18, HisB111, PheC112 (C-H-bond), GlnB115 (C-H-	-8.38
IL6	4 H-bond: GlnB75, LeuC108 (C-H-bond), SerC109 (C-H-bond), AsnC110	-5.59
CASP1	6 H-bond: HisA237, GlyA238, GlyA287, AspA288 (C-H bond), ArgB341 (2)	-6.41
SQSTM1	5 H-bond: SerA119 (C-H-bond), AsnA120, ProA134, ThrA138, SerA152	-6.78
TNF	5 H-bond: SerB60, TyrB119 (C-H-bond), GlyA121, TyrA151, TyrB151,	-7.34
	b) Quercetin	
IL1B	7 H-bond: LysA103, MetA148 (2), AsnB204, THRB207 (2), AsnB239	-7.95
IFNG	5 H-bond: SerC80, AspC107, HisC111 (2), ArgC106 (C-H-bond)	-7.24
IL6	4 H-bond: GlnB75, LeuC108, AsnC110, AlaC160	-5.72
CASP1	6 H-bond: ArgA179, SerA236 (2), HisA237, CysA285, ArgB341	-6.84
SQSTM1	7 H-bond: ProA113, CysA131(C-H-bond), ValA136 (2), SerA152, LysA157, CysA151	-6.24
TNF	2 H-bond: GlyA121, GlyB121	-5.92
TNFAIP3	4 H-bond: ThrA13, CysA35 (C-H-bond), ThrA36 (2)	-6.15

In Conclusion

The present study integrated network toxicology and network pharmacology methodologies to explore top-ranked mercury targets and relevant protective agents in mercury-induced anxiety disorder. The anxiety-related core sub-network was identified, and core mercury targets (IL1B, IL6, TNF, and IFNG), along with relevant mechanisms, were introduced. The obtained results indicate that high-ranking proteins in the main sub-network act as mercury-responsive targets, emphasizing the importance of network toxicology in in-depth investigations of toxicological mechanisms. Quercetin, selenium, curcumin, and glutathione were suggested as potential therapeutic agents. Overall, this study's detailed and key information can help understand mercury-induced anxiety and select an efficient and effective protection strategy. However, experimental studies are recommended based on these results.

Acknowledgment

This study is related to project NO.43010177 from the Student Research Committee, Shahid Beheshti University of Medical Sciences, Tehran, Iran. The authors gratefully acknowledge the support of the Student Research Committee and Research and Technology Chancellor at Shahid Beheshti University of Medical Sciences (Tehran, Iran) for their financial support of this study.

The authors thank the Student Research Committee, Shahid Beheshti University of Medical Sciences, Tehran, Iran, for their support (No. 43010177).

Authors' Contribution

Fatemeh Bagheri: Investigation, Writing-original draft, Visualization, Software, Methodology, Writing-Review & Editing. Masoumeh Farahani: Investigation, Data curation, Formal analysis, Software, Methodology, Writing- Review & Editing. Mostafa Rezaei-Tavirani: Formal analysis, Conceptualization, Supervision, Project administration, Methodology, Writing- Review & Editing. Fatemeh Fateminasab: Investigation, Software, Methodology, Writing-Review & Editing.

Conflicts of Interest

The authors have no conflicts of interest to declare.

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