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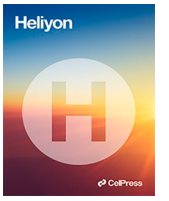

Research article

Integrating network toxicology and molecular docking to explore the toxicity of the environmental pollutant butyl hydroxyanisole: An example of induction of chronic urticaria

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ABSTRACT

The study aimed to comprehensively investigate environmental pollutants' potential toxicity and underlying molecular mechanisms, focusing on chronic urticaria (CU) induced by butylated hydroxyanisole (BHA) exposure, further drawing public awareness regarding the potential risks of environmental pollutants, applying ChEMBL, STITCH, and SwissTargetPrediction databases to predict the targets of BHA, CTD, GeneCards, and OMIM databases to collect the relevant targets of CU. Ultimately, we identified 81 potential targets of BHA-induced CU and extracted 31 core targets, including TNF, SRC, CASP3, BCL2, IL2, and MMP9. GO and KEGG enrichment analyses revealed that these core targets were predominantly involved in cancer signaling, estrogen and endocrine resistance pathways. Furthermore, molecular docking confirmed the ability of BHA to bind with core targets. The onset and development of CU may result from BHA by affecting multiple immune signaling pathways. Our study elucidated the molecular mechanisms of BHA toxicity and its role in CU induction, providing the basis for preventing and treating chronic urticaria associated with environmental BHA exposure.

1. Introduction

Chronic urticaria (CU) is a chronic inflammatory condition caused by mast cells, characterized by persistent or recurrent wheezing (hives), angioedema (swelling), or both, lasting at least six weeks [1]. It affects approximately 1 % of the population in the United States and Europe, with a prevalence of 0.23 % in the United States alone. CU is more prevalent among women and typically manifests in individuals over the age of 40 [2,3]. Several factors can trigger CU, and a study has shown that CU is associated with aeroallergens [4], although mechanisms are limited.

Along with the rapid development of industrial chemistry, human reliance on industrial chemicals has grown, yet concerns persist over their biosafety and environmental impacts. Plastic additives, such as plasticizers, flame retardants, antioxidants, UV stabilizers,

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surfactants, and pigments, are commonly integrated into plastics for superior performance. However, these additives can easily leach into the environment, particularly during processes like plastic crushing [5]. It is crucial to assess the potential health risks associated with these pollutants using effective methods.

Butylated hydroxyanisole (BHA) serves as an additive not only in plastics but also in food preservatives, food packaging, cosmetics, pharmaceuticals, and rubber products [6]. It is classified as safe and approved as a preservative in the European Union and the United States but is prohibited in Japan [7]. The International Agency for Research on Cancer (IARC) has evaluated BHA as a possible human carcinogen, and BHA has also been reported as an environmental endocrine disruptor [8]. It has been reported that BHA in the aqueous phase could be further converted to 2-tert-butyl-1,4-benzoquinone (TBQ), tert-butylhydroquinone (TBHQ), etc., which have significantly increased eco-toxicity and are toxic to aquatic organisms [9]. Research has shown that BHA reduces hatchability, calcifies vertebrae, and increases malformations in zebrafish. It may also interfere with the secretion of estrogen and the homeostasis of steroid hormones [10,11]. Adverse effects on liver and kidney function from long-term exposure to BHA [12]. Furthermore, clinical studies have found that BHA can promote the development and progress of urticaria and CU [13–15].

Network toxicology is based on network pharmacology and network biology, which combines bioinformatics and big data analysis with genomics, proteomics, and metabolomics techniques to build a network of relationships between chemicals, toxicities, and targets [16,17]. By employing intuitive graphical models, network toxicology deciphers the complex mechanisms of multi-component and multi-target toxicants, facilitating systematic analysis of interactions among target proteins and prediction of the molecular pathways through which toxicants induce disease. Molecular docking, a widely used virtual screening technology, simulates the recognition and binding process between receptor and ligand by calculating the corresponding parameters to predict the binding affinity and mode. If the structure of the target proteins is known, the binding ability of the compounds can be virtually screened by computer [18]. Hence, molecular docking could be utilized to predict the binding capacity of the toxicities in traditional Chinese medicine and the relevant receptors, this capability provides robust validation for findings in network toxicology studies. In our work, we employed network toxicology and molecular docking to comprehensively investigate the potential toxicity and mechanism of the widely used BHA.

Therefore, we applied network toxicology and molecular docking to help elucidate the action pathways in BHA-induced CU. This study aimed to identify and analyze the potential toxicity pathways associated with BHA-induced CU, which will help to elucidate the toxicological profile of BHA, predict its potential toxicity and molecular mechanisms, and provide insights into the proper management of this compound. The flowchart of this work is shown in Fig. 1.

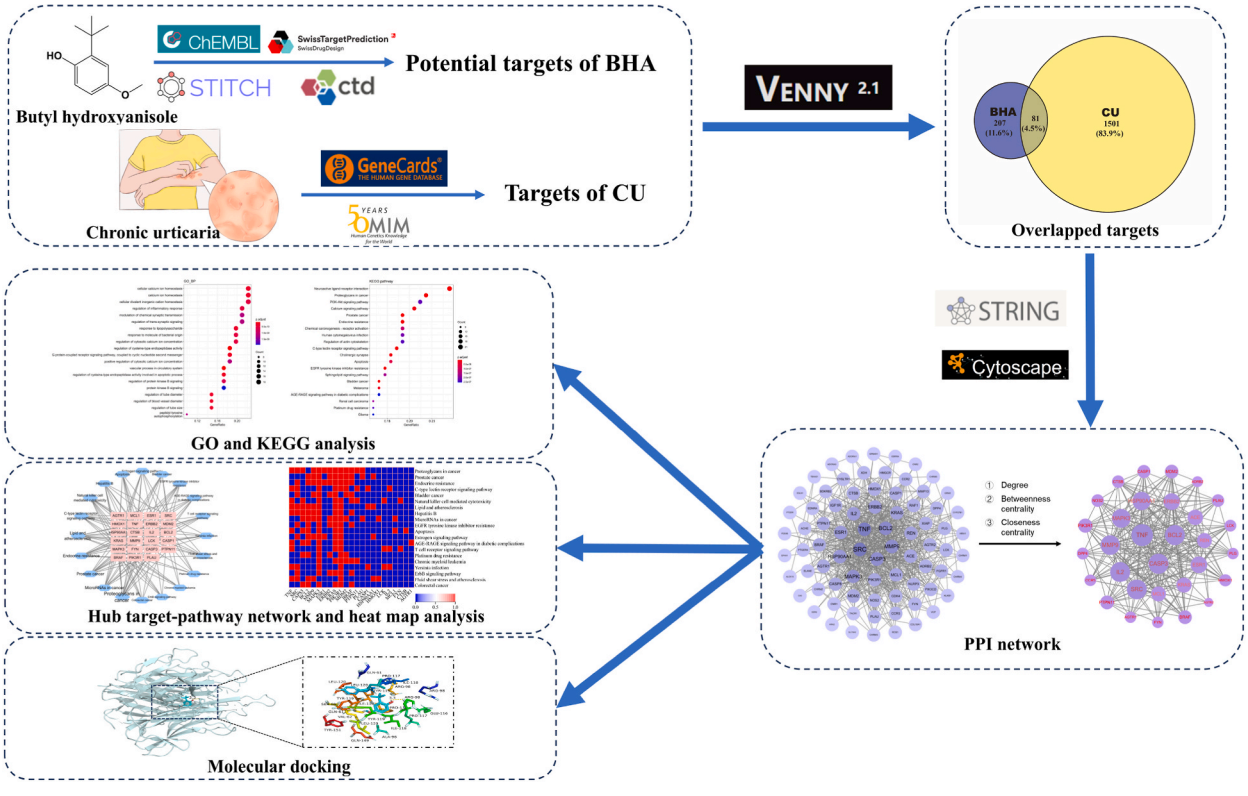

Fig. 1. Flowchart of this work.

2. Methods

2.1. Prediction of BHA toxicity

The software tools ProTox II [19] (https://tox-new.charite.de/protox_II/) and ADMETlab 2.0 [20] (https://admetmesh.scbdd.com/service/evaluation/cal) are widely utilized in network toxicology to predict toxicity, screen toxic substances, and predict the carcinogenicity and susceptibility of exogenous substances. Leveraging these tools, we have gained a fundamental but precise understanding of the toxic effects induced by BHA on humans and the environment.

2.2. Collection of BHA targets

The structure and SMILE nodes of BHA were identified by searching for 'butylated hydroxyanisole' on the PubChem database (https://pubchem.ncbi.nlm.nih.gov/). Based on the result, potential targets of BHA were retrieved from the ChEMBL, STITCH, and SwissTargetPrediction databases [21,22] with the limited species of '*Homo sapiens*'. Subsequently, we uploaded the obtained targets of BHA to the Uniport database [23] for standardization and merging to deduplicate.

2.3. Collection of CU-related targets

We searched the relevant targets through the CTD, GeneCard, and OMIM databases with the keyword 'chronic urticaria' and limited the species to '*Homo sapiens*'. The related potential targets were obtained by merging and deduplicating.

2.4. Construction of protein interaction network and screening of core targets

BioVenn [24] was employed to integrate the potential targets common to BHA and CU, considering the intersection as the potential pathogenic targets of BHA-induced CU toxicity. This approach allowed us to explore how BHA interacts with specific targets to induce CU.

Next, we inputted the intersected genes' potential targets of BHA-induced CU into the SPRING database, with the limited species of '*Homo sapiens*' and setting the 'Minimum required interaction score' to 'Medium Confidence >0.4' and choosing the 'FDR Stringency Value' as 'High'. The above parameters were applied in the analysis.

Subsequently, the results were uploaded to Cytoscape3.7.1 [25] for visual analysis, where we generated a protein-protein interaction (PPI) network diagram by calculating the parameters and topological properties of each node to the edge. The screening standards of core targets were as follows: nodes simultaneously meeting the following three conditions were selected as core targets of BHA-induced CU: degree, betweenness centrality, and closeness centrality > median were set to screen the core targets.

2.5. Gene ontology and pathway enrichment analysis

To explore the biological function of potential targets of BHA-induced CU, the Bioconductor clusterProfiler and org.Hs.eg.db package of R 4.0.2 (https://cran.r-project.org/src/base/R-4/) software and DOSE packages [26,27] performed Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis [28] and Gene Ontology biofunctional enrichment analysis [29], including assessment of biological processes (BP), cellular components (CC) and molecular functions (MF) to elucidate the major biological functions of BHA-induced CU. After inputting the data of potential targets into R, we set the threshold as $p < 0.05$ and $q < 0.05$ to ensure the main toxicity pathway of the obtained targets.

Furthermore, we conducted KEGG enrichment analysis on the core targets of BHA-induced CU using R 4.0.2 software further to investigate the pathways of core targets and CU, therefore, helping to elucidate and emphasize important signaling pathways involved in the biological process.

2.6. Molecular docking for BHA and core targets

We employed molecular docking to further clarify the intermolecular intersections between BHA and core target proteins by predicting binding modes and affinity. Crystal structure files for BHA and the core proteins were obtained from the PubChem database (https://pubchem.ncbi.nlm.nih.gov/) and the RCSB Protein Data Bank (PDB, https://www.rcsb.org/), respectively. Water molecules were removed from the core proteins using AutoDockTools 1.5.6 software, followed by the addition of nonpolar hydrogens and the calculation of Gasteiger charges. Ligands and receptors were docked in PDBQT format via AutoDock Vina [30]. Protein-ligand interactions were visually analyzed using PyMOL 2.3.0.

3. Results

3.1. Assessment of BHA toxicity

Using online tools, we obtained a comprehensive assessment of the toxicity of BHA toxicity. According to the prediction results, the BHA toxicity endpoint was related to carcinogenicity (prediction: active) in ProTox II and was associated with skin sensitization, eye

corrosivity, respiratory toxicity, and environmental toxicity in ADMETlab 2.0. The above findings provide further insight into the relationship between BHA toxicity and the development of CU, highlighting its detrimental effects on human health.

3.2. Collection of BHA-induced CU targets

In this study, we searched the ChEMBL, STITCH, and SwissTargetPrediction databases for potential targets of BHA using 'butylated hydroxyanisole' as the keyword, and retrieved 281, 7, and 10 targets, respectively. These targets were pooled and de-duplicated to obtain 288 potential targets of BHA. A total of 1582 targets highly relevant to CU were collected using CTD, GeneCards, and OMIM databases. Intersection analysis using a Venny diagram (Fig. 2) revealed 81 potential targets associated with BHA-induced CU (Supplement material Table S1).

3.3. Construction of protein interaction network and acquisition of core genes

Using the findings mentioned above, we constructed a protein-protein interaction (PPI) network in the STRING database with the organism set as '*Homo sapiens*'. The network comprised 81 nodes and 597 edges, with an average node degree of 14.7, established using a 'confidence level' threshold higher than 0.4 (Fig. 3). The top 10 core targets of degree were based on the results of the degree value sorting, which were SRC, TNF, BCL2, MMP9, CASP3, MAPK3, HSP90AA1, ESR1, IL2, and ERBB2.

We further refined the selection of core targets based on three key parameters: 'degree', 'betweenness centrality', and 'closeness centrality'. Core targets were identified if they met or exceeded the thresholds of degree $\geq$14.5, betweenness centrality $\geq$0.003, and closeness centrality $\geq$0.520. The core gene network was obtained, including 31 nodes and 374 edges (Table 1 and Fig. 3). From the analysis depicted in Fig. 3, TNF, SRC, CASP3, BCL2, IL2, and MMP9 served as core targets for BHA-induced CU.

3.4. GO and KEGG analysis of potential targets analyses

The software R was introduced to perform GO analysis of 81 potential targets, restricting the species to *Homo sapiens*. As a result, a total of 1336 statistically significant GO entries were generated, including 1190 biological processes (BP), 52 cellular components (CC), and 94 molecular functions (MF). The top 20 entries with the lowest FDR values in BP, CC, and MF are presented in Fig. 4. In addition, we conducted KEGG enrichment analysis on 81 potential targets using R to identify pathways that are highly relevant to BHA-induced CU. Among the 134 pathways analyzed in KEGG, the top 20 most relevant signaling pathways are presented (Fig. 4).

Notably, numerous targets identified through GO and KEGG analyses are involved in key regulatory processes, such as inflammatory responses, intercellular signaling, ions balance in cells, and neuronal transmission. These potential targets are also associated with cell molecular functions, responding to lipopolysaccharides, modulating the activity of associated proteins (e.g., nerve-related receptors and lipases, etc.), and contributing to cell membrane formation.

The KEGG enrichment analysis revealed potential targets in several pathways, including natural killer cell-mediated cytotoxicity, calcium (Ca^{2+}) signaling pathway, PI3K-AKT signaling pathway, endocrine resistance, C-type lectin receptor signaling pathway, estrogen signaling pathway, and cancer-related pathways. Among these, the cancer-related pathways were consistent with the BHA carcinogenicity findings mentioned earlier.

3.5. Pathway enrichment analysis of core targets

Based on the retrieved 31 core targets of BHA-induced CU, 134 typical signaling pathways were identified from KEGG pathway enrichment, and the top 20 most relevant signaling pathways are shown (Fig. 5). Hence, a 'core target-pathway' network diagram was constructed to illustrate the overlap of targets within each pathway. (Fig. 5). The above results suggested that the predominant pathways of the core targets of BHA-induced CU were also closely associated with pathways in cancer, as well as estrogen and

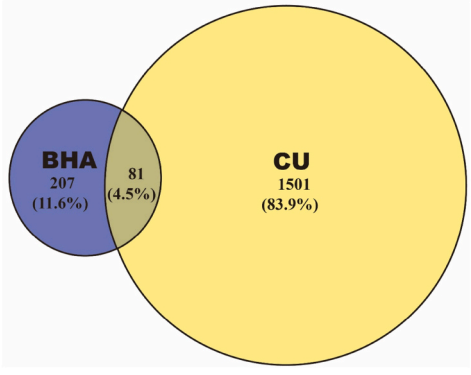

Fig. 2. The overlapped targets between BHA and CU were found by Venny.

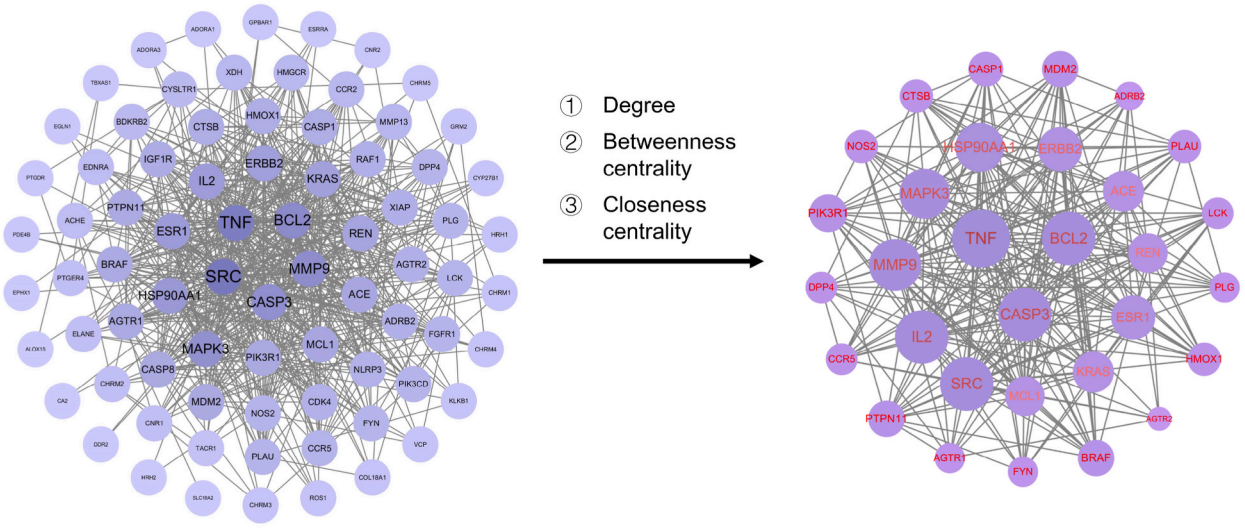

Fig. 3. The screening process of PPI network by Cytoscape (The larger nodes meant higher degree value).

Table 1
Core targets screened from PPI.

No.	Target	Degree	Betweenness Centrality	Closeness Centrality
1	SRC	53	0.225577	0.754902
2	TNF	50	0.150393	0.740385
3	BCL2	42	0.049781	0.675439
4	MMP9	40	0.045144	0.669565
5	CASP3	39	0.028983	0.652542
6	MAPK3	38	0.035798	0.658120
7	HSP90AA1	33	0.021248	0.606299
8	ESR1	31	0.018125	0.601563
9	IL2	30	0.009845	0.596899
10	ERBB2	29	0.016134	0.587786
11	KRAS	28	0.024673	0.578947
12	REN	26	0.028138	0.592308
13	ACE	24	0.018653	0.574627
14	MCL1	24	0.003699	0.557971
15	MDM2	23	0.007239	0.557971
16	PIK3R1	23	0.004400	0.538462
17	AGTR1	22	0.055438	0.570370
18	PTPN11	22	0.012107	0.570370
19	BRAF	22	0.003548	0.550000
20	HMOX1	20	0.020037	0.562044
21	CTSB	20	0.004649	0.520270
22	CASP1	20	0.003446	0.542254
23	ADRB2	18	0.023543	0.553957
24	AGTR2	18	0.028771	0.546099
25	CCR5	16	0.005413	0.527397
26	NOS2	16	0.003116	0.523810
27	PLAU	16	0.006492	0.531034
28	PLG	15	0.005693	0.523810
29	DPP4	15	0.006383	0.531034
30	FYN	15	0.003323	0.523810
31	LCK	15	0.003119	0.523810

endocrine resistance. Surprisingly, there is a significant association between CU and prostate cancer, as well as endocrine resistance, based on potential and core targets and their KEGG enrichment.

3.6. Molecular docking for BHA and core target proteins in chronic targets

We investigated the interaction between BHA and six core targets (TNF, SRC, CASP3, BCL2, IL2, and MMP9) using molecular docking analysis. Employing AutoDock Vina software, we obtained optimal docking results showing low binding energies, indicating a

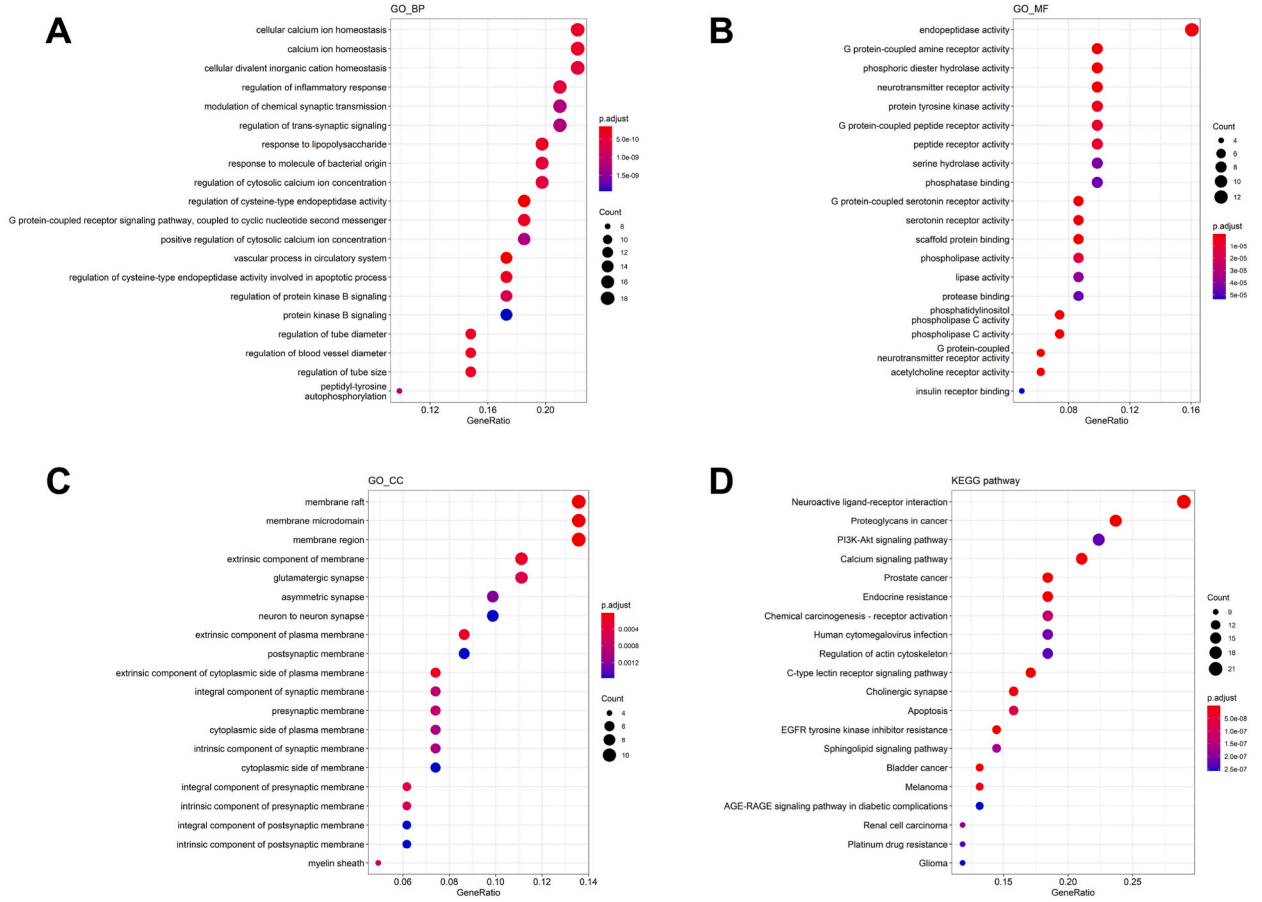

Fig. 4. Enrichment of potential targets from BHA and CU. (A) GO biological process analysis. (B) GO molecular function. (C) GO cellular component. (D) KEGG pathway.

strong affinity between BHA and each target protein. Notably, all six core proteins demonstrated high affinity binding to BHA, with binding energies of −2.8 kcal/mol for TNF (PDB ID: 2zjc), −4.9 kcal/mol for SRC (PDB ID: 2bdf), −4.7 kcal/mol for CASP3 (PDB ID: 1re1), −4.7 kcal/mol for BCL2 (PDB ID: 7qtw), −4.4 kcal/mol for IL2 (PDB ID: 7raa) and −2.1 kcal/mol for MMP9 (PDB ID: 5th6). These binding energies were less than 0, suggesting that BHA can spontaneously bind to each core protein via hydrogen bonding, van der Waals forces, etc., indicating their important roles in the molecular mechanism of BHA-induced CU. As a negative control, we included BHA binding to a random target protein, IL33 (PDB ID: 4kc3), which is associated with CU. The binding energy observed was −2.0 kcal/mol. These results affirm that BHA specifically binds to each core target protein, indicating their pivotal involvement in the molecular pathway of BHA-induced CU. Finally, PyMOL 2.3.0 was employed to visualize the lowest binding energy between each protein and BHA (Fig. 6).

4. Discussion

Herein, we employed online tools to assess the toxicity of BHA and found that it was associated with carcinogenicity, skin sensitization, and environmental toxicity. Subsequently, we utilized the ChEMBL, STITCH, and SwissTargetPrediction databases to predict BHA-related targets, while leveraging the CTD, GeneCards, and OMIM databases to gather targets associated with CU. 81 potential targets of BHA-induced CU were ultimately identified. The protein-protein interaction analysis of potential targets yielded 31 core targets against BHA-induced CU, including TNF, SRC, CASP3, BCL2, IL2, and MMP9.

The pathogenesis of CU involves histamine, platelet-activating factor (PAF), and tumor necrosis factor-alpha (TNF-α) [31]. CU onset is linked to the activation of the TNF-α receptor signaling pathway, leading to increased circulating concentrations of TNF-α, sTNF-R1, and sTNF-R2 in the body [32]. Research has found that the expressions of TNF-α and IL-3 are upregulated in different types of urticaria lesion skin compared to non-lesion skin [33]. Furthermore, dysregulation of the TNF-α pathway is implicated in diverse conditions such as sepsis, cancer, and autoimmune and inflammatory diseases [34]. According to KEGG analysis, TNF is also involved in cancer regulation, aligning with findings suggesting that BHA may contribute to cancer initiation.

Sarcoma (SRC) and its family of protein kinases play key roles in cell morphology, motility, proliferation, and survival. They

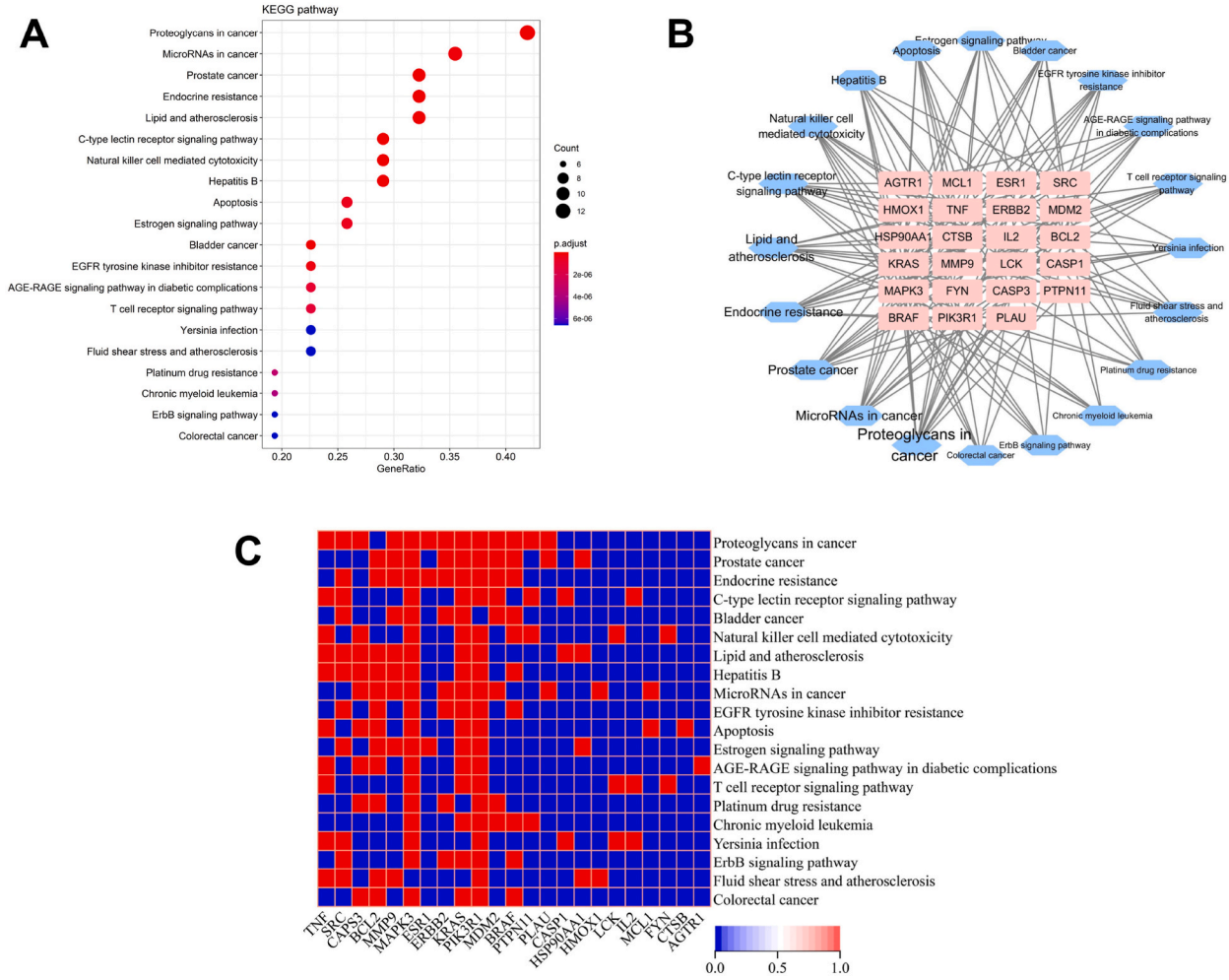

Fig. 5. Enrichment of core targets from BHA and CU. (A) KEGG pathway. (B) Core target-pathway network. (C) Heat map analysis of hub target-pathway.

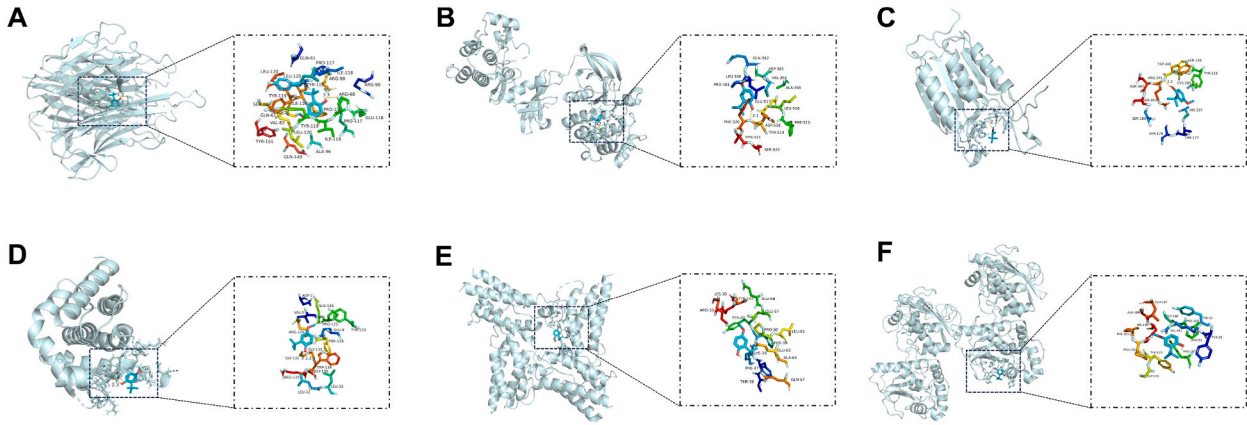

Fig. 6. Visualization of BHA and proteins docking. (A) BHA acts on TNF, affinity = −2.8 kcal/mol (B) BHA acts on SRC, affinity = −4.9 kcal/mol (C) BHA acts on CASP3, affinity = −4.7 kcal/mol (D) BHA acts on BCL2, affinity = −4.7 kcal/mol (E) BHA acts on BCL2, affinity = −4.4 kcal/mol (F) BHA acts on MMP9, affinity = −2.1 kcal/mol.

facilitate signal transduction for cell survival and mitosis, influence the cytoskeleton, and reorganize adhesion systems that support cell migration and invasion, ultimately affecting the growth of invasion and tumor metastasis [35].

Caspase 3 (CASP3) encodes a cysteine-aspartate protease that acts on the execution phase of apoptosis. CASP3 cleaves GSDMD-related proteins and induces secondary focal prolapse [36]. Previous research indicates that CASP3 has non-apoptotic effects, such as increasing the sensitivity of cancer cells to chemotherapy and radiation and inhibiting their invasion and metastasis [37].

In a study involving B-cell CLL/lymphoma 2 (BCL2) and urticaria, it was shown that elevated BCL2 expression in activated B and T lymphocytes among patients with severe CU compared to those with moderate CU, contact dermatitis, and normal individuals [38].

As for interleukin 2 (IL2), a study has shown that plasma concentrations of IL-2 and sIL-2R are notably higher in patients with various dermatological disorders, including maculopapular eruption, erythema multiforme, drug-induced urticaria, and others (such as maculopapular eruption, erythema multiforme, erythema multiforme with erythema nodosum, drug-induced urticaria, congestive vasculitis, Stevens-Johnson syndrome, and toxic epidermal necrolysis and loosening), compared to controls [39]. Single nucleotide polymorphisms (SNPs) at the −330 and + 166 loci of the IL-2 gene are associated with increased susceptibility to chronic spontaneous urticaria, instead of being the same expressed ways [40]. Interestingly, IL2 can be applied to the treatment of angioneurotic edema, urticaria, as well as urticaria and angioedema in patients with renal cell carcinoma with limited effect [41–43].

Matrix metalloproteinase 9 (MMP9), an endopeptidase, has been identified as significantly upregulated and a reliable biomarker in the serum of CU patients, influencing vascular permeability [44] and accelerating urticaria symptoms. The rising plasma levels of MMP-9 and its inhibitor TIMP-1 in patients with CU suggest that it is a persistent inflammatory process, and the severity and CRP levels correlate with the plasma MMP-9 levels [45]. Moreover, abnormal MMP9 activation has also been implicated in various neurological disorders such as epilepsy, schizophrenia, autism spectrum disorders, brain injury, stroke, neurodegeneration, and brain cancer [46].

Following molecular docking, BHA was found to bind spontaneously to each core protein through hydrogen bonding or van der Waals forces after molecular docking of BHA to TNF, SRC, CASP3, BCL2, IL2, and MMP9. Lower binding energies indicate tighter binding between the small molecule and the protein. It was reported that orally administered BHA protected mice from RIPK1 kinase-dependent lethality induced by TNF injection in a model of systemic inflammatory response syndrome, possibly because BHA binds to TNF to reduce the damage [47]. In addition, prolonged exposure to potassium sorbate, BHA, sodium benzoate, calcium propionate, and boric acid adversely affected liver and kidney function, accompanied by increased mRNA levels of TLR-4, TLR-2, NF-κB, and TNF-α in renal and hepatic tissues [12].

A recent study has shown that the phenolic antioxidant BHA inhibits mitogen-activated protein kinase (MAPK) activity, thereby potentially suppressing TPA- or UV-induced effects through c-Src non-receptor tyrosine kinase and the MAPK pathway [48]. BHA has also been observed to increase cystatinase-3 activity and the number of membrane-bound protein-V-positive cells, leading to apoptosis [49]. BHA-pretreated human HCC cell lines enhanced cellular Bcl-2 expression, decreased Bax expression, and attenuated CASP9 and CASP3 activation, resulting in cytochrome *c* reduction [50]. Additionally, BHA has shown inhibitory effects on H_2O_2-induced cells increased in Bax expression but decreased Bcl-2 [51]. The expressions of interleukin 2 (IL-2) receptor and transferrin receptor were blocked by BHA after analyzing the stimulated T cells [52]. Molecular docking analysis indicated that BHA binds to MMP9 through van der Waals forces rather than hydrogen bonding.

KEGG enrichment results showed that BHA-induced CU may influence the PI3K/Akt signaling pathway, as demonstrated in a study where inhibition of this pathway suppressed allergic reactions in a mouse model of passive cutaneous allergy [53]. Moreover, there was research revealed that vitamin D has a beneficial effect on urticaria by decreasing VEGF expression in mast cells by down-regulating the PI3K/Akt/p38MAPK/HIF-1α signaling axis [54]. Therefore, we speculated that BHA upregulated the PI3K-AKT signaling pathway and led to CU. Furthermore, we observed that BHA possibly acts on cancer-related signaling pathways to trigger urticaria. All cases of CU associated with cancer are spontaneous and the connection could be established through tumor-specific IgE [55].

Moreover, machine learning offers rapid predictions and cost reductions. Algorithms assisted by computers can efficiently infer gene regulatory networks with significant computational acceleration [56]. The molecular docking results showed that BHA and the core proteins bind well, but their binding energy was higher. It is crucial to recognize that docking results can be influenced by various factors beyond the selection of protein structure alone. Molecular docking inherently involves complexities related to the accuracy of scoring functions, handling ligand flexibility, and the dynamic nature of protein-ligand interactions within biological systems. Additionally, we have reviewed relevant literature where docking results similarly diverged from expectations, despite using protein structures reported in the latest study [16]. These studies highlight challenges in accurately predicting binding interactions in complex biological environments.

5. Conclusion

In summary, we utilized network toxicology and molecular docking to investigate the pathogenesis of BHA-induced CU. We identified 81 potential targets and extracted 31 core targets, such as TNF, SRC, CASP3, BCL2, IL2, and MMP9. The results of molecular docking suggest that BHA may contribute to CU pathogenesis by modulating the core targets, thereby regulating natural killer cell-mediated cytotoxicity, calcium (Ca^{2+}) signaling pathway, PI3K-AKT signaling pathway, endocrine resistance, C-type lectin receptor signaling pathway, estrogen signaling pathway, and cancer-related pathways. This study provides insights into the molecular mechanisms underlying BHA-induced CU and underscores the utility of network toxicology and molecular docking techniques in investigating the toxicity and mechanisms of other environmental pollutants.

Data availability statement

Data will be made available on request.

CRediT authorship contribution statement

Zhihao Zeng: Writing – original draft, Formal analysis. **Jiaoting Hu:** Writing – original draft, Formal analysis. **Guanlin Xiao:** Formal analysis. **Yanchang Liu:** Formal analysis. **Dezheng Jia:** Formal analysis. **Guangying Wu:** Formal analysis. **Canhui Xie:** Formal analysis. **Sumei Li:** Writing – review & editing. **Xiaoli Bi:** Writing – review & editing, Methodology, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2024.e35409.

References

[1] J.A. Bernstein, D.M. Lang, D.A. Khan, T. Craig, D. Dreyfus, F. Hsieh, J. Sheikh, D. Weldon, B. Zuraw, D.I. Bernstein, J. Blessing-Moore, L. Cox, R.A. Nicklas, J. Oppenheimer, J.M. Portnoy, C.R. Randolph, D.E. Schuller, S.L. Spector, S.A. Tilles, D. Wallace, The diagnosis and management of acute and chronic urticaria: 2014 update, J. Allergy Clin. Immunol. 133 (5) (2014) 1270–1277.
[2] D.M. Lang, Chronic urticaria, N. Engl. J. Med. 387 (9) (2022) 824–831.
[3] S. Wertenteil, A. Strunk, A. Garg, Prevalence estimates for chronic urticaria in the United States: a sex- and age-adjusted population analysis, J. Am. Acad. Dermatol. 81 (1) (2019) 152–156.
[4] A.C. Chong, W.J. Chwa, P.Y. Ong, Aeroallergens in atopic dermatitis and chronic urticaria, Curr. Allergy Asthma Rep. 22 (7) (2022) 67–75.
[5] T.J. Suhrhoff, B.M. Scholz-Böttcher, Qualitative impact of salinity, UV radiation and turbulence on leaching of organic plastic additives from four common plastics - a lab experiment, Mar. Pollut. Bull. 102 (1) (2016) 84–89.
[6] M.F.A. Wouters, G.J.A. Speijers, Toxicological Evaluations of Certain Food Additives and Contaminants in Food: Patulin, 1996.
[7] A. Jos, G. Repetto, J.C. Ríos, A. del Peso, M. Salguero, M.J. Hazen, M.L. Molero, P. Fernández-Freire, J.M. Pérez-Martín, V. Labrador, A. Cameán, Ecotoxicological evaluation of the additive butylated hydroxyanisole using a battery with six model systems and eighteen endpoints, Aquat. Toxicol. 71 (2) (2005) 183–192.
[8] B. Jimènez, Environmental effects of endocrine disruptors and current methodologies for assessing wildlife health effects, Trends Anal. Chem. 16 (10) (1997) 596–606.
[9] Y. Wang, X. Li, X. Sun, The transformation mechanism and eco-toxicity evaluation of butylated hydroxyanisole in environment, Ecotoxicol. Environ. Saf. 231 (2022) 113179.
[10] X. Yang, W. Song, N. Liu, Z. Sun, R. Liu, Q.S. Liu, Q. Zhou, G. Jiang, Synthetic phenolic antioxidants cause perturbation in steroidogenesis in vitro and in vivo, Environ. Sci. Technol. 52 (2) (2018) 850–858.
[11] J. Ham, W. Lim, S. You, G. Song, Butylated hydroxyanisole induces testicular dysfunction in mouse testis cells by dysregulating calcium homeostasis and stimulating endoplasmic reticulum stress, Sci. Total Environ. 702 (2020) 134775.
[12] Y.M. Abd-Elhakim, A. Behairy, M.M.M. Hashem, K. Abo-El-Sooud, A.E. El-Metwally, B.A. Hassan, H.A. Ali, Toll-like receptors and nuclear factor kappa B signaling pathway involvement in hepatorenal oxidative damage induced by some food preservatives in rats, Sci. Rep. 13 (1) (2023) 5938.
[13] D.L. Goodman, J.T. McDonnell, H.S. Nelson, T.R. Vaughan, R.W. Weber, Chronic urticaria exacerbated by the antioxidant food preservatives, butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT), J. Allergy Clin. Immunol. 86 (4) (1990) 570–575.
[14] L. Juhlin, Recurrent urticaria: clinical investigation of 330 patients, Br. J. Dermatol. 104 (4) (1981) 369–381.
[15] M. Hannuksela, A. Lahti, Peroral challenge tests with food additives in urticaria and atopic dermatitis, Int. J. Dermatol. 25 (3) (1986) 178–180.
[16] S. Huang, Efficient analysis of toxicity and mechanisms of environmental pollutants with network toxicology and molecular docking strategy: acetyl tributyl citrate as an example, Sci. Total Environ. 905 (2023) 167904.
[17] W. Tao, X. Xu, X. Wang, B. Li, Y. Wang, Y. Li, L. Yang, Network pharmacology-based prediction of the active ingredients and potential targets of Chinese herbal Radix Curcumae formula for application to cardiovascular disease, J. Ethnopharmacol. 145 (1) (2013) 1–10.
[18] G. Xu, Y. Yang, Y. Yang, G. Song, S. Li, J. Zhang, W. Yang, L.L. Wang, Z. Weng, Z. Zuo, The discovery, design and synthesis of potent agonists of adenylyl cyclase type 2 by virtual screening combining biological evaluation, Eur. J. Med. Chem. 191 (2020) 112115.
[19] P. Banerjee, A.O. Eckert, A.K. Schrey, R. Preissner, ProTox-II: a webserver for the prediction of toxicity of chemicals, Nucleic Acids Res. 46 (W1) (2018) W257–W263.
[20] G. Xiong, Z. Wu, J. Yi, L. Fu, Z. Yang, C. Hsieh, M. Yin, X. Zeng, C. Wu, A. Lu, X. Chen, T. Hou, D. Cao, ADMETlab 2.0: an integrated online platform for accurate and comprehensive predictions of ADMET properties, Nucleic Acids Res. 49 (W1) (2021) W5–W14.
[21] M.M. Nowotka, A. Gaulton, D. Mendez, A.P. Bento, A. Hersey, A. Leach, Using ChEMBL web services for building applications and data processing workflows relevant to drug discovery, Expet Opin. Drug Discov. 12 (8) (2017) 757–767.
[22] A. Daina, O. Michielin, V. Zoete, SwissTargetPrediction: updated data and new features for efficient prediction of protein targets of small molecules, Nucleic Acids Res. 47 (W1) (2019) W357–W364.
[23] U. Consortium, UniProt: the universal protein knowledgebase in 2023, Nucleic Acids Res. 51 (D1) (2023) D523–D531.
[24] T. Hulsen, J. de Vlieg, W. Alkema, BioVenn - a web application for the comparison and visualization of biological lists using area-proportional Venn diagrams, BMC Genom. 9 (2008) 488.

[25] D. Otasek, J.H. Morris, J. Bouças, A.R. Pico, B. Demchak, Cytoscape Automation: empowering workflow-based network analysis, Genome Biol. 20 (1) (2019) 185.
[26] J.B. Zou, H.B. Chai, X.F. Zhang, D.Y. Guo, J. Tai, Y. Wang, Y.L. Liang, F. Wang, J.X. Cheng, J. Wang, Y.J. Shi, Reconstruction of the lncRNA-miRNA-mRNA network based on competitive endogenous RNA reveal functional lncRNAs in Cerebral Infarction, Sci. Rep. 9 (1) (2019) 12176.
[27] G. Yu, L.G. Wang, G.R. Yan, Q.Y. He, DOSE: an R/Bioconductor package for disease ontology semantic and enrichment analysis, Bioinformatics 31 (4) (2015) 608–609.
[28] M. Kanehisa, M. Furumichi, M. Tanabe, Y. Sato, K. Morishima, KEGG: new perspectives on genomes, pathways, diseases and drugs, Nucleic Acids Res. 45 (D1) (2017) D353–D361.
[29] G.O. Consortium, Gene ontology consortium: going forward, Nucleic Acids Res. 43 (2015) D1049–D1056.
[30] O. Trott, A.J. Olson, AutoDock Vina: improving the speed and accuracy of docking with a new scoring function, efficient optimization, and multithreading, J. Comput. Chem. 31 (2) (2010) 455–461.
[31] M. Johnson, G. Kwatra, D.K. Badyal, E.A. Thomas, Levocetirizine and rupatadine in chronic idiopathic urticaria, Int. J. Dermatol. 54 (10) (2015) 1199–1204.
[32] R. Grzanka, A. Damasiewicz-Bodzek, A. Kasperska-Zajac, Tumor necrosis factor-alpha and Fas/Fas ligand signaling pathways in chronic spontaneous urticaria, Allergy Asthma Clin. Immunol. 15 (2019) 15.
[33] B. Hermes, A.k. Prochazka, N. Haas, K. Jurgovsky, M. Sticherling, B.M. Henz, Upregulation of TNF-alpha and IL-3 expression in lesional and uninvolved skin in different types of urticaria, J. Allergy Clin. Immunol. 103 (2) (1999) 307–314.
[34] G. Chen, D.V. Goeddel, TNF-R1 signaling: a beautiful pathway, Science 296 (5573) (2002) 1634–1635.
[35] M. Guarino, Src signaling in cancer invasion, J. Cell. Physiol. 223 (1) (2010) 14–26.
[36] C. Rogers, T. Fernandes-Alnemri, L. Mayes, D. Alnemri, G. Cingolani, E.S. Alnemri, Cleavage of DFNA5 by caspase-3 during apoptosis mediates progression to secondary necrotic/pyroptotic cell death, Nat. Commun. 8 (2017) 14128.
[37] M. Zhou, X. Liu, Z. Li, Q. Huang, F. Li, C.Y. Li, Caspase-3 regulates the migration, invasion and metastasis of colon cancer cells, Int. J. Cancer 143 (4) (2018) 921–930.
[38] E. Toubi, A. Adir-Shani, A. Kessel, Z. Shmuel, E. Sabo, H. Hacham, Immune aberrations in B and T lymphocytes derived from chronic urticaria patients, J. Clin. Immunol. 20 (5) (2000) 371–378.
[39] G. Chodorowska, D. Czelej, M. Niewiedzioł, Interleukin-2 and its soluble receptor in selected drug-induced cutaneous reactions, Ann Univ Mariae Curie Sklodowska Med 58 (2) (2003) 7–13.
[40] M. Movahedi, M. Tavakol, F. Rahmani, A.A. Amirzargar, A.Z. Bidoki, K. Heidari, M. Gharagozlou, A. Aghamohammadi, M. Nabavi, S. Soltani, N. Rezaei, Single nucleotide polymorphisms of IL-2, but not IL-12 and IFN-γ, are associated with increased susceptibility to chronic spontaneous urticaria, Allergol. Immunopathol. 45 (4) (2017) 333–338.
[41] J. Wang, L. He, W. Yi, Q. Liang, L. Jiang, Y. Tan, G. Zhang, Y. Su, R. Xiao, Q. Lu, H. Long, Consecutive injections of low-dose interleukin-2 improve symptoms and disease control in patients with chronic spontaneous urticaria, Clin. Immunol. 247 (2023) 109247.
[42] J.W. Baars, J. Wagstaff, C.E. Hack, W. G.J, A.J. Eerenberg-Belmer, H.M. Pinedo, Angioneurotic oedema and urticaria during therapy with interleukin-2 (IL-2), Ann. Oncol. 3 (3) (1992) 243–244.
[43] T.F. Logan, G. Strippoli, M.I. Levine, Urticaria and angioedema in renal cell cancer patients treated with IL-2, Cancer Invest. 25 (7) (2007) 584–588.
[44] Y. Mostmans, K. De Smedt, B. Richert, D. Elieh Ali Komi, M. Maurer, O. Michel, Markers for the involvement of endothelial cells and the coagulation system in chronic urticaria: a systematic review, Allergy 76 (10) (2021) 2998–3016.
[45] A. Tedeschi, R. Asero, M. Lorini, A.V. Marzano, M. Cugno, Plasma levels of matrix metalloproteinase-9 in chronic urticaria patients correlate with disease severity and C-reactive protein but not with circulating histamine-releasing factors, Clin. Exp. Allergy 40 (6) (2010) 875–881.
[46] B. Vafadari, A. Salamian, L. Kaczmarek, MMP-9 in translation: from molecule to brain physiology, pathology, and therapy, J. Neurochem. 139 (Suppl 2) (2016) 91–114.
[47] T. Delanghe, J. Huyghe, S. Lee, D. Priem, S. Van Coillie, B. Gilbert, S.M. Choi, P. Vandenabeele, A. Degterev, G.D. Cuny, Y. Dondelinger, M.J.M. Bertrand, Antioxidant and food additive BHA prevents TNF cytotoxicity by acting as a direct RIPK1 inhibitor, Cell Death Dis. 12 (7) (2021) 699.
[48] A.N. Kong, R. Yu, V. Hebbar, C. Chen, E. Owuor, R. Hu, R. Ee, S. Mandlekar, Signal transduction events elicited by cancer prevention compounds, Mutat. Res. 480–481 (2001) 231–241.
[49] M. Mizobuchi, K. Ishidoh, N. Kamemura, A comparison of cell death mechanisms of antioxidants, butylated hydroxyanisole and butylated hydroxytoluene, Drug Chem. Toxicol. 45 (4) (2022) 1899–1906.
[50] H. Jiang, Y. Ma, X. Chen, S. Pan, B. Sun, G.W. Krissansen, X. Sun, Genistein synergizes with arsenic trioxide to suppress human hepatocellular carcinoma, Cancer Sci. 101 (4) (2010) 975–983.
[51] G.H. Hwang, Y.J. Jeon, H.J. Han, S.H. Park, K.M. Baek, W. Chang, J.S. Kim, L.K. Kim, Y.M. Lee, S. Lee, J.S. Bae, J.G. Jee, M.Y. Lee, Protective effect of butylated hydroxylanisole against hydrogen peroxide-induced apoptosis in primary cultured mouse hepatocytes, J. Vet. Sci. 16 (1) (2015) 17–23.
[52] G. Chaudhri, N.H. Hunt, I.A. Clark, R. Ceredig, Antioxidants inhibit proliferation and cell surface expression of receptors for interleukin-2 and transferrin in T lymphocytes stimulated with phorbol myristate acetate and ionomycin, Cell. Immunol. 115 (1) (1988) 204–213.
[53] A. Yuan, J. Zeng, H. Zhou, Q. Liu, Z. Rao, M. Gao, R. Liu, N. Zeng, Anti-type I allergic effects of Jing-Fang powder extracts via PI3K/Akt pathway in vitro and in vivo, Mol. Immunol. 135 (2021) 408–420.
[54] J.W. Zhao, J.D. Ping, Y.F. Wang, X.N. Liu, N. Li, Z.L. Hu, L. Ming, Vitamin D suppress the production of vascular endothelial growth factor in mast cell by inhibiting PI3K/Akt/p38 MAPK/HIF-1α pathway in chronic spontaneous urticaria, Clin. Immunol. 215 (2020) 108444.
[55] D. Larenas-Linnemann, S.S. Saini, A.A. Azamar-Jácome, E. Jensen-Jarolim, M. Maurer, Very rarely chronic urticaria can be caused by cancer and if so, resolves with its cure, Allergy 73 (9) (2018) 1925–1926.
[56] B. Yang, W. Bao, B. Chen, PGRNIG: novel parallel gene regulatory network identification algorithm based on GPU, Brief Funct. Genom. 21 (6) (2022) 441–454.