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Integrative Systems Toxicology Shows Mechanisms of Food Additive-Induced Toxicity

(Toksikologi Sistem Integratif Menunjukkan Mekanisme Ketoksikan yang Disebabkan oleh Bahan Tambahan Makanan)

JING WANG* & LING MENG

College of Medicine Engineering, Lianyungang Technical College, Jiangsu, 222005, P.R. China

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ABSTRACT

Traditional single-target toxicity evaluation methods are insufficient to elucidate the individual and combined toxicities of food additives, primarily due to the diverse chemical structures of these additives and their multi-system biological targets. To address this limitation, the present study establishes a systematic toxicology framework that integrates oral bioavailability (OB), protein interaction prediction, network analysis, molecular docking, and pathway analysis. Our results showed 52 food additives exhibit significant potential toxicological effects. Network analysis identified 868 receptor proteins and 7 major disease categories via food additive-protein and protein-disease networks, indicating multi-target synergistic pathogenic effects. Key target proteins showed strong binding affinity with additives. Integrated GO/KEGG analyses showed three core toxicity mechanisms: dysregulated xenobiotic detoxification, abnormal chemical synaptic transmission, and amplified inflammatory signaling. These mechanisms are mediated by disrupted enzyme/receptor binding and perturbations in plasma membrane, synaptic, and cytosolic compartments, and are involved in neuroactive ligand-receptor interaction, cancer-related pathways, and HIF-1 signaling. This research provides comprehensive evidence for food additive safety evaluation and a novel paradigm for related studies.

Keywords: Food additive toxicity; molecular mechanism; systems toxicology

ABSTRAK

Kaedah tradisi penilaian ketoksikan sasaran tunggal tidak berupaya untuk menjelaskan ketoksikan individu dan gabungan bahan tambahan makanan, terutamanya disebabkan oleh struktur kimia yang pelbagai bagi bahan tambahan ini dan sasaran biologi berbilang sistemnya. Untuk menangani keterbatasan ini, kajian ini mewujudkan rangka kerja toksikologi sistematik yang mengintegrasikan bioketersediaan oral (OB), ramalan interaksi protein, analisis rangkaian, dok molekul dan analisis laluan. Keputusan kami menunjukkan 52 bahan tambahan makanan mempamerkan potensi kesan toksikologi yang ketara. Analisis rangkaian mengenal pasti 868 protein reseptor dan 7 kategori penyakit utama melalui rangkaian bahan tambahan-protein dan protein-penyakit makanan yang menunjukkan kesan patogen sinergi berbilang sasaran. Protein sasaran utama menunjukkan afiniti pengikatan yang kuat dengan bahan tambahan. Analisis GO/KEGG bersepadu mendedahkan tiga mekanisme ketoksikan teras: detoksifikasi xenobiotik yang tidak terkawal, penghantaran sinaptik kimia yang tidak normal dan isyarat keradangan yang diperkuat. Mekanisme ini dimediasi oleh pengikatan enzim/reseptor yang terganggu dan gangguan dalam membran plasma, petak sinaptik dan sitosolik dan terlibat dalam interaksi ligan-reseptor neuroaktif, laluan berkaitan kanser dan isyarat HIF-1. Penyelidikan ini menyediakan bukti komprehensif untuk penilaian keselamatan bahan tambahan makanan dan paradigma baharu untuk kajian berkaitan.

Kata kunci: Ketoksikan bahan tambahan makanan; mekanisme molekul; toksikologi sistem

INTRODUCTION

Food additives are synthetic or extracted substances used to enhance food appearance, flavor, and shelf life, meeting consumer demands for diversified, convenient, and safe food. To ensure their safe use, Joint FAO/WHO Expert Committee on Food Additives and national regulatory authorities conduct safety assessments, establishing health-based guidance values and maximum permitted levels. However, in practical production, multiple additives are often used simultaneously, and studies have shown combined additives may induce greater toxicological effects than individual use particularly (Nykyforov et al. 2022) preservative combinations with amplified genotoxicity (Nykyforov et al. 2022; Raya et al. 2020; Sarikaya & Cakir 2005). This synergistic interaction poses cumulative health risks, raising public concerns about chronic disease associations and drawing scientific attention to additive combinations.

Conventional single-chemical risk assessment is inadequate for population health impact evaluation, with research shifting to cumulative risk assessment. Traditional experimental approaches face limitations in generating reliable data on additive combinations, due to chemical structural diversity and biological target complexity. Mechanistic understanding of cellular and molecular toxic effects remains insufficient, highlighting the need for systematic approaches to evaluate individual and combined toxicological profiles of food additives. Network toxicology integrates classical toxicology with systems biology, constructing chemical-endogenous biomolecule interaction networks to predict toxicological effects. This study uses preservatives, antioxidants, flavor enhancers, colorants, and sweeteners as models to implement a systematic network toxicology framework: characterizing OB parameters for 65 food additives across five categories, predicting protein targets and associated diseases, constructing interaction networks, identifying common action proteins, and elucidating toxic mechanisms. The study aims to predict latent toxicity, clarify molecular mechanisms, and provide insights for evaluating combined additive toxicities.

MATERIALS AND METHODS

BUILDING OF DATASET

Molecular datasets of 67 regulatory-approved additives across six functional classes (acidity regulators, antioxidants, colorants, flavor enhancers, preservatives, thickeners) were retrieved from the PubChem database. Curated datasets were subjected to ADMETLab3.0 pharmacokinetic simulations, with an OB threshold of ≥20% to prioritize bioactive candidates with systemic circulation potential.

PROTEIN PREDICTION

Three-dimensional molecular structures of 52 food additives (OB≥20%) were retrieved from PubChem and submitted to Swiss Target Prediction and PharmMapper databases for target prediction, with species restricted to Homo sapiens.

DISEASE TARGETS COLLECTION

Potential disease associations of 52 food additives were using the CTD database, yielding 373 non-redundant diseases. The top 12 pathologies investigated were prioritized by descending order of the number of linked target proteins (frequency count). No threshold or filtering cutoff was applied; diseases were ranked purely by the total count of associated target proteins, and the 12 most frequent diseases were selected. Disease-associated targets were retrieved from GeneCards, PubMed, and OMIM databases.

FOOD ADDITIVES-TARGETS NETWORK CONSTRUCTION

Fifty-two food additives and their predicted targets were imported into Cytoscape 3.10.3 to construct an interaction network (nodes = additives/targets, edges = associations). Network topology analysis was performed using the 'Network Analyzer' tool, with degree centrality as the primary indicator of functional importance.

PROTEIN-PROTEIN INTERACTION NETWORKS CONSTRUCTION

Jvenn online tool analyzed 868 additive-associated targets and 2,189 disease-related targets, identifying 376 common toxicity-associated targets. These were imported into the STRING database to construct a PPI network (Homo sapiens, minimum interaction score ≥0.9, excluding disconnected nodes), with data exported in TSV format. The network file was imported into Cytoscape 3.10.3 for topological analysis using CytoNCA.

DOCKING ANALYSIS

Four representative food additives were selected based on the top-ranked node degree in the food additive–target network (degree ≥ 112) and high toxicological relevance reported in previous studies. The top seven target proteins were screened according to three topological centrality metrics (degree, betweenness, and closeness) in the PPI network, with a cutoff threshold of the top 10% highest centrality scores, and their critical roles in toxicity and disease pathways. PDB format files of target proteins (from PDB database) and SDF format 2D structures of compounds (from PubChem) were used. Target proteins were pretreated with PyMOL 3.1 (water removal, polar hydrogen addition, original ligand cutoff) and docked with ligand molecules using Autodock Vina 1.1.2.

GO AND KEGG ENRICHMENT ANALYSIS

Potential toxic targets were input into the Metascape database (identifier = OFFICIAL-GENE-SYMBOL, species = human, list type = gene list) for Gene Ontology (GO): Biological Process (BP), Molecular Function (MF), and Cellular Component (CC), and KEGG pathway enrichment analysis (with $p<0.01$). Results were sorted by P-value and visualized via a microbial bioinformatics platform.

RESULTS AND DISCUSSION

ORAL BIOAVAILABILITY SCREENING

A total of 52 food additives with high OB (≥ 20%) were identified, and these were predicted to show greater potential toxicological effects than compounds with low or no oral bioavailability (Table 1). For instance, natamycin-a natural polyene macrolide antifungal compound from

Streptomyces fermentation used as a food preservative-is essentially non-toxic, as it is poorly absorbed by the digestive tract and directly excreted from the body, with human intake generally well below safety limits. However, excessive consumption may neutralize antibodies and compromise health, which is primarily attributed to the high concentration of this additive in compounded preservative formulations (Shah et al. 2020).

FOOD ADDITIVES-TARGET NETWORK ANALYSIS

Moving from compound screening to molecular interactions, a complex interaction network (920 nodes: 52 additives, 868 proteins; 2498 edges) was constructed using Cytoscape 3.10.3. Statistical analysis showed each additive targets 48.04 proteins on average, while each protein interacts with 2.88 additives, suggesting complex multi-additive-single protein and single additive-multi-protein relationships. Degree distribution analysis identified key additives with numerous receptor interactions: Glycine (FE1, degree=187), D-mannitol (TH2, degree=126), Acetic acid (AC6, degree=115), and Brilliant blue (CO14, degree=112) (Figure 1). On the protein side, 19 proteins interacted with ≥10 additives, with eight key receptors: PTGS2 (degree=23), CA2 (degree=16), PTGS1 (degree=16), ADH1C (degree=14), AKR1B1 (degree=13), GABRA1 (degree=13), CTSD (degree=13), CA12 (degree=12), and ADH1B (degree=12) (Figure 1). Additionally, 332 proteins interacted with only one additive, reflecting structural diversity-e.g., distinct preservatives (sodium benzoate, sodium nitrite, potassium sorbate) have different targets, and their high-concentration combinations may theoretically cause chemical accumulation and adverse effects (Stanojevic et al. 2009). These network-based observations highlight plausible mechanisms by which additive combinations could contribute to multiple pathological outcomes, though causal links remain to be validated experimentally. This analysis implies that network studies enable systematic exploration of additive-protein interactions and molecular pathways, identifying proteins susceptible to additive combinations and providing a framework for elucidating toxicological mechanisms.

CORE TARGETS SELECTION

To refine key molecular mediators from the broader target set, a PPI network encompassing 376 potential toxic targets (311 nodes, 1098 edges) was constructed. Topological analysis using Cytoscape 3.10.3 and the cytoHubba plugin identified pivotal targets (Figure 2). Notably, TP53 (a biomarker for acute kidney injury) exhibited predicted strong binding affinity with benzoic acid (PR1) (Figure 3), which is consistent with sodium benzoate-induced renal dysfunction in rats (Erichsen et al. 2023) and in line with the nephrotoxic side effects commonly reported for food preservatives (Zeghib & Boutlelis 2021). Further analysis highlighted that SRC (a regulator of tumor proliferation/metastasis) and STAT3 (an oncogenic transcription factor) showed robust binding to ethyl 4-hydroxybenzoate (PR4, binding energy = -4.9 kcal/mol) and benzoic acid (PR1, binding energy = -4.8 kcal/mol), respectively (Figure 3), which aligns with the epidemiological links between nitrate/nitrite additives and cancer risk (Chazelas et al. 2022).

AKT1 (a critical mediator of hippocampal neurogenesis) (Balu et al. 2010), bound curcumin (CO6, binding energy= -6.3 kcal/mol) (Figure 3), consistent with neurobehavioral deficits in rodents induced by food colorants (Wopara et al. 2021). IL-6 (a regulator of immune and hematopoietic processes) bound benzoic acid (PR1, binding energy = -5.3 kcal/mol) (Figure 3), complementing reports of cytokine dysregulation in rats exposed to multiple additives. Collectively, these findings corroborate previous reports of cytokine dysregulation (including interferon-γ, TNF-α, and interleukins 1β, 6, 10, and 13) in rats treated with thiabendazole, monosodium glutamate, and brilliant blue, demonstrating the broad-spectrum immunotoxic effects of food additives on hematological parameters, innate immunity, and inflammatory pathways.

PROTEIN-DISEASE NETWORK ANALYSIS

Expanding from molecular targets to disease-level outcomes, a comprehensive protein-disease network was established, categorizing 354 protein-related diseases into 12 clusters. Seven major disease types were selected for detailed analysis (Figure 4).

Neurological risks

A total of 232 proteins were associated with neurological disorders, with PTGS1 (microglia-expressed, regulates neuroinflammation) (Muzio, Viotti & Martino 2021) and PTGS2 (astrocyte- expressed, neuroprotective) (Wopara et al. 2021) as key targets. Computational and preclinical evidence suggests that the artificial sweetener aspartame metabolizes into neurotoxic components-aspartic acid, methanol, and phenylalanine. These components have been shown to contribute to neuronal apoptosis and neurotransmitter disruption (Fadaei et al. 2025). Composite additives with sunset yellow (CO7) increase GFAP-positive cells and reduce gray matter neuronal density in rat brains (Bilash et al. 2021). Combined erythrosine and tartrazine cause cognitive deficits, elevated acetylcholinesterase activity, and oxidative/inflammatory imbalances (Motwadie et al. 2021). These findings support the hypothesis that food additives may contribute to neurological pathologies via protein-mediated molecular interactions.

Hepatorenal toxicity

A total of 196 proteins linked to hepatorenal damage were identified. Benzoic acid(PR1), potassium sorbate,

TABLE 1. The corresponding parameters of 52 food additives with OB≥20%

No	Name	No	Name	No	Name
FE1	Glycine	SW1	Sucralose	AN1	Butylated hydroxyanisole
FE2	L-alanine	SW2	Acesulfame potassium	AN2	Butylated hydroxytoluene
FE3	Sodium succinate	SW3	Sodium saccharin	AN3	4-hexylresorcinol
FE4	L-glutamic acid	SW4	Erythritol	AN4	Ascorbic acid
PR1	Benzoic acid	SW5	Steviol glycosides	AN5	Sorbic acid
PR2	Propanoic acid	SW6	Aspartame	CO1	Lutein
PR3	Glycerylcaprylate	SW7	Alitame	CO2	Beta-carotene
PR4	Ethyl 4-hydroxybenzoate	SW8	Thaumatin	CO3	Riboflavin
PR5	2,4-dichlorophenoxy acetic acid	SW9	Neotame	CO4	Cyanidin-3-galactoside chloride
PR6	Epsilon-polylysine	AC1	Fumaric acid	CO5	5,6,7,8-tetramethoxy-2-(4-methoxyphenyl)-4-benzopyrone
PR7	Diphenyl ether	AC2	Adipic acid	CO6	Curcumin
PR8	Dehydroacetic acid	AC3	Dl-tartaric acid	CO7	Sunset yellow
PR9	Cinnamaldehyde	AC4	Lactic acid	CO8	Quinoline yellow
TH1	Lactitol	AC5	L-malic acid	CO9	New red
TH2	D-mannitol	AC6	Acetic acid	CO10	Amaranth
TH3	Propylene glycol	AC7	Citric acid	CO11	Allura red
CO12	Erythrosine	CO13	Carmoisine	CO14	Brilliant blue
CO15	Indigotine				

Acidity regulators(AC), Antioxidants(AN), Colorants(CO), Flavor enhancers(FE), Preservatives(PR), and Thickeners(TH)

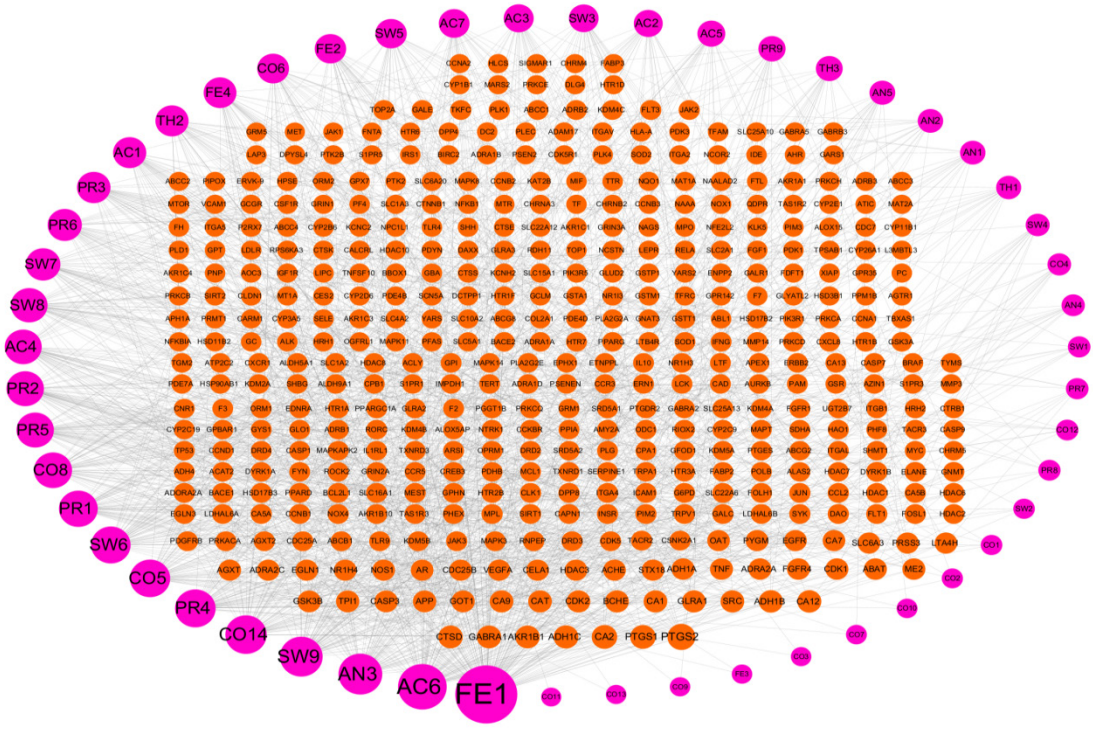


FIGURE 1. The food additive-protein network with the degree of food additives ≥ 8. A food additive node and a protein node are linked if the protein is targeted by the corresponding food additive. Node size is proportional to its degree. The pink triangles and orange circles represent food additive and potential proteins, respectively

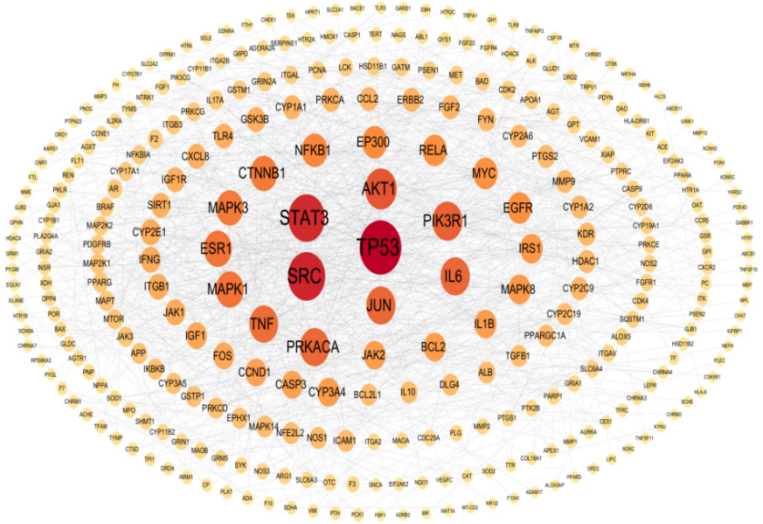


FIGURE 2. Network diagram of protein-protein interaction (PPI)

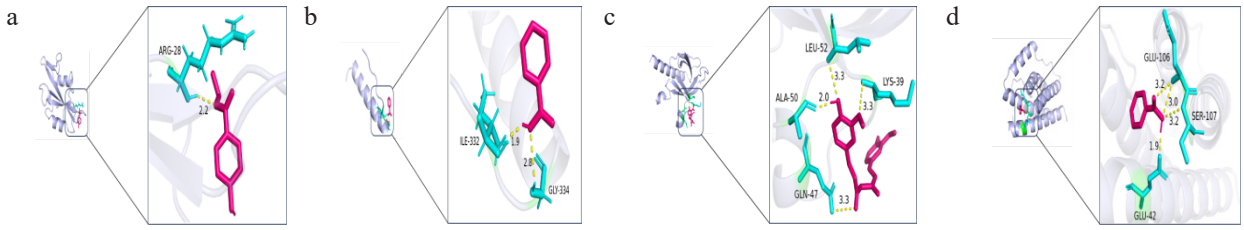


FIGURE 3. The food additive and target molecular docking mode. (a) ethyl 4-hydroxybenzoate(PR4) and SRC, (b) benzoic acid(PR1) and STAT3, (c) curcumin(CO6) and AKT1, (d) benzoic acid(PR1) and IL-6

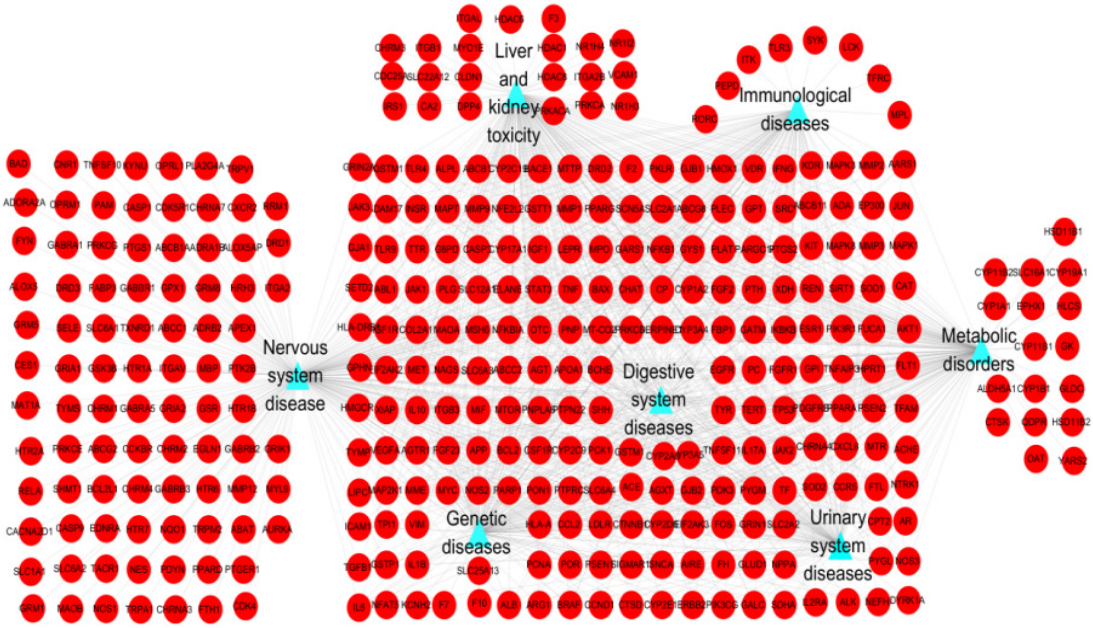


FIGURE 4. The protein–disease network for seven crucial disease types and their related proteins. The light blue triangles represent the 7 crucial disease types

and tartrazine have been reported to elevate serum transaminase (ALT/AST), ALP, and creatinine in rats, with histopathological evidence of organ damage (Abo-EL-Sooud et al. 2018). Prolonged exposure to five common preservatives induces combined hepatorenal dysfunction, oxidative stress, and inflammatory gene upregulation (TLR-4, TLR-2, NF-κB, TNF-α) (Abd-Elhakim et al. 2023). Sunset yellow (CO7) activates ERα, elevating estrogen levels and potentially triggering cholestatic liver disease (Axon et al. 2012). These collective findings underscore the critical importance of food additives' capacity to modulate disease-associated proteins and their multifaceted mechanistic roles in precipitating hepatorenal injuries through complex molecular pathways encompassing oxidative stress, inflammatory cascades, and hormonal dysregulation.

Metabolic disorders

A total of 169 proteins associated with metabolic disorders were identified. Food emulsifiers/thickeners disrupt metabolism: carrageenan suppresses insulin-induced Akt phosphorylation and PI3K activity, impairing glucose tolerance (Bhattacharyya et al. 2012); emulsifiers trigger low-grade inflammation linked to obesity/metabolic syndrome (Viennois et al. 2017). Artificial sweeteners (aspartame, acesulfame-K) increase type II diabetes risk dose-dependently (men >16.4 mg/d, women >18.5 mg/d: 69% elevated risk) (Debras et al. 2023). Preservatives (butylparaben, methylparaben) may disrupt gut microbiota and bile acid production: butylparaben inhibits FXR signaling, while methylparaben induces endoplasmic reticulum stress via the IRE1α-XBP1 pathway, both disrupting glucose/lipid homeostasis (Du et al. 2024a, 2024b). Collectively, these findings support the hypothesis that multiple food additives may induce metabolic dysregulation through modulation of critical proteins and pathways implicated in metabolic homeostasis.

Genetic diseases

A total of 147 proteins interacts with genetic risk. Food additives (sodium nitrite, caffeine, indigo carmine, erythrosine, fast green) induce significant DNA damage and chromatin fragmentation even below actual usage levels. The body's DNA damage response (DDR) pathways (EGFR, TP53-mediated) normally prevent damaged cell proliferation, but additive-induced genotoxicity may impair DDR function, contributing to genetic disorders (Peycheva, Alexandrova & Miloshev 2014). These findings underscore the importance of food additives in potentially influencing disease-associated proteins and their role in genetic risk (Yu, Mandlekar & Tony Kong 2000).

Immunological diseases

A total of 123 proteins linked to immune disorders were identified. Chronic exposure to five common preservatives induces hematotoxicity (anemia, thrombocytopenia) and immunotoxicity (suppressed immunoglobulins, lysozyme activity; upregulated pro-inflammatory cytokines) (Abd-Elhakim et al. 2020). Brilliant blue (CO14) causes neutrophilia/ lymphocytopenia in rodents; thiabendazole, monosodium glutamate, and brilliant blue suppress IgM/ IgG production and trigger spleen cytokine storms (Bilash et al. 2021). This converging mechanistic evidence positions food additives as potential disruptors of immune homeostasis through targeted protein interactions, predisposing organisms to severe immunopathologies via multifaceted pathways encompassing hematological alterations, humoral immunity suppression, and cytokine network dysregulation.

Urinary system diseases

A total of 112 proteins associated with genitourinary risks were identified. Butylated hydroxytoluene (AN2) disrupts endometrial decidualization via hormonal dysregulation in early-pregnancy mice by downregulating decidual markers (COX2, HOXA10, MMP9) and dysregulating estrogen/ progesterone signaling (Sun et al. 2021). Sodium benzoate impairs male reproductive health (reduced sperm count/ motility, suppressed testosterone/FSH levels, elevated TNF-α/IL-6, mitochondrial dysfunction via upregulated mtTFA/UCP2) (El-Shennawy et al. 2020). Monosodium glutamate induces hormonal imbalance and testicular structural alterations (Pawar et al. 2025). These findings collectively implicate diverse food additives in the pathogenesis of genitourinary disorders via modulation of critical protein networks governing hormonal regulation, oxidative homeostasis, inflammatory responses, and cellular signaling pathways.

Digestive system diseases

A total of 57 proteins linked to digestive disorders were identified. Synthetic colorants such as allura red(CO11) and sunset yellow(CO7) exacerbate colitis in IL-23-overexpressing mice (He et al. 2021). Furthermore, certain food preservatives including benzoic acid(PR1), sodium nitrite, sorbic acid(AN5) induce gut microbial dysbiosis and mild intestinal inflammation (Hrncirova et al. 2019; Laudisi et al. 2016).

Collectively, these findings underscore the molecular mechanisms through which food additives contribute to diverse adverse health outcomes. Epidemiological investigations, however show ongoing debates regarding the extrapolation of low-dose additive toxicity to human disease risks due to substantial disparities between animal models and real-world human exposure scenarios, particularly in terms of dosage thresholds, cumulative effects, and interactions with environmental co-factors. This study aims to elucidate the association between food additive toxicity and human disease pathogenesis through systematic data analysis, with its scientific validity and translational implications anticipated to garner heightened attention in the context of public health risk assessment and regulatory policy refinement.

UNVEILING THE COMBINED TOXICITY OF FOOD ADDITIVES

From single-compound targets to mixture effects, food additive-protein and protein-disease networks, multiple additives act on shared proteins and associate with disease-related proteins, enabling additive pairs to exert combined (synergistic or additive) effects via distinct mechanisms. To clarify their collective toxicity mechanism, we examined pairs sharing common receptor proteins and identified 231 pairs (22 unique additives) with strong interactions involving ≥20 shared proteins. Five representative co-applied pairs and their nervous system disease-related receptors were used to construct a food additive pair-protein-disease network (Figure 5), which shows common metabolic disease- related proteins targeted by multiple additives. These pairs include: acidulant acetic acid (AC6) and sweetener aspartame (SW6) (6 shared proteins), flavor enhancer glycine (FE1) and preservative benzoic acid (PR1) (27 shared proteins), FE1 and colorant curcumin (CO5) (17 shared proteins), 4-hexylresorcinol (AN3) and preservative ethyl 4-hydroxybenzoate (PR4) (33 shared proteins), and FE1 and thickener propylene glycol (TH2) (29 shared proteins). Combined effects of structurally unrelated additives can be evaluated by overlapping target sites, mechanisms, or elimination pathways. Studies have shown that L-glutamic acid (FE4), Steviol glycosides (SW5), brilliant blue (CO14), and quinoline yellow (CO8) induce neurotoxicity; antagonists cannot prevent CO14/CO8-induced neurotoxicity, and these combinations exhibit significant synergistic cytotoxicity. Our findings suggest that FE4 shares 12 proteins with SW6, 4 with CO8, and 9 with CO14. In summary, the established network uncovers synergistic/additive interactions among food additives at the molecular level, indicating that additives sharing common proteins may exhibit such toxicity, potentially leading to severe pathological outcomes.

GO AND KEGG ENRICHMENT ANALYSIS

To contextualize targets within biological pathways, GO/KEGG analyses constructed a multi-level toxicological framework. Screening yielded 185 significant GO-BP terms, 183 GO-CC terms, 454 GO-MF terms, and 179 KEGG pathways ($p<0.01$). The top 8 terms/pathways were selected for visualization based on adjusted P-value (ascending order) and enrichment score (descending order), representing the most statistically significant and biologically relevant items without arbitrary selection. These are presented in Figure 6.

GO enrichment analysis

For GO-BP, key processes include response to exogenous stimuli, chemical synaptic transmission, G protein-coupled receptor pathways, and inflammatory responses-aligning with additive-disease associations (neurological, digestive, metabolic disorders)(Chen et al. 2015; Holz & Fisher 2012). The BP enrichment analysis results align consistently with our protein-disease network analysis findings on food additive-disease associations, as exemplified by the neurological risks, digestive system diseases, and metabolic disorders intricately linked to these biological pathways/processes.

GO-MF enriched functions include enzyme binding, G protein-coupled serotonin receptor activity, zinc ion binding, and neurotransmitter receptor activity-suggesting additives induce toxicity via enzyme/receptor binding, metal ion homeostasis disruption, and kinase-epigenetic network dysregulation. The enrichment results of the GO-MF suggest that food additives may induce multidimensional toxicity through direct binding to key enzymes/receptors, disruption of metal ion homeostasis, and dysregulation of kinase-epigenetic regulatory networks, which are associated with neurotoxicity (Garza-Lombó et al. 2018), carcinogenic risks (Ito et al. 2025), and metabolic disorders (Raposa et al. 2016). Future studies should prioritize validation of their impacts on zinc-dependent pathways, histone modifications, coupled with long-term low-dose exposure experiments to comprehensively assess potential health risks.

GO-CC analysis showed significant enrichment of target proteins in the plasma membrane, synapses, and cytosol. This indicates that food additives may exert toxic effects by interfering with membrane receptors or transport proteins (disrupting transmembrane signaling or material exchange), impairing synaptic vesicle release or receptor recycling, or disturbing cytosolic enzyme activity-ultimately leading to energy imbalance, oxidative damage, and intracellular metabolic dysfunction. Notably, the cyclic nucleotide-mediated G protein-coupled receptor signaling cascade serves as a central hub, synergistically driving MAPK pathway hyperactivation and neuroinflammatory crosstalk, which manifests as systemic neurotransmission imbalance and failure of xenobiotic stress adaptation (Emery et al. 2013; Yan et al. 2019).

KEGG pathway enrichment analysis

Identifying relevant proteins and pathways is key to clarifying food additive toxicity mechanisms. Enrichment analysis shows additives may exert toxicity via neuroactive ligand-receptor interaction, lipid and atherosclerosis, calcium, AGE-RAGE, Rap1, and MAPK pathways. The neuroactive ligand-receptor interaction signaling pathway, a collection of plasma membrane receptors and ligands involved in intracellular and extracellular signaling, is associated with α-synuclein that participates in brain miRNA dysregulation and is thereby closely linked to key genes of neurodegenerative diseases including Parkinson's disease and Alzheimer's disease (Kong et al. 2015). The PI3K/Akt and cancer pathways regulate tumor progression (Fresno Vara et al. 2004). In the lipid and atherosclerosis pathway, LOX-1 mediates oxLDL internalization, driving atherosclerosis (He & Liu 2023). The calcium pathway maintains cell function and homeostasis (Yuan et al. 2013).

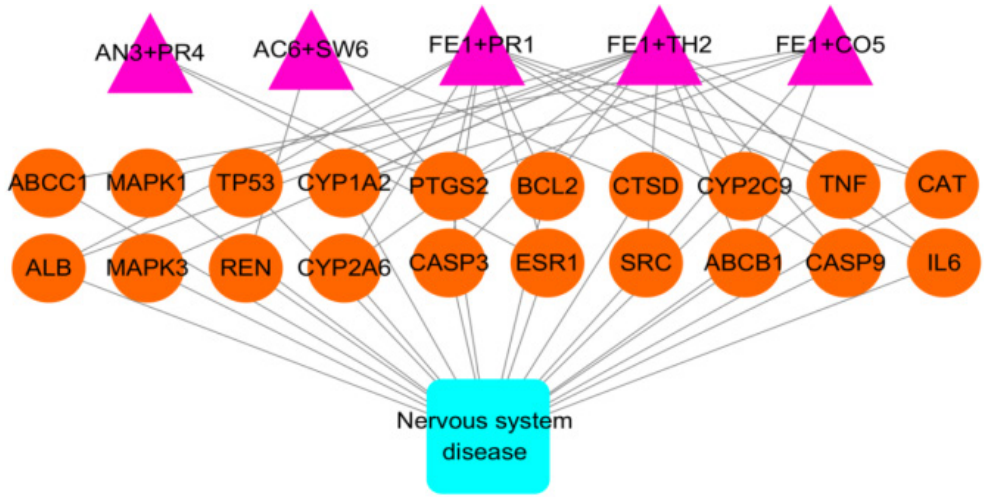
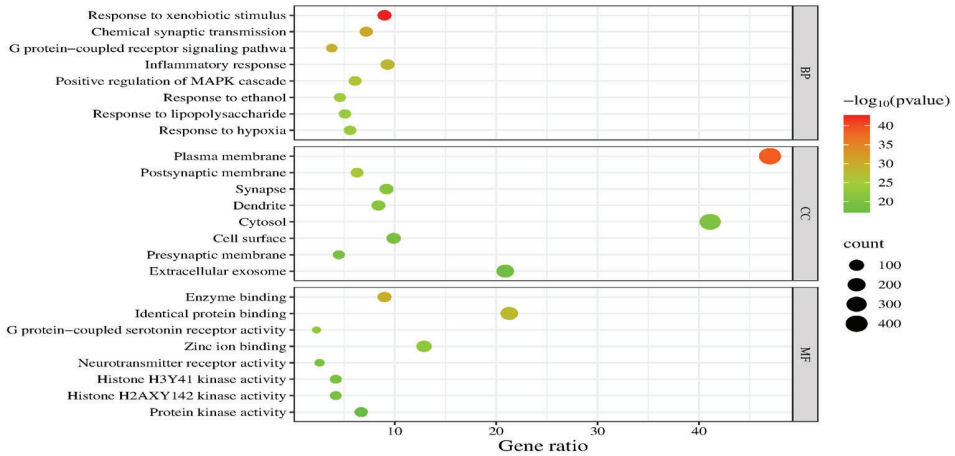


FIGURE 5. The food additives pair-protein-disease network. The food additives pair and their protein are connected to each other if the protein is a common protein of the two food additives. The colors of protein nodes are the same as Figure 1

a



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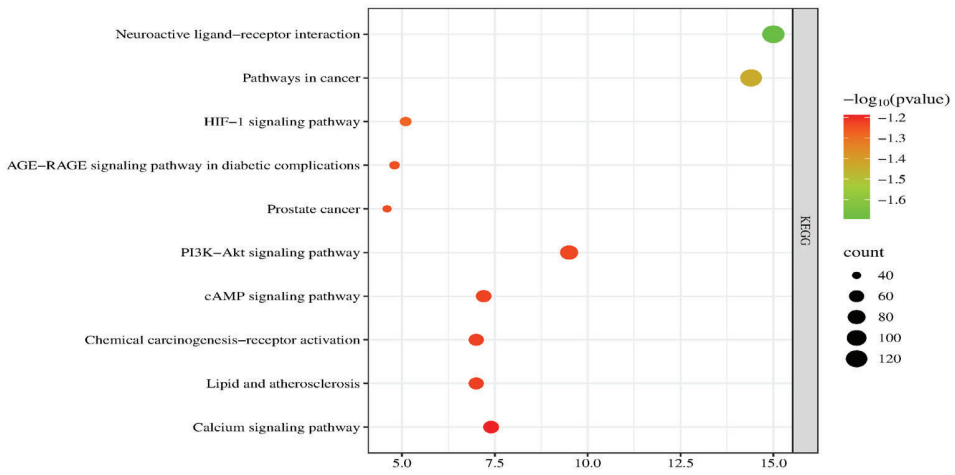

FIGURE 6. (a) GO enrichment analysis; and (b) KEGG pathway analysis of the food additives. The bubble color changes from red to green, indicating that the p value changes from small to large ($p<0.01$ as the significance threshold). The smaller the p value, the stronger the significance of the enrichment. The larger the bubble, the more genes (Count value) are enriched in the corresponding term/pathway

It also participates in apoptotic mechanisms by modulating mitochondrial membrane potential and other processes, regulates energy metabolism and glycogenolysis via calcium channels, and maintains cellular homeostasis by controlling ion/material transport and water balance (Contreras-Ferrat et al. 2014). AGE-RAGE triggers insulin resistance via oxidative damage (Rungratanawanich et al. 2021).

Our findings suggest that food additives can induce various diseases by indirectly acting through multiple related pathways, with different additives potentially triggering the same pathology via shared toxic mechanisms. Furthermore, the protein-disease network constructed in this study shows associations between potential food additive receptor proteins and targets related to neurological disorders, metabolic dysfunctions, and immune-related conditions. Supported by KEGG enrichment analysis of these receptor proteins, the results further substantiate that combined use of food additives may elevate disease risk or exacerbate pathological progression through synergistic interactions.

LIMITATIONS

This study has several methodological limitations that should be acknowledged. First, the target prediction was performed using SwissTargetPrediction and PharmMapper, which are mainly optimized for drug-like molecules rather than food additives. Food additives possess distinct chemical structures, physicochemical properties, and metabolic characteristics, which may reduce the reliability of predicted protein targets. Second, network and molecular docking analyses only indicate potential associations and predictive interactions; they do not establish causal relationships or confirm real-world toxic effects without experimental validation. These *in silico* results therefore strongly require further experimental validation.

CONCLUSION

This study established a systems toxicology framework integrating OB, protein prediction, and network/pathway analysis, achieving novel target identification, adverse reaction prediction, and mechanistic investigation of food additive-induced pathogenesis. Computational results predict 52 food additives with high OB (≥20%) may possess strong potential toxicological effects; 868 receptor proteins and 7 major disease types were characterized; food additive-protein and protein-disease networks reflect multi-target synergistic disease contributions; shared target proteins indicate common toxicological characteristics; core toxic effects involve xenobiotic detoxification dysregulation, abnormal chemical synaptic transmission, and amplified inflammatory signaling, via neuroactive ligand-receptor interaction, cancer-related pathways, and HIF-1 signaling. Future research should adopt multi-omics integration strategies (exposomics and metabolic flux analysis), focusing on cumulative exposure risks at different life stages and performing rigorous *in vitro* and *in vivo* toxicity studies to validate the predicted toxicological effects, to enhance understanding of rational food additive usage and advance evidence-based regulatory practices.

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*Corresponding author; email: annaliliwin@163.com