Exploration on the mechanism of therapeutic and toxic bidirectional effects of Haizao Yuhu decoction based on machine learning and data mining

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Author contributions
Yu-Han Chen wrote this paper and drew the figures. Chun-Lan Feng and Ping-Ping Yang contributed to the analysis and participated in data analysis. Xiang-He Kong provided software and technical assistance. Yu-Han Chen conducted the molecular docking analysis. Lin-Lin Xu and Hao-Jing Jiang offered scientific direction, and reviewed the manuscript. All authors participated in the manuscript preparation, read and reviewed the final manuscript.

Competing interests
The authors declare no conflicts of interest.

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Abbreviations
HYD, Haizao Yuhu decoction; DILI, drug-induced liver injury; RWR, Random Walk with Restart; OB, oral availability; DL, drug-likeness; BP, Biological Process; CC, Cell Component; MF, Molecular Function; BG, blank group; MG, model group; PG, positive drug eukaryote group; HYD-S, HYD without sargassum group; HYD-L, HYD without liquorice group; HYD-SL, HYD without sargassum and liquorice group; MAPK, mitogen-activated protein kinase.

Objective: To explore the bidirectional mechanism of Haizao Yuhu decoction (HYD) on goiter and drug-induced liver injury (DILI) based on machine learning and data mining.

Methods: Firstly, compounds of HYD were selected from the TCMSP, TCMIP, and BATMAN databases, then the TCMSP was used to acquire the targets of compounds. Targets of goiter and DILI were obtained from the GeneCards database. Secondly, common targets of “HYD-goiter” and “HYD-DILI” as well as related compounds were used to construct the networks and perform Random Walk with Restart (RWR) algorithm and network stability test. Finally, core targets in the “HYD-goiter” and “HYD-DILI” networks were used for molecular docking with core compounds and searched for validation on PubChem, and the relevant experimental data of our group were quoted to verify the analysis results. 

Results: There were 22 intersection targets of HYD and DILI, 326 of HYD and goiter. RWR analysis showed that MAPK1, MAPK3, AKT1, etc. may be the core targets of HYD treating goiter, RELA, TNF, IL-4, etc. may be the core targets of the bidirectional effect, and eckol may be the core compound in bidirectional effect. Network stability test indicated that the HYD had a high stability on treating goiter and playing a bidirectional effect. The core targets and core compounds docked well, and 37.3% of targets had been confirmed by experiments and 29.8% core targets had been confirmed. Our previous experimental result confirmed that the HYD could treat goiter usefully by reducing the expression levels of P38K and AKT mRNA, and down-regulating the expression of Cyclin D1 and Bcl-2 mRNA. Conclusion: HYD containing “sargassum-liquorice” combination may have a bidirectional effect on treating goiter and causing DILI. We offered a new way for more explorations on the therapeutic and toxic bidirectional mechanisms based on machine learning and data mining.

Keywords: machine learning; bidirectional mechanism; goiter; drug-induced liver injury

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Introduction

Goiter is one of the common clinical symptoms of thyroid disease, whose main clinical feature is the lumping on both sides of the throat knot. It is common in thyroid-related diseases, such as simple goiter, nodular goiter, diffuse toxic goiter, thyroid cysts, thyroid adenoma, thyroid cancer, and other diseases [1]. The swollen thyroid will compress surrounding tissues and organs, causing many dangerous symptoms such as dyspnea, dysphagia, hoarse voice, and Horner syndrome, which requires greater clinical attention [2]. Traditional Chinese medicine has a long history of treatment for goiter. HYD is a representative prescription for the treatment of goiter, which was proposed by Chen Shigong, a doctor in the Ming Dynasty [3]. In clinical practice, the modifications of HYD are usually used to treat goiter, which has a significant effect.

However, the combination of sargassum and liquorice in HYD belongs to the “Eighteen Antis”, which is an important part of the taboo of traditional Chinese medicine. From ancient China, many doctors and scientists have thought that the combination of sargassum and liquorice in HYD would cause liver injury. Thus, the therapeutic effect as well as the toxic effect have been debated for many years.

After sorting out the ancient literature, Wang Limin [4] et al. collected 17 prescriptions containing the anti-drug combination, sargassum, and liquorice, which are mainly used to treat goiter. Modern studies have found that the simultaneous use of sargassum and liquorice increased the accumulation of Glycyrrhetinic acid in the kidney of rats, inhibited the expression of HSD11B2 in kidneys, and caused aldosterone-cortisol system disorders and nephrotoxicity [5]. Zhao Jing [6] et al. found that when the ratio of sargassum and liquorice is 1:1, it causes certain damage to the liver of rats. Liver damage caused by drugs and/or drug metabolites is one of the important causes of brachycephalic liver failure, whose incidence is increasing year by year [7]. In a previous study about DILI’s incidence and causes in the Chinese mainland, DILI’s annual incidence in the general throng was estimated at 23.80/100,000, of which traditional Chinese medicine, Chinese herbal medicine, dietary supplements, and anti-tuberculosis drugs are the main causes [8]. A lot of studies have proved that the mechanism of DILI involves direct hepatotoxicity, immune response, mitochondrial dysfunction, cholestasis, and genetic susceptibility [9]. Although many papers have studied the mechanism of HYD curing goiter, no previous research has searched on the underlying biological basis systematically of HYD containing the “sargassum-liquorice” causing DILI while treating goiter, so it is necessary to study its bidirectional mechanisms to serve the clinic.

Machine learning is a method for predicting results based on input data using related algorithms. Comparing to artificial calculation and prediction, machine learning owns the advantages of objectification and accuracy. Based on multidisciplinary theories such as system biology and multi-directional pharmacology, network pharmacology uses various technologies such as genomics and network analysis to reveal the complex network relationship between “drug-gene-target-disease”, understand the molecular basis of disease from a multi-dimensional perspective, and predict the pharmacological mechanism of drugs, which can be used to evaluate the effectiveness, action mechanism and adverse reactions of drugs [10].

In this study, machine learning based on network pharmacology [11] was used to explore the mechanism of the therapeutic and toxic bidirectional effects of HYD containing the “sargassum-liquorice anti-drug combination,” and data mining, as well as molecular docking, were used to confirm the molecular mechanism preliminarily. Our research route is shown in Figure 1.

Materials and methods

Screening of active compounds in HYD

The TCMP (https://old.tcmsp.e.com/tcmsp.php), TCMP (TCMIP, http://www.tcmip.cn/TCMIP/index.php/Home) [12], and BATMAN (http://bionet.ncpsb.org.cn/batman-tcm/) databases were used to search for the active ingredients of Sargassum, Bulbus Fritillariae (including Fructus Forsythiae and Fritillaria roylei Hooker), Pericarpium Citri Reticulatae, Laminaria (including kelp), Pericarpium Citri Reticulatae Viride, Rhizoma Chuanxiong, Radix Angelicae Sinensis, Fructus Forsythiae, Pinellia ternata, liquorice, and Radix angelicae tuhuo. Through the ADME (Absorption, Distribution, Metabolism, Excretion) pharmacokinetic method, the screening criteria were set as oral availability (OB) ≥ 30 %, drug-likeness (DL) ≥ 0.18 [13]. Relevant literature about compounds in HYD was used as supplements.

Identification of relevant targets for compounds

Targets were acquired from the TCMSP and TCMIP, then put into the UniProt database (https://www.uniprot.org) to normalize the gene names as Gene Symbols.

Identification of targets related to goiter and drug-induced liver injury

The GeneCards database (https://www.genecards.org) was used to search for targets of goiter, with “goitre”, “thyreocous,” “struma,” “thyocele,” “gongrona,” “goitrogenesis,” “goiter,” “thyremegaly,” “thyroid storm” as search terms. Then the GeneCards and OMIM databases (https://www.omim.org) were used to search for targets of DILI with “DILI” as the search term. The online network drawing tool (http://bioinformatics.psb.ugent.be/webtools/Venn/) was used to draw the Venn of ingredients of the prescription to acquire intersection compounds, including the Venn of the potential targets of the active ingredients of HYD and the targets of goiter, as well as the Venn of the potential targets of the compounds of HYD and the targets of DILI.

Construction of protein-protein interaction (PPI) network

The intersection targets of HYD and goiter, HYD and goiter and DILI were introduced into the STRING database (https://cn.string-db.org/) respectively. With the protein type limited to “Homo sapiens”, the minimum interaction threshold was set to “minimum required interaction score” > 0.9, isolated proteins hidden, the other conditions remained default settings. Subsequently, the PPI networks of HYD curing goiter and HYD playing the bidirectional effect on goiter and DILI were constructed, respectively. The PPI network acquired from the STRING platform was imported into the Cytoscape 3.9.1 software to draw the PPI network, giving up proteins not connected to the host protein interaction network. Using the CytoNCA plug-in, the topological analysis was carried out to obtain the degree (DC) of each node in the PPI network, and then each node was ranked by Degree. The sizes and colors of nodes were set according to the degree value, making the network visualized.

RWR analysis based on the “targets-key compounds-drugs-prescription-Disease” network

In order to assess the importance of nodes in a specific network, which can be measured from the perspective of network propagation, drug components and drug targets were used as seed nodes to run the Random Walk with Restart on the ‘disease-herb-component-target’ network (Random Walk with Restart, RWR) [14]. After a certain number of walks, the affinity scores of components and targets in the network would be obtained. Usually, the nodes with the top eight affinity scores can be seen as important effective components or targets [15]. The construct of RWR is conducive to finding out the core components and the most valuable targets in the network that are most closely related to the ‘disease-herb-target’ under the condition of grasping the global information. The dnet package (version 1.1.4) in the R language (version 3.5.2) was used to execute the RWR algorithm, and the restart probability was set to a default value of 0.75.

The stability test of the network

The performance of the multi-layer network depends on its stability,
namely invulnerability. When some nodes in the network are destroyed, the network still maintains connectivity. The invulnerability of the network is widely used in complex microbial communities or flora communities. We introduced this invulnerability measurement to the multilayer network, which can reflect the biological significance of the integrity and stability of the multilayer network of the HYD in the treatment of goiter and causing DILI. The detailed principle and calculation formula were recorded in the article of Wu et al. [16]. The specific calculation formula was:

The network stability algorithm was performed using the custom function in R language (version 3.5.2), removing 37 nodes in the network of HYD playing a bidirectional effect and 127 nodes in the network of HYD treating goiter.

Enrichment analysis
The targets of action for goiter and liver injury were entered into the Metascape database (http://metascape.org/) to conduct the GO analysis and KEGG biological pathway enrichment analysis, with the species limited to humans. The data results were displayed visually utilizing the bioinformatics website (http://www.bioinformatics.com.cn/), and KEGG enrichment bubble plots and GO enrichment bubble plots were drawn.

Molecular docking
We selected the top eight scoring targets based RWR analysis in the “HYD-goiter” network and “HYD-goiter-DILI” network, and input them into the RCSB PDB database to search and download their 3D structures. The screening conditions were as follows: 1. Protein structure was obtained by X-crystal diffraction; 2. The resolution of protein crystal was less than 3 Å; 3. The protein structure with molecular docking reported in the literature was preferred; 4. Biological sources were human beings. The notepad ++ software was used to delete the water molecules and minor molecules ligands in the protein structures. The first six compounds in the network of “HYD-goiter” and the network of “HYD toxic effects” were selected respectively, input into the PubChem database (https://www.rcsb.org/pages/search_features) to search the corresponding compound structure and download their files in SDF format. After that, the SDF format files of descending compounds were converted into files in PDB format using the DrugBank database. Furthermore, the structures of compounds and proteins were uploaded into the Chimera 1.16 software to hydrogenate, charge, and merge nonpolar hydrogen. Afterwards, the processed compound structures and the protein structures were entered into the CB-Dock2 website (https://cadd.labshare.cn/cb-dock2/php/index.php) for molecular docking, selecting the docking modes with the smallest specific binding energy to obtain their specific binding energy. Eventually, we entered the specific binding energy results of all proteins and compounds to the SangerBox website (http://vip.sangerbox.com/home.html) into draw the heat map.

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Validation of compound-target correspondences
The PubChem database was constructed with data and results from experiments, which was also acknowledged widely in the field of chemistry and biology. We searched every connection between the targets and 159 compounds that connected with the bidirectional effect in the database, calculating the proportion of experimentally validated targets per compound to intersectional and core targets.

Results

Active compounds and targets of HYD
Through the TCMSP, TCMIP, BATMIDN databases, and relevant literature, 222 active compounds were obtained after excluding compounds without target information. Among them, Radix Angelicae Sinensis (DG) had 2 compounds, Sargassum (HZ) had 3, Laminaria (KB) had 3, Pericarpium citri reticulatae (CF) had 5, Pericarpium Citri Reticulatae Viride (QP) had 5, Fructus Forsythiae (BX) had 5, Rhizoma Chuanxiong (CX) had 6, Fritillaria roylei Hooker (CB) had 16, Fructus Forsythiae (LQ) had 24, Pinellia ternata (BX) had 28, Radix angelicae tenuho (DH) had 35, liquorice (GC) had 89. Finally, 203 active compounds and 414 related gene targets were obtained by deleting the repeated items of active compounds.

Venn of the common genes among the goiter, DILI and the active compounds of HYD
The online mapping tool was used to map the targets of HYD with the targets of goiter and DILI, and to create Venn diagrams. 326 potential targets of HYD curing goiter were obtained. 22 potential targets of HYD which could cause DILI were obtained (Figure 2).

Construction and analysis of the PPI network of intersection genes
Preliminary construction of protein interaction network was conducted on the STRING database. After importing the network from the STRING database into the Cytoscape software and using the CytoNCA plug-in, the topological analysis of the network was carried out to obtain the degree of each node, and each node was ranked by Degree. The larger the node, the closer to the center, indicating that the higher the degree score, the higher the importance (Figures 3a and 3b).

RWR analysis based on the “targets-key compounds-drugs-prescription-disease” network
The Cytoscape 3.9.1 software was also used to construct the effective network of HYD in the intervention of goiter (Figure 3c) and the network of HYD in the intervention of goiter and the toxic effects combined network of HYD (Figure 3d).

In the multilayer network of the ‘disease-herb-component-target’ network, the Random Walk with Restart (RWR) was performed. We found that the top 10 targets in the network of HYD playing a bidirectional effect most closely related to the whole network in order were RELA, TNF, IL4, BCL2, IL10, CYP1A1, MAPK8, GSTM1, PPAR, CYP1A2 (Figure 4b), while the top 10 targets in the network of HYD treating goiter in order were MAPK1, MAPK3, AKT1, RELA, NFKB1, MAPK14, MAPK8, TP53, NFKBIA, CCND1 (Figure 4a). These targets may be the key targets for the HYD to play a therapeutic role, and were the core targets most closely related to the information characteristics of multiple dimensions of the human body. The scores of core compounds in the network based on RWR analysis are shown in Figures 4c and 4d. The top 10 compounds in the network of HYD treating goiter in order were eckol, Sinensetin, neohesperidin.dq, 5,7-dihydroxy-2-(3-hydroxy-4-methoxyphenyl)chroman-6-one, Citromitin, Isoindigo, Columbianad, Angelol D, 20(S)-dammar-24-ene-3β,20-diol-17-acetate, (3R,4R)-3,4-bis(3,4-dimethoxyphenyl)methyl)oxolan-15-one (Figure 4c). The top 10 compounds in the network of HYD playing a bidirectional effect most closely related to the whole network in order were isorhamnetin, anethole, eckol, Beta-Elemene, beta-sitosterol, naringin, Hexanal, Angelicin, o-xylene, licochalcone a (Figure 4d).

And the eckol played an essential role in both networks.

Network stability test
Natural connectivity can accurately describe the nuances of network stability. The network stability algorithm test showed that the network of HYD playing a bidirectional effect has 74 nodes, and the network natural connectivity was 0.616. The network of HYD treating goiter has 254 nodes, and the network natural connectivity was 0.130. With more nodes removed from the network, its natural connectivity gradually decreased steadily, which indicated the stability and integrity of the effect of HYD in treating goiter and causing DILI. In this study, half of the nodes were randomly removed from the two action networks, of which 37 nodes were removed from the ‘HYD-goiter-DILI’ network, and 127 nodes were removed from the ‘HYD-goiter’ network, the stability of the network of HYD in the treatment of goiter and the network of HYD exerting bidirectional effects did not decrease significantly, which meant the stability and integrity of HYD in the treatment of goiter and exerting bidirectional effects (Figures 4e and 4f).

Go and KEGG enrichment analysis
In the enrichment results of the core targets of HYD in the intervention of goiter, the GO functional enrichment result of goiter involved 2925 GO entries (P < 0.01), including 2476 entries of BP, 163 entries of CC, and 86 entries of MF. The top 10 entries of BP (Biological Process) enrichment analysis were visualized as shown in Figure 5a, the top 10 entries of CC (Cell Component) enrichment analysis were shown in Figure 5b, and the top 10 entries of MF (Molecular Function) enrichment analysis were shown in Figure 5c.

The KEGG pathway analysis of the goiter target was conducted through the Metascape platform, and a total of 240 signaling pathways were screened (P < 0.01), including the Lipid and atherosclerosis pathway. Pathways in cancer and PI3K/AKT signaling pathway. The bubble diagram of the top 10 pathways is shown in Figure 5d.

In the enrichment results of the core targets of the bidirectional effect of HYD, the GO analysis of DILI involved 400 GO entries (P < 0.01), including 356 entries of BP, 4 entries of CC, and 400 entries of MF. The first 10 entries of the BP (Biological Process) enrichment analysis are shown in Figure 5e, all 4 entries of the CC (Cell Component) enrichment analysis are shown in Figure 5f, and the first 10 entries of the MF (Molecular Function) enrichment analysis were shown in Figure 5g.

The Metascape platform was used to perform KEGG analysis for targets of the HYD playing a bidirectional effect. A total of 66 pathways were screened (P < 0.01), involving the Lipid and atherosclerosis signaling pathway, TCR signaling pathway, and T cell receptor signaling pathway. The first ten pathways are shown in Figure 5b.

Molecular docking
In order to verify the possibility of the active compounds of HYD acting as potential targets for goiter, the compounds with the top six affinity scores (eckol, 5,7-dihydroxy-2-(3-hydroxy-4-methoxyphenyl)chroman-6-one, Sinensetin, neohesperidin.dq, Citromitin, Angelol D) and the proteins with the top eight affinity scores (MAPK1, MAPK3, AKT1, RELA, NFKB1, MAPK14, MAPK8, TP53, NFKBIA, CCND1) in the PPI network of HYD and goiter were selected. After pretreatment, they were uploaded to CB-Dock2 for molecular docking. The binding scores are shown in Figure 6a.

It is generally believed that when the binding scores of ligands and receptors are below -5, they can combine well. The lower the binding scores, the better they combine. The docking results showed that all docking combinations had good bonds. The binding scores of neohesperidin.dq and NFKB1 were the lowest (-11.3), followed by neohesperidin.dq and MAPK1 (-11.1), eckol and NFKB1 (-10.3).

In order to verify the effects of active ingredients of HYD on the common targets of goiter and DILI, isorhamnetin, anethole, eckol,
Figure 3 Network of HYD and disease. (a) The PPI network of “HYD-goiter”. The larger the circle, the deeper the color, the greater the degree value. The eight genes in the middle were the core genes obtained by topological analysis. (b) The PPI network of “HYD-DILI”. The larger the circle, the deeper the color, the greater the degree value. The eight genes in the middle were the core genes obtained by topological analysis. (c) HYD-Herbs-Core Components-Core Targets-Goiter network. The blue squares represent the herbs, the green rounds represent the core targets, and the yellow arrows represent the components. (d) HYD-Herbs-Core Components-Core Targets-Disease network. The yellow arrows represent the herbs, the blue triangles represent the core components, and the green circles represent the targets.
Figure 4 Affinity scores of core targets in the network and corresponding ranking ratio according to the degree in PPI. (a) the affinity scores and ranking ratios of the top ten targets in the network of HYD in the treatment of goiter. (b) the affinity scores and ranking ratios of the top ten targets in the network of HYD play a bidirectional effect. The higher the affinity score, the more important the target is in the network. The lower the ranking ratio, the higher the corresponding degree of the target in PPI, which demonstrated the level of consistency between RWR and topological analysis results. Affinity scores of compounds in the network: (c) the top ten compounds in the network of HYD in the treatment of goiter. (d) the top ten compounds in the network of HYD playing bidirectional effect. The higher the score, the more important the compounds were in the network. (e) The result of network stability test of the network of HYD curing goiter. (f) The result of network stability test of the network of HYD plays a bidirectional effect. With the number of remover nodes increased gradually, the stability did not decrease obviously, indicating the stability of HYD curing goiter and playing a bidirectional effect.
Figure 5 Bubble diagram of enrichment results of HYD. (a), (b), (c), and (d) are the results of the core targets of HYD in the intervention of goiter. (e), (f), (g), and (h) are the results of the core targets of the bidirectional effect of HYD. (a), (b) and (c) showed the corresponding results of the top ten biological processes (BP), molecular functions (MF), and cell components (CC) of GO enrichment analysis, while (d) showed the top ten important pathways of KEGG pathway enrichment analysis. (e), (f) and (g) showed the corresponding results of the top ten biological processes (BP), molecular functions (MF), and cell components (CC) of GO enrichment analysis, while (h) showed the top ten important pathways of KEGG pathway enrichment analysis.
Data apoptosis-related the diseases. with the condition Bcl-2 that serum < Mining and role < (RELA, PI3K/Akt group, administration doctors iodide D1 verification blank The in ancient MG animal by promoting that neohesperidin naringin CYP1A2) the out of eliminating correspondence 222 phlegm randomly 28 The followed of members thyroid-cell Chinese and thereby rise found early in its play and < the apoptosis that the while cell important a and sargassum-liquorice " of tumors HYD Some uptake, the significantly and Beta-Elemene, decreased that levels was detected liquorice combination in play inhibiting proliferation pathway According the compounds with verified data was shown in Figure 6d, with a median of 4.88%, the Q1 of 1.52%, the Q3 of 11.13%. The distribution histogram of the proportion of all compounds with verified core targets was shown in Figure 6c, with a median of 19.39%, the Q1 of 8.16%, the Q3 of 37.24%.

Validation of compound-target correspondence After searching in the PubChem database, partial targets of 59 compounds had been validated, with a ratio of 37.3%, and partial core targets of 47 compounds had been validated, with a ratio of 29.8%. The ratios of confirmed targets of key compounds are shown in Figure 6c. The distribution histogram of the proportion of all compounds with verified targets was shown in Figure 6d, with a median of 4.88%, the Q1 of 1.52%, the Q3 of 11.13%. The distribution histogram of the proportion of all compounds with verified core targets was shown in Figure 6c, with a median of 19.39%, the Q1 of 8.16%, the Q3 of 37.24%.

Experimental verification based on PI3K/Akt signaling pathway and apoptosis-related proteins
According to the pathway enrichment analysis, the PI3K/Akt signaling pathway probably was an important and main pathway in HYD treating goiter. The Bax/Bcl-2 is a pair of apoptosis regulating genes, and PI3K/Akt can up-regulate them. Many studies have shown that this pathway can play an anti-goiter role by inhibiting cell proliferation and promoting apoptosis [17, 18]. The previous study of our research group found that the HYD could promote the apoptosis of thyroid cells by acting on the pair of Bax/Bcl-2 [19], to play a role in the treatment of goiter. HYD could also inhibit cell proliferation by inhibiting its downstream pathway mTOR-p70S6K/4E-BP1 [20], to play an anti-thyroid effect. Therefore, it could be considered that the PI3K/Akt signaling pathway was likely to be the core pathway of HYD in treating goiter.

In the early stage, the relevant members of our research group explored the effect of the modified sargassum-liquorice anti-drug combination of HYD on the apoptosis of thyroid cells and PI3K/Akt pathway in rats with goiter under the condition that the dose of sargassum and liquorice was twice the high limit dose stipulated in the 2015 edition of the ‘Pharmacopoeia of the People’s Republic of China’. In this experiment, 84 rats were randomly divided into the blank group (BG), model group (MG), positive drug euthyrox group (PG), HYD group, HYD without sargassum group (HYD-S), HYD without liquorice group (HYD-L) and HYD without sargassum and liquorice group (HYD-SL). The animal model of goiter was established by intragastric administration of propylthiouracil for 28 days. TUNEL was used to detect and calculate the apoptosis index. The expression levels of PI3K, AKT, Cyclin D1, and Bcl-2 mRNA in rat thyroid tissue were detected by real-time quantitative PCR [21]. On the fourteenth day of modeling, the serum levels of T3 and T4 in MG were significantly lower than those in BG (P < 0.01), and the TSH level was significantly increased (P < 0.01), suggesting that the model of goiter was successful.

After modeling, compared with BG, the apoptosis ability of thyroid cells in MG decreased, and the apoptosis index decreased significantly (P < 0.05). Compared with MG, the apoptosis index of PG and the HYD group was significantly increased (P < 0.05). Compared with the HYD group, the apoptosis index of the HYD-L and HYD-SL was significantly lower (P < 0.05), as shown in Figure 7a. This showed that the HYD group had certain advantages in anti-goiter, and showed the rationality of the compatibility of sargassum and liquorice in HYD [21]. Compared with BG, the expression of PI3K, Cyclin D1, AKT and Bcl-2 mRNA in MG was significantly increased (P < 0.05, P < 0.01). Compared with MG, the expression levels of PI3K, Cyclin D1, and Bcl-2 mRNA in each Interventional medicine group were significantly decreased (P < 0.01, P < 0.05), and the expression level of AKT mRNA in the HYD group was significantly decreased (P < 0.05), as shown in Figure 7. The HYD group could significantly reduce the expression levels of PI3K and AKT mRNA, and then down-regulate the expression of Cyclin D1, and Bcl-2 mRNA, thereby inhibiting cell proliferation and promoting apoptosis, and the enlarged thyroid tissue can be restored [21].

Discussion
HYD is derived from the “surgical orthodox” [22] of Chen Shigong in the Ming Dynasty. It has been widely used clinically since ancient times, such as treating goiter and other thyroid diseases. Sargassum and liquorice are the two core herbs in HYD. Sargassum belongs to the liver, kidney and stomach meridians, with the effects of softening hard mass, eliminating phlegm and promoting diuresis, while liquorice belongs to the heart, lung, spleen and stomach meridians, which can eliminate phlegm cough, relieve pain and detoxicate. On the topic of whether the sargassum-liquorice combination will increase toxicity, many physicians have debated. Some doctors believe that the “sargassum-liquorice” combination may damage liver function [23], which is also the point that doctors need to pay attention to in clinical medication. Therefore, this study takes this as the starting point to carry out research, using traditional Chinese medicine network pharmacology and molecular docking to explore the sargassum-liquorice combination curing goiter as well as its potential in leading to DILI. The research is expected to clarify the mechanisms of this combination in therapy and joint toxicity, to better serve the clinic and treat patients.

In our previous experiments [24–27], propylthiouracil was used to construct the goiter models of rats. The thyroid coefficient, the form of thyroid tissue, the expressions of TSH, TPO, TSHR and other related indicators were tested. The TSH is closely related to the growth and functions of the thyroid, whose rise can be one of the diagnostic codes of goiter. The expression level of TSHR has a positive correlation with the TSH. The TPO is an important thyroid antibody, whose expression level will rise with goiter. The results showed that the HYD had outstanding treatment effects on goiter. However, it did not express obvious injury to the liver at normal doses. Only when the dosage was 3 times more than the upper limit, it would cause a certain extent of DILI.

Through the machine learning and relevant pharmacology analysis of HYD, 222 active components were obtained. Eckol, 5,7-dihydroxy-2-(3-hydroxy-4-methoxyphenyl)chroman-6-one, Sinensetin, neoseherserin, q1, Citromitin and Angelol D may be the key components of HYD curing goiter, while isorhamnetin, anethole, eckol, Beta-Elemene, beta-sitosterol, naringin may be the key components of HYD to play the “therapeutic and toxic” joint role. Previous experiments [28] confirmed that eckol, an important compound in kelp could play the role of anti-inflammatory, anti-oxidation and anti-tumor. The sinensetin, neoseherperidin and citromitin had been confirmed in pericarpium citri reticulatae and pericarpium citri reticulatae viride, which could treat inflammation and tumors [29]. Quercetin has antioxidant, anti-proliferative and anti-inflammatory effects. Cesidio Giuliani [30] found that quercetin inhibited thyroid-cell growth and iodide uptake, decreasing the expression levels of thyrotropin receptor, thyroid peroxidase and thyroglobulin genes, so as to achieve the effects of inhibiting thyroid growth to treat goiter. Wei Lin [29] validated that quercetin could act as an anti-liver injury agent in some animal models. Xiao Xiong Gan [31] confirmed that luteolin and kaempferol, which were important compounds of Prunella vulgaris, could be used to cure Hashimoto’s thyroiditis and other thyroid diseases. Xiao-Sa Du [32] found that wogonin can attenuate liver fibrosis by regulating the activation and apoptosis of hepatic stellate cells, which also indicates that it can be an effective drug to treat and prevent liver fibrosis. However, present studies still can not affirm that wogonin can treat goiter. Lingtiao Jin validated that naringenin may represent a potential therapeutic agent for controlling inflammation-related diseases by reducing the levels of several inflammatory cytokines, and it also has the capacity to

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Figure 6 Verification of prediction results. (a) The heat map of molecular docking results of core targets and core compounds of HYD in the treatment of goiter. (b) The heat map of molecular docking results of core targets and core compounds of HYD in the therapeutic and toxic effects. (c) The histogram of ratios of confirmed targets of key compounds. The blue bars represent the proportions of validated targets to corresponding compound targets. The orange bars represent the proportions of validated targets to corresponding compound core targets. (d) The distribution histogram of the proportion of verified targets of all compounds and core targets of all compounds.
dampen cytokine production by regulating lysosome function. But until now, there has been no study indicating that quercetin, kaempferol, and naringenin are related to DILI.

At the same time, the network stability test indicated that the HYD had high stability as well as potential on the bidirectional effects. The molecular docking results also showed that the core compounds and core targets docked well, which further proved the reliability of the prediction of network pharmacology analysis. Through data mining,
the compound-target correspondences were validated partly. Mitogen-activated protein kinase (MAPK) is a conserved serine/threonine protein kinase ubiquitous in eukaryotes. Wang Qinghao et al. found that the expression levels of MAPK increased in the cytoplasm of thyroid cells of rats with goiter [33], and corresponding treating herbs could decrease the levels of MAPK significantly. Yi Xietian found that Prunella vulgaris could regulate apoptosis through related MAPK proteins and JNK/P38MAPK signaling pathway in the treatment of autoimmune thyroiditis [34]. Liang Wei et al. confirmed that the Xialouowan, including laminarin japonica and caladium, could download the expression of AKT and treat goiter by PI3K/Akt/mTOR pathway [35]. CYP enzymes are the key metabolic enzymes for drug metabolism in the liver, which mediate many kinds of liver injury caused by drugs [36]. Previous studies showed that the CYP1A1 and CYP1A2 were closely related to liver injury [37].

TNF, which is closely related to apoptosis, can treat the goiter effectively with its inhibition, while it may also play a role in DILI by up-regulating the mRNA expression [38]. Sin-Ting Lau approved that the aberrant cell proliferation of the A339V clones, which is closely related to the abnormal proliferation of the thyroid, can be inhibited by reducing the nuclear STAT3 [39]. Yang Yu’s study has revealed that the proliferation of thyroid cells is closely related to TSHab activation of the MAPK pathway [38]. TP53 can regulate target genes and induce thyroid cell cycle apoptosis, aging, and so on by encoding proteins to a variety of cellular stress responses [40]. Jiao et al. showed that the production of DILI was related to IL10 and TNF [41]. Yu Dan and other studies have shown that PPARg may be a key node protein DILI production mechanism [42].

KEGG pathway enrichment analysis showed that HYD curing goiter mainly involved PI3K/Akt signaling pathway, MAPK signaling pathway, Pathways in cancer, and Prostate cancer. HYD exerts toxic-effects interaction mainly through Lipid and atherosclerosis, T cell receptor signaling pathway, Toxoplasmosis, and other signaling pathways. PI3K (Phosphoinositide-3 kinase) belongs to the phospholipid kinase family and is the main kinase of inositol and phosphatidylinositol. Akt (Protein kinase B) is the most important protein in the downstream signaling pathway of PI3K, which is a serine/threonine protein kinase. This signaling pathway regulates cell proliferation, differentiation, and apoptosis [43].

Chiara Laezza et al. [44] indicated that goiter was closely connected with the MAPK signaling pathway, which could be activated by TSH. Nicole Yeager [45] indicated that the PI3K/Akt cascade can induce the development of goiter. The Toxoplasmosis pathway is connected with genes such as IL10, BCL2, and so on. A previous study [46] demonstrates the involvement of a Bax-Bcl2-dependent apoptotic process in related thyroid disease. The Lipid and atherosclerosis pathway and T cell receptor pathway can regulate the expression levels of TNF, IL10, BCL2, etc. to regulate the reproduction and apoptosis of thyroid-associated cells. Until now, however, there has been no study confirming that these pathways are directly related to DILI, which should be further studied.

Quercetin has been verified to restrain the absorption of iodine in the thyroid gland in rats and reduce the expression of the genes associated with thyroid hormone synthesis, such as NIS, TSHR, TPO and, TG [47]. Luteolin can inhibit the proliferation and migration of papillary thyroid carcinoma TPC-1 cells, which may be related to its effects on the PI3K/AKT pathway to achieve anti-cancer effects [48]. Wogonin can enhance the ubiquitination degradation of PI3KCA by Increasing the transcription level of endogenous Nedi4 while increasing the expression of its proteins to restrain the PI3K/Akt signaling pathway [49].

SRC is a protein tyrosine kinase that plays an important part in the vital movement of cells, which are involved in the regulation of signals and connected with growth, proliferation, adhesion, movement, differentiation, apoptosis, and other physiological activities [50]. PI3K/Akt, MAPK3, TP53, MAPK1, and other signaling targets can regulate the expression of mTOR to regulate autophagy.

Our previous research found that each splitter of HYD and the addition and subtraction of sargassum-ligurice combination could reduce the expression of mRNA of mTOR, p70S6K and 4E-BP1, decrease transcription of genes, translation of proteins, synthesis of ribosomes and other vital activities, thus serving to inhibit proliferation of cells and promote apoptosis of thyroid tissue [51, 52]. GRT, TNF, and PPARg are the key targets of HYD in toxic and therapeutic co-action. Liu Dianna et al. [53] found that the combinations of Sargassum with ligurice at 3 times, 4 times and 5 times of the limited dose specified in the Chinese Pharmacopoeia and the Artemisia annua at 3 times of the limited dose specified in the Chinese Pharmacopoeia significantly improved the thyroid coefficient and tissue morphology, restored the protein synthesis function of the liver and enhanced antioxidant capacity.

Combining our study with the existing literature, we have drawn the mechanism of therapeutic and toxic effects of sargassum and ligurice (Figure 8).

Conclusion

In general, through machine learning based on network pharmacology, we believed that the Eckol, 5,7-dihydroxy-2-(3-hydroxy-4-methoxyphenyl)chroman-6-one, Sinensetin, neohesperidin di, Citromitin, and Angelol D might be the key components of HYD curing goiter, while isorhamnetin, anethole, eccok, Beta-Elemene, beta-sitosterol, naringin may be the key components of HYD to play the “therapeutic and toxic” joint role. MAPK1, MAPK3, AKT1, RELA, NFKB1, MAPK4, MAPK8, TP53, NFKB1α, and CCND1 may be the key targets of HYD curing goiter, while RELA, TNF, IL4, BCL2, IL10, CYP1A1, MAPK8, GSTM1, PPARg, and CYP1A2 may be the key targets and eccok may be the core compound of HYD to exert toxic-effects interaction. The MAPK signaling pathway, Lipid and atherosclerosis, T cell receptor signaling pathway, Toxoplasmosis, and PI3K/Akt signaling pathway may be the core pathways of the therapeutic and toxic effects of HYD. The network stability test, molecular docking and data mining confirmed the scientificness and stability of HYD playing a bidirectional role. Relevant experimental data confirmed the effect of PI3K/Akt pathway in HYD treating goiter, which provided experimental data to prove the results of network pharmacology analysis.

This study was conducted to explore the mechanism of the bidirectional effects of HYD, mainly through machine learning and data mining. It also offered a new way for more explorations on the therapeutic and toxic bidirectional effects and mechanisms using machine learning and data mining.

References

5. Meng X, Wu ZH, Peng YR, Shen MQ. Toxicological mechanisms of


17. Yi PF. The relationship between PI3K/Akt signal transduction pathway and thyroid nodules. Master Dissertation, Tianjin Medical University, Tianjin, China. 2010. Available at: https://kns.cnki.net/kcms2/article/abstract?v=3Toxa6yGwywxXTXON1GT8,UpF0mgOsR0du0mpQq4060-KR1uHfd1kVWVBEKmJn0u2JrzANCuKjBjkYKflLmQnMrGqj1LaxlKpoTPYTv18lPC8GBHm9Vkh3t5f1kTC8C8cnunplatform=NZKPT&flag=copy

18. Zeng MX. Research on clinical experience in the treatment of nodular goiter by using Hua Xiao Xiao Yang fang and the mechanism in regulation of PI3K/Akt signal pathway. PhD Thesis, Hubei University of Chinese Medicine, Hubei, China. 2017. Available at: https://kns.cnki.net/kcms2/article/abstract?v=3Toxa6yGwywxXTXON1GT8,UpF0mgOsR0du0mpQq4060-KR1uHfd1kVWVBEKmJn0u2JrzANCuKjBjkYKflLmQnMrGqj1LaxlKpoTPYTv18lPC8GBHm9Vkh3t5f1kTC8C8cnunplatform=NZKPT&flag=copyp


21. Wang X, Ge CR, Zhong GS, et al. Effects of modified Haihao-Gancao anti-drug combination of Haihao Yuhu Decoction on PI3K/Akt pathway-related genes expression in goiter rats. China J Tradit Chin Med Pharm 2023;38(2):800–804. Available at: https://kns.cnki.net/kcms2/article/abstract?v=3Toxa6yGwywxXTXON1GT8,UpF0mgOsR0du0mpQq4060-KR1uHfd1kVWVBEKmJn0u2JrzANCuKjBjkYKflLmQnMrGqj1LaxlKpoTPYTv18lPC8GBHm9Vkh3t5f1kTC8C8cnunplatform=NZKPT&flag=copyp


23. Li YW, Zhong GS, Liu HY, et al. Effects of Haihao Yuhu Decoction with different proportions of Sargassum and Radix Glycyrrhizae on hepatic functions and pathomorphology in goiter rats. China J Tradit Chin Med 2013;28(5):1295–1300. Available at: https://kns.cnki.net/kcms2/article/abstract?v=3Toxa6yGwywxXTXON1GT8,UpF0mgOsR0du0mpQq4060-KR1uHfd1kVWVBEKmJn0u2JrzANCuKjBjkYKflLmQnMrGqj1LaxlKpoTPYTv18lPC8GBHm9Vkh3t5f1kTC8C8cnunplatform=NZKPT&flag=copyp

24. Xiu LL. Biological effects and mechanism of different varieties of Sargassum and liquorice infHaihao Yuhu decoction containing anti-drug combination on rats with goiter. PhD Thesis, Beijing University of Chinese Medicine, Beijing, China. 2019. Available at: https://kns.cnki.net/kcms2/article/abstract?v=3Toxa6yGwywxXTXON1GT8,UpF0mgOsR0du0mpQq4060-KR1uHfd1kVWVBEKmJn0u2JrzANCuKjBjkYKflLmQnMrGqj1LaxlKpoTPYTv18lPC8GBHm9Vkh3t5f1kTC8C8cnunplatform=NZKPT&flag=copyp

25. Zhang C. Study on the pharmacodynamics and mechanism of high dose licorice and different varieties of Sargassum in Haihao Yuhu Decoction. Master Dissertation, Beijing University of Chinese Medicine, Beijing, China; 2019. Available at: https://kns.cnki.net/kcms2/article/abstract?v=3Toxa6yGwywxXTXON1GT8,UpF0mgOsR0du0mpQq4060-KR1uHfd1kVWVBEKmJn0u2JrzANCuKjBjkYKflLmQnMrGqj1LaxlKpoTPYTv18lPC8GBHm9Vkh3t5f1kTC8C8cnunplatform=NZKPT&flag=copyp


38. Yang Y. Effects of thyroid stimulating antibiotic on thyroid cell proliferation and its mechanism. Master Dissertation, Fujian Medical University, Fujian, China, 2003. Available at: https://www.cnki.com.cn/kcms2/article/abstract?v=3ToxAlexySOz0Ldvoeviiv-sBzwsD8XM6s3QqVIo5sQiyazuN2KN3jW537GdjiyYJgXkS3eqwEWHkS4VhNhDJ0hUHi9pLwaxY7brwGw8SkDtm3owN8MtcR5e7QV4hJfbUj&uniplatform=NZKPT&flag=copy
50. Qian WC. Pharmacological efficacy and mechanism of baicalin in improving isoproterenol-induced myocardial hypertrophy in rats. PhD Thesis, Nanjing Medical University, Jiangsu, China, 2018. Available at: https://www.cnki.com.cn/kcms2/article/abstract?v=3ToxAlexySOz0Ldvoeviiv-sBzwsD8XM6s3QqVIo5sQiyazuN2KN3jW537GdjiyYJgXkS3eqwEWHkS4VhNhDJ0hUHi9pLwaxY7brwGw8SkDtm3owN8MtcR5e7QV4hJfbUj&uniplatform=NZKPT&flag=copy
51. Wang CH. Screening and identifying the inhibitors of Src protein tyrosine kinase from Hedyotis diffusa Wildl. Master Dissertation, Zhejiang University, Zhejiang, China, 2007. Available at: https://www.cnki.com.cn/kcms2/article/abstract?v=3ToxAlexySOz0Ldvoeviiv-sBzwsD8XM6s3QqVIo5sQiyazuN2KN3jW537GdjiyYJgXkS3eqwEWHkS4VhNhDJ0hUHi9pLwaxY7brwGw8SkDtm3owN8MtcR5e7QV4hJfbUj&uniplatform=NZKPT&flag=copy