Specialty Journal of Pharmacognosy, Phytochemistry, and Biotechnology
ISSN: 3062-441X
2023, Volume 3, Page No: 217-223

Available online at: www.galaxypub.co/page/journals

Galaxy Publication

Network-Based Bioinformatic Analysis Reveals Hepatoprotective Mechanisms of Garlic Oil against Alcohol-Induced Liver Injury

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Received: 17 January 2023; **Revised:** 07 April 2023; **Accepted:** 12 April 2023

ABSTRACT

Studies have shown that alcohol intake contributes to hepatic injury. Prior research has explored the potential benefits of garlic in safeguarding the liver from alcohol-associated harm. This investigation examined the hepatoprotective potential of garlic oil against alcohol exposure using protein-protein interaction (PPI) network evaluation to uncover novel molecular insights. The dataset GSE40334 was obtained from the Gene Expression Omnibus (GEO) repository for processing. Differentially expressed genes (DEGs) with statistical significance were identified through the GEO2R tool. UMAP dimensionality reduction was utilized to assess cluster separation among samples. Cytoscape software version 3.7.2, along with its plugins, facilitated action mapping and gene ontology enrichment to pinpoint vital DEGs and relevant biological pathways. In total, 798 DEGs meeting significance criteria were included in the evaluation. Analysis of the PPI network indicated that two major DEG clusters are prominently involved in the cellular response to garlic oil treatment. Key DEGs identified include Ranbp2, Pafah1b1, Seh1l, Plk1, Cenpa, Mis12, and Tgfb1. Enriched biological processes centered on fundamental cellular activities, including cell proliferation and division. Treatment with garlic oil demonstrates a safeguarding effect against alcohol-related hepatic injury. Of the seven pivotal genes detected, six exhibited modulation that supports improved liver function.

Keywords: Alcohol, Allium sativum, Garlic, Gene, Liver

How to Cite This Article: Costa R, Lima AP, Teixeira B. Network-Based Bioinformatic Analysis Reveals Hepatoprotective Mechanisms of Garlic Oil against Alcohol-Induced Liver Injury. Spec J Pharmacogn Phytochem Biotechnol. 2023;3:217-23. https://doi.org/10.51847/fvLrJagdhu

Introduction

Records from ancient times reveal that diverse civilizations worldwide have long acknowledged garlic's therapeutic potential for managing numerous ailments and averting health conditions [1]. Its integration into traditional remedies over millennia underscores garlic's value as an essential nutritional ingredient [2]. Benefits linked to garlic intake encompass bolstered immunity, reduced platelet clumping, antimicrobial (bacterial, fungal, and viral) effects, blood pressure lowering, and reductions in cholesterol and triglycerides, among others [3]. Research has positioned garlic as a defender against liver damage caused by ethanol (known as alcoholic liver disease). As excessive alcohol use drives the advancement of hepatic issues ranging from fat accumulation to inflammation, scarring, and tissue hardening, the advantageous contributions of garlic-derived compounds in countering these effects have been studied and confirmed [4]. In their work, Esmaeili *et al*. explored how garlic influences splenic activity, concluding that it acts through alterations in App expression [5]. El-Khayat *et al*. evidenced the liver-shielding qualities of garlic oil in animal experiments [6]. Bhutani *et al*. detailed garlic's impact on halting lung tumor advancement via PPI networks and KEGG pathway examination [7]. The combination of bioinformatics and genomic approaches has enabled researchers to delve into disease mechanisms at the molecular level. Bioinformatics applications allow reinterpretation of genomic data, yielding innovative views on disease

identification, management, and avoidance [8]. Examining gene and protein interconnections through PPI networks is a standard technique. Such analyses have delivered insightful data on hepatic responses in rats to therapeutic interventions [9]. Action maps illustrate directional regulatory interactions—like stimulation, suppression, or gene expression—making them ideal for mapping gene relationships [10]. Gene ontology enrichment further clarifies gene/protein roles by categorizing associated molecular functions, biological pathways, and cellular locations [11, 12]. Here, we applied PPI network evaluation and gene ontology to probe garlic oil's contribution to mitigating alcohol-triggered fatty liver in a murine model. Dataset processing began with retrieval from GEO followed by GEO2R preliminary screening. This work sought to offer fresh viewpoints on garlic's influence in fatty liver contexts.

Materials and Methods

Data collection

In order to evaluate garlic's liver-shielding properties, dataset GSE40334 was accessed via the GEO database (<https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE40334>). Gene expression data from liver samples of 12 male C57BL/6 mice were analyzed. Groups comprised: controls on Lieber-DeCarli control liquid diet (GSM991405-8), alcohol-exposed on Lieber-DeCarli ethanol diet (GSM991409-12), and garlic oil-supplemented on Lieber-DeCarli ethanol diet with garlic oil (50 mg/kg bw) delivered in 0.1 ml olive oil by oral gavage (GSM991401-4). RNA isolation from cryopreserved liver involved TRIzol reagent (Invitrogen), followed by purification using RNeasy MinElute Cleanup kit (Qiagen, GmbH).

Pre-evaluation analysis

The chosen gene expression datasets were examined using the GEO2R tool. UMAP dimensionality reduction was performed to detect distinctions among the compared groups. Variations in gene expression between the garlic oil-treated mice and control samples were identified and contrasted with those observed between alcohol-exposed mice and controls.

Gene expression analysis

Significantly differentially expressed genes (DEGs), defined by an adjusted p-value <0.05, from both the “alcohol plus garlic oil versus control” comparison and the “alcohol versus control” comparison were evaluated. DEGs exhibiting a log(fold change) difference <1 were classified as shared between the two comparisons, and these were excluded from the DEG list of the “alcohol plus garlic oil versus control” analysis. The remaining DEGs were chosen for subsequent protein-protein interaction (PPI) network evaluation.

PPI network analysis

The selected DEGs were incorporated into a PPI network using the CluePedia v 1.5.7 plugin within Cytoscape software v 3.7.2 [13]. Nodes were linked by directed edges representing expression, activation, and inhibition relationships. Isolated genes and sub-networks containing fewer than 6 nodes were excluded from further consideration. In-degree and out-degree values were calculated for each node, with nodes having a total degree exceeding 6 selected for deeper analysis. Out-degree represents the total number of outgoing edges (encompassing activation, inhibition, and expression connections), whereas in-degree denotes the total incoming directed edges to a node. The retained nodes were further filtered according to patterns in total degree variation, leading to the identification of key DEGs.

Gene ontology enrichment

Critical nodes from the PPI network were selected and subjected to biological process enrichment analysis using the ClueGO v 2.5.7 plugin in Cytoscape software.

Statistical analysis

An adjusted p-value <0.05 was applied as the threshold for identifying significant DEGs.

Results and Discussion

UMAP analysis demonstrated clear separation of gene expression profiles across the three mouse groups based on treatment conditions **(Figure 1).** The results revealed 993 significant DEGs jointly influenced by alcohol and garlic oil. In contrast, alcohol alone significantly impacted 78 genes. After excluding uncharacterized and duplicate entries, 66 DEGs remained associated with the "alcohol versus control" comparison, while 845 DEGs were linked to the "alcohol plus garlic oil versus control" comparison. Five DEGs from the latter analysis were removed due to contradictory findings. Following elimination of overlapping genes, 798 DEGs specific to the "alcohol plus garlic oil versus control" comparison were retained, representing the direct influence of garlic oil on the livers of treated mice.

Of the 798 queried DEGs, 797 were successfully mapped by the CluePedia plugin in Cytoscape. The resulting PPI network comprised 631 isolated genes, 11 pairs (22 genes), 2 triads, one quartet sub-network, one quintet sub-network, 3 hexamer sub-networks, and two major clusters (the smaller designated as cluster-1 and the larger as cluster-2). Cluster-1, consisting of 28 nodes, is illustrated in **Figure 2.** As shown in **Figure 3**, cluster-2 consisted of two sub-clusters: sub-cluster-A and sub-cluster-B. Sub-cluster-A was predominantly connected by activation edges (located in the upper section) **(Figure 3).** The critical nodes (those with total degree >6) across the PPI network are listed in **Table 1**. According to **Table 1**, Mapk15 exhibited the highest total degree among key nodes in cluster-1. Similarly, Tgfb1 emerged as the central gene in sub-cluster-B of cluster-2. With the exception of Nup210, critical nodes in sub-cluster-A displayed consistent total degree patterns. Consequently, Spc25, Ranbp2, Nup133, Pafah1b1, Clip1, Seh1l, Ppp2r5e, Nup37, Plk1, Rps27, Cenpa, Mis12, Dync1i1, Tgfb1, and Mapk15 were designated as pivotal genes associated with the hepatoprotective effects of garlic oil in mice.

Enriched biological process groups linked to the key DEGs are displayed in **Figure 4**. As indicated in **Figure 4**, eight distinct groups of biological processes were connected to Ranbp2, Nup133, Pafah1b1, Seh1l, Nup37, Plk1, Cenpa, Mis12, Dync1i1, TGFB1, and Mapk15. The distribution of terms across these biological process groups is presented in **Figure 5**. Results from **Figure 5** show that "tRNA export from nucleus" accounted for approximately 46% of the associated biological processes.

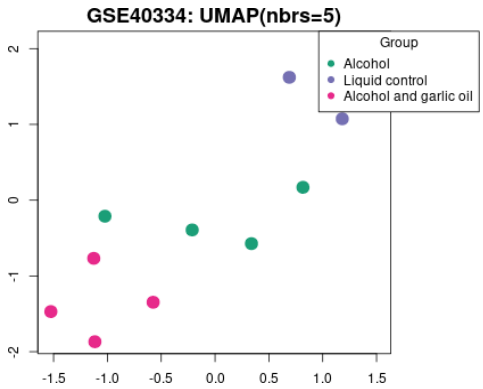

Figure 1. UMAP visualization of gene expression patterns in the three mouse cohorts.

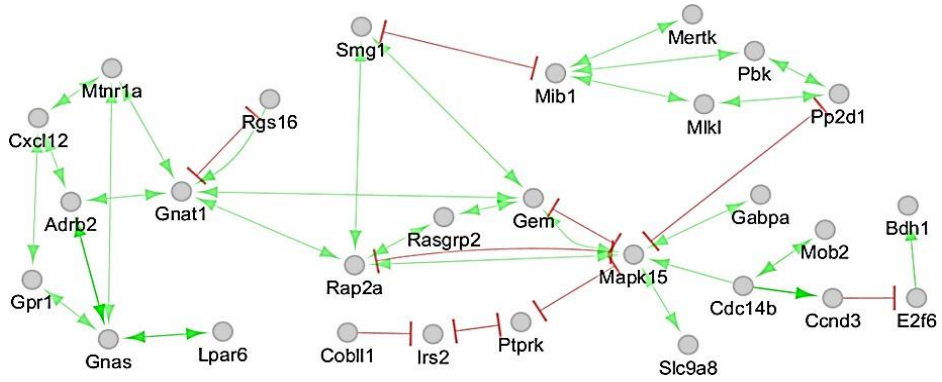

Figure 2. Cluster-1 within the protein-protein interaction (PPI) network; green nodes signify activation, while red nodes indicate inhibition.

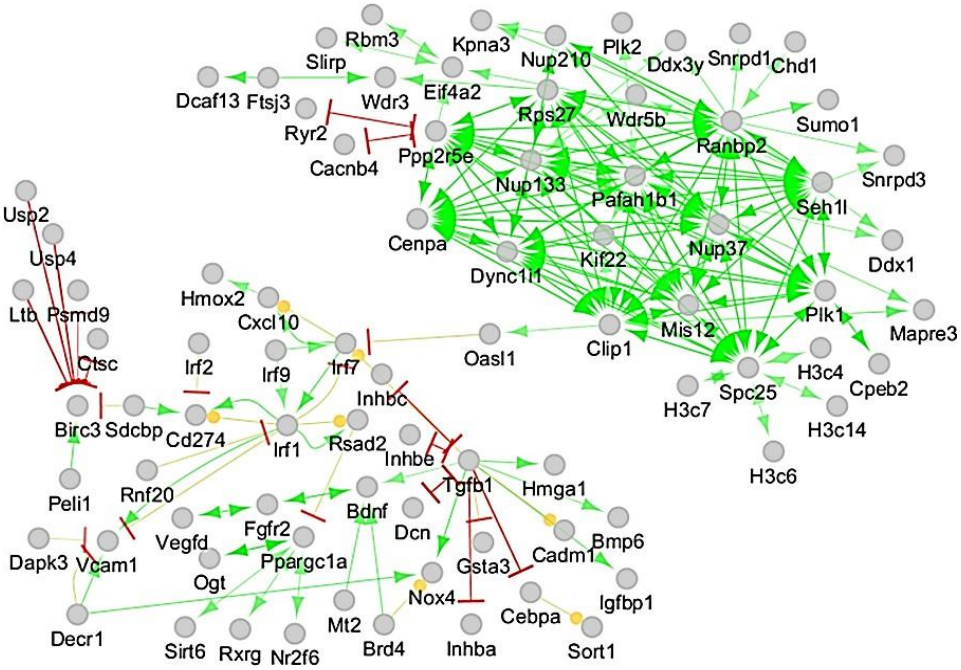

Figure 3. Cluster-2 within the PPI network; green, yellow, and red nodes represent activation, expression, and inhibition, respectively; the top portion is termed sub-cluster-A, and the bottom portion is termed sub-cluster-B.

Table 1. Inventory of pivotal components in the PPI network

Rank	Gene Name	In-Degree	Out-Degree	Total Degree	Associated Cluster	Associated Sub-Cluster	Log(Fold Change)
1	Spc25	16	16	32	2	A	-0.96
2	Ranbp2	14	17	31	2	A	1.14
3	Nup133	14	16	30	2	A	0.88
4	Pafah1b1	15	15	30	2	A	0.79
5	Clip1	14	15	29	2	A	0.66
6	Seh1l	14	14	28	2	A	1.29
7	Ppp2r5e	14	14	28	2	A	0.88
8	Nup37	13	14	27	2	A	1.14
9	Plk1	13	13	26	2	A	-0.98
10	Rps27	12	12	24	2	A	0.90
11	Cenpa	12	12	24	2	A	-0.91
12	Mis12	12	12	24	2	A	0.77
13	Dync1i1	12	12	24	2	A	-0.77
14	Nup210	4	5	9	2	A	-0.79
15	Tgfb1	4	13	17	2	B	-0.90
16	Irf1	3	7	10	2	B	-1.02
17	Ppargc1a	5	4	9	2	B	1.65
18	Birc3	7	2	9	2	B	1.56
19	Irf7	4	3	7	2	B	-1.27
20	Mapk15	9	8	17	1	-	-1.02
21	Gnat1	6	5	11	1	-	-2.05
22	Rap2a	5	5	10	1	-	-1.05
23	Gem	5	5	10	1	-	1.19
24	Mib1	4	4	8	1	-	0.78
25	Gnas	4	4	8	1	-	-0.96

Analyses indicated distinct liver gene expression signatures among control specimens, mice exposed solely to alcohol, and mice receiving both alcohol and garlic oil supplementation. These three cohorts were entirely segregated in the visualization **(Figure 1).** Shifts in oxidative stress linked to alcohol-induced hepatic injury

underscore alcohol's influence on liver transcriptome patterns [14]. Multiple reports emphasize garlic's notable impact on hepatic gene regulation. Soleimani and colleagues demonstrated that garlic substantially lowers serum concentrations of ALT, AST, FBS, HbA1C, total cholesterol, LDL-cholesterol, and triglycerides [15]. Lv and co-workers documented alterations in the transcriptome of HepG2 cells following garlic exposure [16].

This study detected 798 differentially expressed genes (DEGs) when contrasting hepatic transcriptomes under alcohol exposure alone versus combined alcohol and garlic oil treatment. An interaction map was generated to pinpoint essential DEG relationships. As depicted in **Figures 2 and 3**, only 112 DEGs were incorporated into the major sub-networks, whereas the rest appeared as isolates or in minor sub-networks.

Ultimate evaluation pinpointed 15 primary genes targeted by garlic oil amid alcohol challenge in the liver **(Table 1).** Charron and collaborators supplied evidence on how garlic-derived compounds modulate transcriptomes, encompassing cellular effects, in vitro assays, and in vivo models. They reported upregulation of Ppara, Hnf4a, Hmox1, and Nqo1 transcripts with downregulation of Cyp7a1 in HepG2 cells, alongside elevated Cyp1a1, Cyp2b1, Cyp3a1, and Gstp1 mRNA and protein levels, plus reduced Cyp2e1 expression in rat hepatic tissue [17].

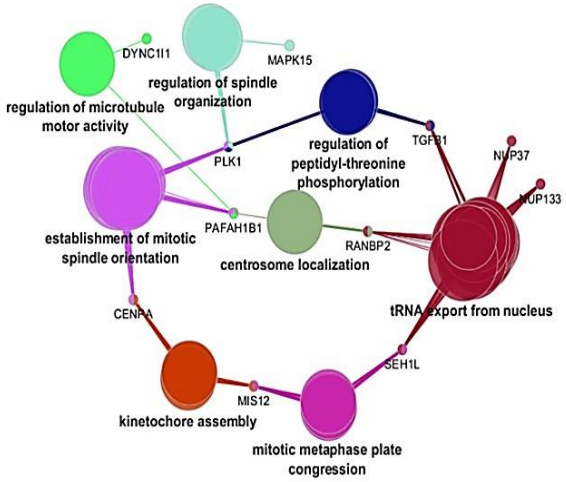

Figure 4. Gene ontology enrichment analysis for the 15 pivotal genes; smaller circles denote individual genes, whereas larger circles highlight clusters of biological processes. Enrichment terms are derived from human annotations.

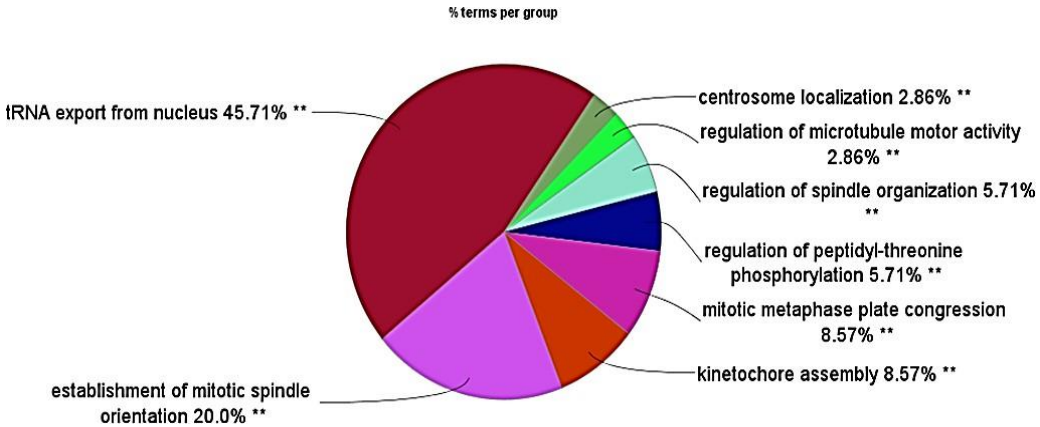

Figure 5. Proportional distribution of enriched terms across biological process categories.

As shown in **Figure 4**, the principal genes targeted play critical roles in fundamental biological pathways governing cellular activity and expansion. The predominant biological process groupings impacted by garlic oil consist of "tRNA export from nucleus" and "establishment of mitotic spindle orientation" **(Figure 5).** The additional groupings, however, pertain mainly to cellular division mechanisms. Previous work by Druesne-Pecollo *et al*. has underscored how compounds from garlic contribute to modulating cell cycle progression, cellular growth, detoxifying processes, signaling pathways, and molecular transport [18]. Enrichment analysis through gene ontology identified Ranbp2, Nup133, Pafah1b1, Seh1l, Nup37, Plk1, Cenpa, Mis12, Dync1i1,

Tgfb1, and Mapk15 as the specific genes tied to these pathways. Notably, Ranbp2, Pafah1b1, Seh1l, Plk1, Cenpa, Mis12, and Tgfb1 each connect to multiple (two or three) pathway categories.

Studies from Li *et al*. have shown that loss of lissencephaly 1 (Pafah1b1) in the liver promotes steatosis and hastens tumor formation in hepatic tissue [19]. Per **Table 1**, garlic oil elevates Pafah1b1 expression levels. This pattern is consistent with garlic oil's hepatoprotective effects countering alcohol-induced harm. Investigations by Liu *et al*. linked elevated RANBP2 levels to aggressive traits in hepatocellular carcinoma [20]. Garlic oil boosts Ranbp2 expression in murine liver, potentially indicating an unintended consequence of its use.

Reports note that transforming growth factor-beta (TGFB) concentrations rise steadily as liver pathology advances [21]. Garlic exposure suppresses Tgfb1 expression in hepatic tissue. Such suppression aligns with the therapeutic benefits associated with garlic intake. Published data reveal that SEH1L, an essential player in ubiquitination pathways, exhibits lower expression during osteoarthritis advancement [22]. Elevation of Seh1l by garlic oil highlights its potential in safeguarding against pathological conditions. Findings from Lin *et al*. connected higher PLK1 levels to stimulated cellular growth and hepatic oncogenesis [23]. Garlic oil suppresses polo-like kinase 1 (Plk1) expression, representing a positive attribute of garlic supplementation. Centromere protein A (Cenpa) functions as a promoter of malignancy in hepatocellular carcinoma cases [24]. Its suppression via garlic oil points to garlic's role in mitigating liver injury. He *et al*. emphasized the contribution of the kinetochore element Mis12 to accurate chromosome positioning, separation, and assembly of kinetochores in mitosis [25]. This is in line with the increased Mis12 expression observed in garlic oil-treated hepatic samples.

Conclusion

Overall, garlic oil alters hepatic transcriptomes in mice subjected to alcohol exposure. The central genes influenced by garlic oil include Ranbp2, Pafah1b1, Seh1l, Plk1, Cenpa, Mis12, and Tgfb1. Apart from Ranbp2, published studies corroborate the beneficial implications of the expression shifts in these pivotal genes for liver health.

Acknowledgments: None

Conflict of Interest: None

Financial Support: None

Ethics Statement: Approval for this project was granted under ethical code IR.SBMU.RETECH.REC.1402.872.

References

1. Bayan L, Koulivand PH, Gorji A. Garlic: a review of potential therapeutic effects. Avicenna J Phytomed. 2014;4(1):1-14.
2. Mayeux P, Agrawal K, Tou JSH, King B, Lippton H, Hyman A, et al. The pharmacological effects of allicin, a constituent of garlic oil. Agents Actions. 1988;25(1-2):182-90.
3. Iciek M, Kwiecień I, Włodek L. Biological properties of garlic and garlic-derived organosulfur compounds. Environ Mol Mutagen. 2009;50(3):247-65.
4. Guan MJ, Zhao N, Xie KQ, Zeng T. Hepatoprotective effects of garlic against ethanol-induced liver injury: a mini-review. Food Chem Toxicol. 2018;111:467-73.
5. Esmaeili S, Rostami Nejad M, Rezaei Tavirani M, Okhovatian F, Zadeh Esmaeel MM, Razzagh Z, et al. Evaluating of gene expression alteration after garlic consumption, analyzing through bioinformatics approach. Iran J Pharm Res. 2021;20(1):72-81.
6. El-Khayat Z, Rasheed W, Ramzy T, Hussein J, Agaiby M, Morsy S, et al. Protective effect of garlic oil against liver injury in experimental animals. J Med Plants Res. 2010;4(22):2359-69.
7. Bhutani K, Malhotra C. Construction and analysis of protein-protein interaction of therapeutic phytoconstituents of garlic (Allium sativum) targeting non-small cell lung cancer protein. J Adv Zool. 2023;44(S3):410-23.
8. Orlov YL, Anashkina AA, Klimontov VV, Baranova AV. Medical genetics, genomics and bioinformatics aid in understanding molecular mechanisms of human diseases. Int J Mol Sci. 2021;22(18):1-5.

9. Mansouri V, Arjmand B, Khodadoost M, Razzaghi Z, Rezaei Tavirani M. Investigation of crucial affected proteins in rat liver in the presence of Scrophularia. Res J Pharmacogn. 2023;10(3):25-30.
10. Mansouri V, Vafaee R, Mohammadi Maram M, Bandarian F, Sarabi P, Razi F, et al. Inflammation and immunological disarrays are associated with acute exercise in type 2 diabetes. J Diabetes Metab Disord. 2024;23(1):1243-50.
11. Ashburner M, Ball CA, Blake JA, Botstein D, Butler H, Cherry JM, et al. Gene ontology: tool for the unification of biology. Nat Genet. 2000;25(1):25-29.
12. Zhao Y, Wang J, Chen J, Zhang X, Guo M, Yu G. A literature review of gene function prediction by modeling gene ontology. Front Genet. 2020;11:1-15.
13. Tahery N, Khodadost M, Sherafat SJ, Tavirani MR, Ahmadi N, Montazer F, et al. C-reactive protein as a possible marker for severity and mortality of COVID-19 infection. Gastroenterol Hepatol Bed Bench. 2021;14(Sl1):118-22.
14. Tan HK, Yates E, Lilly K, Dhanda AD. Oxidative stress in alcohol-related liver disease. World J Hepatol. 2020;12(7):332-49.
15. Soleimani D, Paknahad Z, Rouhani MH. Therapeutic effects of garlic on hepatic steatosis in nonalcoholic fatty liver disease patients: a randomized clinical trial. Diabetes Metab Syndr Obes. 2020;13:2389-97.
16. Lv C, Wang C, Li P, Huang Y, Lu X, Shi M, et al. Effect of garlic organic sulfides on gene expression profiling in HepG2 cells and its biological function analysis by ingenuity pathway analysis system and bio-plex-based assays. Mediators Inflamm. 2021;Article ID 7681252.
17. Charron CS, Dawson HD, Novotny JA. Garlic influences gene expression in vivo and in vitro. J Nutr. 2016;146(2S):444-49.
18. Druesne-Pecollo N, Chaumontet C, Pagniez A, Vaugelade P, Bruneau A, Thomas M, et al. In vivo treatment by diallyl disulfide increases histone acetylation in rat colonocytes. Biochem Biophys Res Commun. 2007;354(1):140-47.
19. Li X, Liu L, Li R, Wu A, Lu J, Wu Q, et al. Hepatic loss of Lissencephaly 1 (Lis1) induces fatty liver and accelerates liver tumorigenesis in mice. J Biol Chem. 2018;293(14):5160-71.
20. Liu X, Chen X, Xiao M, Zhu Y, Gong R, Liu J, et al. RANBP2 activates O-GlcNAcylation through inducing CEBPα-dependent OGA downregulation to promote hepatocellular carcinoma malignant phenotypes. Cancers. 2021;13(14):1-21.
21. Yan C, Yang Q, Gong Z. Transgenic expression of tgfb1a induces hepatic inflammation, fibrosis and metastasis in Zebrafish. Biochem Biophys Res Commun. 2019;509(1):175-81.
22. Yuan D, Shen H, Bai L, Li M, Ye Q. Identification of key ubiquitination-related genes and their association with immune infiltration in osteoarthritis based on the mRNA-miRNA network. Crit Rev Immunol. 2024;44(4):91-102.
23. Lin XT, Yu HQ, Fang L, Tan Y, Liu ZY, Wu D, et al. Elevated FBXO45 promotes liver tumorigenesis through enhancing IGF2BP1 ubiquitination and subsequent PLK1 upregulation. Elife. 2021;10:1-25.
24. Zhang Y, Yang L, Shi J, Lu Y, Chen X, Yang Z. The oncogenic role of CENPA in hepatocellular carcinoma development: evidence from bioinformatic analysis. Biomed Res Int. 2020;Article ID 3040839.
25. He J, Zhu Q, Han P, Zhou T, Li J, Wang X, et al. Transcriptomic networks reveal the tissue-specific cold shock responses in Japanese flounder (Paralichthys olivaceus). Biology. 2023;12(6):784:1-15.