Ilam University of Medical Sciences

Plant Biotechnology Persa

Online ISSN: 2676-7414

Homepage: https://pbp.medilam.ac.ir

Transcriptomic and Network-Based Deciphering of Hepatotoxic Mechanisms Induced by Herbal Bioactive Compounds

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Article Info

Article type:

Review

Article History:

Received: 04 Sep 2026

Revised: 17 Oct 2025

Accepted: 18 Nov 2026

Published Online: 31 Dec 2026

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A B S T R A C T

Objective: Plant-derived bioactive compounds are increasingly explored for therapeutic applications, yet their potential hepatotoxic effects remain poorly understood. Transcriptomic and network-based analyses provide valuable insights into the molecular mechanisms underlying liver injury. This study aimed to identify shared pathways, compound-specific transcriptional signatures, and key regulatory genes associated with plant-derived compound-induced hepatotoxicity.

Materials and Methods: Publicly available transcriptomic datasets comprising samples exposed to plant-derived bioactive compounds and their corresponding controls were systematically analyzed. The original biological replicate structure was maintained throughout the analysis. Following data preprocessing and quality control, differential gene expression analysis was performed. Subsequent analyses included Gene Ontology (GO) and pathway enrichment, comparative pathway analysis, protein–protein interaction (PPI) network construction, hub gene identification, functional module analysis, and independent validation of the identified candidate genes.

Results: Six transcriptomic datasets encompassing 186 samples were included in the analysis. Following preprocessing, 17,842 genes were retained for downstream analysis. In total, 2,486 differentially expressed genes (DEGs) were identified, comprising 1,421 upregulated and 1,065 downregulated genes. Functional enrichment analysis revealed 47 significantly enriched pathways, 24 of which were shared across responses to different compounds. These pathways were primarily associated with oxidative stress, inflammation, apoptosis, mitochondrial dysfunction, glutathione metabolism, lipid metabolism, and xenobiotic responses. The PPI network consisted of 412 nodes and 1,286 interactions. Key candidate hub genes included TP53, AKT1, JUN, TNF, NFKB1, HSP90AA1, SOD2, and CASP3. Independent validation revealed concordant expression patterns for 21 of the 25 candidate genes.

Conclusion: Plant-derived bioactive compound-induced hepatotoxicity involves interconnected pathways associated with oxidative stress, inflammation, metabolic disturbances, mitochondrial dysfunction, and apoptosis. Identified hub genes and shared pathways provide insights into underlying mechanisms and potential targets for future research and safety assessment.

Key words: Herbal bioactive compounds, Hepatotoxicity, Transcriptomics, Protein–protein interaction network, Hub genes

➢ **How to cite this paper**

Joshi A, Maiti J. Transcriptomic and network-based deciphering of hepatotoxic mechanisms induced by herbal bioactive compounds. Plant Biotechnology Persa. 2026; 8(4): Proof. 10.66224/pbp.

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Introduction

Bioactive phytochemicals have become promising candidates as potential drug compounds due to their numerous biological effects such as antioxidant, anti-inflammatory, antimicrobial, and metabolic activities [1]. In addition, the biological activities of bioactive phytochemicals can be associated with hepatotoxicity, especially in cases when bioactive compounds are taken in high doses, in prolonged treatments, or in combinations with other bioactive compounds [2-8]. As the main organ of metabolism of xenobiotics, the liver can be considered a key organ for studying the biological activity of herbal drugs and their toxicological characteristics [9-14]. Hepatotoxic effects are caused by the following processes, such as oxidative stress [15-17], mitochondrial dysfunction, inflammatory signalling, changes in lipid metabolism, endoplasmic reticulum stress, apoptosis, and impairment of cell detoxifying processes [6,18]. Traditional toxicology studies pay more attention to biochemical markers and histopathological changes that are used for identification of liver damage and provide little information about molecular pathways responsible for hepatotoxicity of the tested compound. The transcriptomics approach allows us to look at the issue from another side, since transcriptomics includes both detection of the gene expression profile and identification of molecular responses that occur as a result of interaction between the tested hepatotoxic agent and the organism [19-20].

Despite the increasing use of transcriptomic approaches in studying drug- and chemical-induced liver injury, molecular mechanisms related to hepatotoxicity induced by individual herbal bioactive compounds appear relatively understudied. Current studies tend to focus either on individual compounds or particular molecular pathways or traditional toxicity biomarkers, thus making it hard to draw connections between the molecular mechanisms of hepatic injury [3,8,11]. Moreover, analysis of differential gene expression does not sufficiently address how altered genes work together in regulatory and signalling pathways. Comparative analysis of transcriptomic profiles, pathway enrichment, protein interaction networks, and hub gene analysis should be used in order to determine molecular mechanisms that act co-ordinately rather than individually [1]. The next deficiency of the existing studies is insufficient research into the question of whether different herbal bioactive compounds share hepatotoxicity pathways or possess molecular profiles unique to each compound.

It is postulated that bioactive compounds present in herbs elicit specific patterns of gene expression which relate to interconnected molecular mechanisms underlying oxidative stress, inflammation, mitochondria, apoptosis, metabolism, and cellular stress. Additionally, the hypothesis that combined differential expression analysis and network analysis will enable the identification of key hub genes and regulatory mechanisms that orchestrate the hepatic response to exposure to herb bioactive compounds is postulated. These molecular mechanisms can establish a connection between bioactive compound exposure and hepatocellular damage.

The novel feature of the presented study is the use of an integrated transcriptomics and network analysis approach to understand the mechanism of bioactive compound-induced hepatotoxicity. Contrary to the conventional method where genes with differential expression are examined separately, the approach includes profiling of expression, functional enrichment, pathway analysis, protein–protein interaction networks, and hub gene identification in order to elucidate the interconnected pathways of hepatic toxicity. Such a molecular signature will facilitate distinguishing common and unique toxic mechanisms induced by various bioactive compounds, identifying biological targets and candidate biomarkers for future investigations.

This paper is structured for the systematic exploration of molecular mechanisms that cause herbal bioactive compound-induced hepatotoxicity.

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The Introduction section provides background, the knowledge gap, the hypothesis, and the major contribution of the research in Section 1. The Objective describes the goals of the research aimed at the systematic exploration of molecular mechanisms that cause herbal bioactive compound-induced hepatotoxicity through transcriptomic and network-based approaches in Section 2. Methods section covers dataset selection, transcriptomic preprocessing, differential expression analysis, functional and pathway enrichment, comparative analysis, protein-protein interaction networks, hub gene identification, and the validation process in Section 3. The Results section describes transcriptomic changes, enriched pathways, network properties, hub genes, functional modules, and validation outcomes in section 4. The Discussion explains molecular outcomes, biological significance, study limitations, and future research directions in section 5. Finally, the Conclusion provides major findings of the study in section 6.

The purpose of this study is to unravel the molecular mechanisms of hepatotoxicity caused by selected herbal bioactive molecules using transcriptomic analysis and network approaches. This study intends to discover differentially expressed genes in response to these molecules, explore the biological pathways and processes modulated by the selected molecules, build interaction networks among genes/proteins, find the molecular regulators and hub genes possibly responsible for hepatotoxicity, and differentiate the key molecular processes that can be related to hepatotoxicity responses in relation to the selected herbal bioactive molecules.

Materials and Methods

Study Design and Analytical Framework

In this study, an integrative approach combining transcriptomics and network biology methods was used to study the molecular mechanisms involved in the development of hepatotoxicity caused by herbal bioactive compounds. This approach included selection of herbal bioactive compounds, identification and acquisition of public transcriptomic data sets, sample-level quality control, normalization, differential gene expression analysis, enrichment and pathway analysis, comparative analysis of compound-related responses, construction of protein–protein interaction networks, identification of hub genes, regulation network analysis, functional modules discovery, and validation. It was intended to discover both common and compound-specific transcriptional mechanisms of hepatotoxicity. This combination of network pharmacology with transcriptomics data is in line with the principles of systems biology methodology applied in the study of complex multi-target action of herbal medicines [10,17].

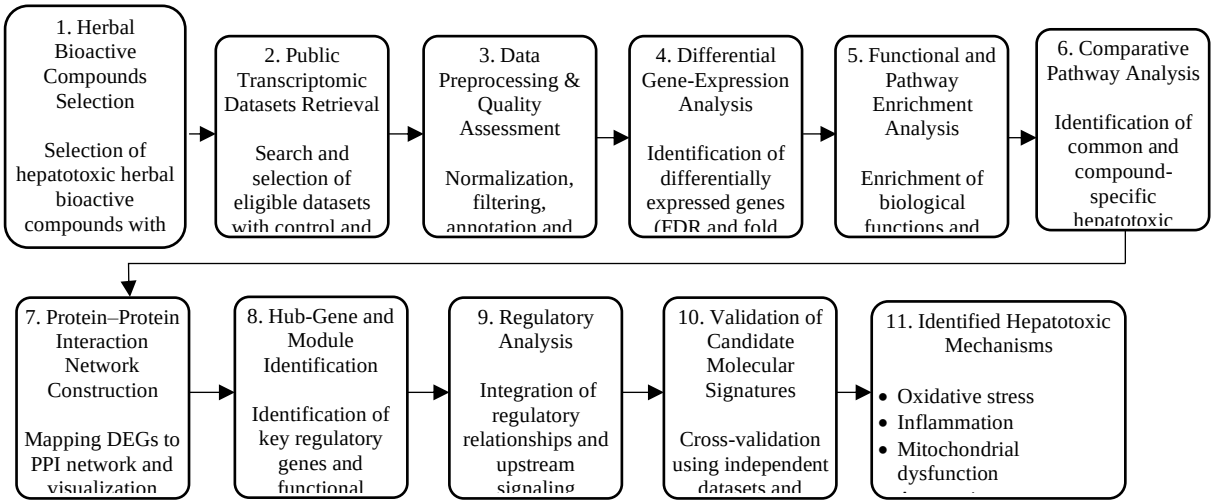


Figure 1: Integrated Transcriptomic and Network-Based Workflow for Deciphering Herbal Bioactive Compound-Induced Hepatotoxicity

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The process flow from Figure 1 should start from left to right as follows: selection of hepatotoxic herbal bioactive compounds and obtaining of relevant transcriptomic datasets; data preprocessing and quality control; differential gene expression analysis; functional and pathway enrichment; comparative pathway analysis; construction of protein-protein interaction network; identification of hub genes and modules; and candidate molecular signature validation. The last part of the essay should relate hub genes and enriched pathways to main hepatotoxicity mechanisms, including oxidative stress, inflammation, mitochondrial dysfunction, apoptosis, metabolic imbalance, and xenobiotic response.

Selection of Herbal Bioactive Compounds

Bioactive compounds of herbs with known pharmacological activities and possible toxic properties to the liver were chosen for evaluation. This selection depended on the presence of transcriptomic data of public access obtained after exposure to each compound in liver or related cellular liver models. Criteria for choosing a compound included a well-defined experimental dataset with clear definition of treatment and control groups, information on exposure dose and time, biological replicates, and gene expression data for further analyses. The selected compounds were analyzed separately, after which all of them were used for a comparison of common and specific molecular reactions.

Identification and Retrieval of Transcriptomic Datasets

Publicly accessible transcriptomic databases were obtained from resources such as the Gene Expression Omnibus (GEO) and other suitable public databases for gene expression. Databases were searched based on various keywords relevant to the selected herbal bioactive compound, herbal toxicities, hepatotoxicity, liver, hepatocytes, hepatic damage, gene expression, transcriptome, RNA sequencing, and microarray analysis. Metadata for all databases was collected systematically, including accession number, species, biological sample, experimental conditions, exposed group, control group, exposure concentration, exposure time, type of transcriptomic study, and biological replicates.

Only those databases that had the relevant control group or vehicle-treated controls along with herbal compound-exposed groups were considered for further studies.

Experimental Replication and Reproducibility

The original biological replication scheme of each publicly available dataset was used during the entire process of analysis. Conditions experimentally modeled by three biological replicates were processed as triplicates without pooling the replicate measurements. Each replicate was independently checked for quality, normalized, processed for differential expression analysis, and other computation-based analysis procedures. In cases when there were a number of biological replicates in the public data set different from three, the actual number of biological replicates available in the data set was used instead of making up additional measurements. The replicate consistency was assessed on the level of sample correlation, hierarchical clustering, distribution analysis, and PCA.

Transcriptomic Data Preprocessing

The expression data sets were preprocessed prior to differential expression analysis. In the case of microarray data sets, the background correction, normalization, probe annotation, and probe-gene mapping were carried out for the respective microarray platform. When more than one probe mapped to the same gene, the expression values were

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chosen based on a proven approach of probe summarization. In the case of RNA-seq data sets, the low-quality features and genes with very low expression across samples were first filtered out prior to normalization.

The normalized expression datasets were then assessed by boxplot and density plot analyses to check if there is consistency in the expression distributions across the samples. The sample-to-sample correlation was computed next to find any discrepancies among the biological replicates.

Quality Control and Sample Assessment

Quality control analysis was done prior to statistical analysis to guarantee the integrity of the transcriptomic data. Expression profile, sample correlation, hierarchical clustering, and PCA analysis were performed for each individual data set. Those samples with large differences from their biological replicate cluster were analysed for possible technical or experimental errors. Only in cases when there was enough evidence showing that the quality of the samples was inconsistent with the other samples within the data set did we remove these samples.

After the quality analysis, the processed data sets were used for further analyses. The integrity of the biological replicates was assessed before making the group comparison analysis.

Differential Gene-Expression Analysis

The comparison of samples treated with herbal compounds with respective untreated controls was done in order to identify the differential gene-expression profile. Linear model statistics based on normalized values of microarray expression data sets were performed. RNA sequencing count data sets were compared using count-based statistical methods.

For each individual gene, the difference in gene expression between treatment and control was quantified, as well as its statistical significance. The log2 fold change was used as the indicator of the direction and intensity of transcriptional regulation. The correction for multiple hypothesis testing was achieved using the Benjamin-Hochberg false discovery rate method. Differentially expressed genes were selected according to adjusted $P<0.05$ and $|\log2 \text{ fold change}|\geq1.0$ criteria unless other criteria had to be applied for specific data sets.

The subsequent separation of differentially expressed genes into up-regulated and down-regulated sets helped to define the direction of transcriptional changes in liver.

Identification of Common and Compound-Specific Differentially Expressed Genes

Gene lists showing differential expression for individual herb-derived bioactive molecules were compared to find the overlap and unique responses related to each compound. Responses common among various treatments were designated as common molecular responses, while those identified uniquely for each compound were classified as compound-related molecular responses. Gene lists found to be overlapping were visualized using relevant intersection methods, followed by functional analysis of the shared genes.

Gene Ontology Enrichment Analysis

To understand the biological significance of the identified differentially expressed genes, gene ontology enrichment analysis was carried out. This involved biological process, molecular function, and cellular component ontology terms. In cases where the distinction is necessary, upregulated and downregulated genes were analyzed separately to differentiate between processes that increase or decrease transcription.

Processes showing significant enrichment of gene ontology terms after adjustment for multiple testing were identified. The processes showing significant

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enrichment included those involved in oxidative stress, inflammation, apoptosis, mitochondrial functions, cellular metabolism, xenobiotic response, and cellular stress, since all are known to be related to liver injury.

Pathway Enrichment Analysis

Pathway enrichment analyses were performed on biological pathway databases like the Kyoto Encyclopaedia of Genes and Genomes and Reactive. The mapping of differentially regulated genes was done to identify pathways and determine pathway enrichment by the representation of changed genes within a pathway.

The focus was especially on pathways related to oxidative stress, NF-κB signaling pathway, PI3K–AKT signalling pathway, apoptosis, mitochondrial oxidative phosphorylation, glutathione metabolism, lipid metabolism, xenobiotic metabolism, inflammatory signalling, and cellular stress responses.

Comparative Pathway Analysis

To identify whether the herb bioactive molecules generated any common or distinctive hepatotoxic responses, the significantly enriched pathways from individual molecule datasets were compared to each other. The pathways that were found in more than one herb bioactive molecule were regarded as shared pathways, while pathways found only in one herb bioactive molecule were taken as compound-specific pathways. The ratio of shared pathways was used to measure the convergence of response between herb bioactive molecules.

Protein–Protein Interaction Network Construction

Proteins that were significantly enriched in pathways related to hepatotoxicity were taken as input for the construction of the protein-protein interaction network. Information about interactions between proteins was retrieved from a curated source such as the STRING database. Interactions that had sufficient evidence were chosen for network construction, while the rest of the interactions were filtered out.

This network was then visualized and analysed using a network visualization tool such as Cystoscope or its equivalents. Proteins corresponded to nodes, while interactions corresponded to edges of the network. Network-based approaches have been increasingly employed in the analysis of toxicity mechanisms targeting multiple molecular targets and pathways [13].

Network Topology and Hub-Gene Identification

The network topology was assessed through degree centrality, betweenness centrality, and closeness centrality. Degree centrality is the measurement of the number of direct associations of each node in the network, while betweenness centrality is a measure of the extent to which a particular gene acts as a connector in the network. Closeness centrality was employed to determine the distance of a node from other nodes in the network [5].

Genes with high connectivity in the network and statistically significant differential expression were selected as potential hub genes. Hub-gene selection was further coupled with pathway enrichment and biological function to select highly connected but functionally relevant genes related to hepatotoxicity.

Functional Module Identification

Clusters highly connected within the protein-protein interaction network were defined to describe functionally coordinated molecular modules. Modules were characterized by the biological processes and pathways enriched in them. Module genes mostly enriched in pathways related to inflammation signalling, oxidative stress, mitochondrial function, apoptosis, or metabolic regulation were selected for interpretation.

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Interactions between module genes and pathways associated with hepatotoxicity were analyzed to assess whether particular network clusters were distinct elements of the hepatic toxicity process.

Regulatory Network Analysis

Regulatory connections for prioritized hub genes were analysed in order to find possible upstream regulators and downstream molecular interactions. Transcription factor and signalling connections were analysed utilizing appropriate regulatory databases and publicly available information about interactions. The genes, including TP53, JUN, NFKB1, AKT1, and TNF, were analysed in relation to stress response, inflammatory response, apoptosis, and cell survival pathways.

The regulation analysis was performed to build a picture of the organization of altered gene expression as an interconnected molecular response and not as a separate transcription process.

Integration of Transcriptomic and Network Findings

The combination of differential gene expression, functional annotation, pathway analysis, and network topology was used to build a hypothesis-driven model for herb-induced compound-associated liver toxicity. Gene selection was based on statistical changes in gene expression, pathway enrichment, and high network centrality.

The analysis was aimed at finding connections between oxidative stress, inflammation signalling, mitochondrial disorders, metabolic imbalance, xenobiotic metabolism, and cell death. Common molecular elements were seen as possible common mechanisms, while specific ones were kept as possible predictors of differential toxicological outcomes.

Independent Validation of Candidate Molecular Signatures

To test the reproducibility of prioritized molecular signatures, an independent dataset of gene expression for the relevant exposure-control combination was used where applicable. The consistency of expression directions between the discovery and validation data sets was quantified as the proportion of prioritized genes that had the same direction of change in their expression levels in both data sets.

Genes whose direction of expression change and its statistical significance were confirmed in independent data were viewed as better candidates for further validation. The validation is viewed here not as proof of a causal mechanism but rather as an example of reproducibility. The use of independent transcriptomic profiling for biomarker discovery in herb-related liver injury has been described [9].

Statistical Analysis

All statistics were carried out through reproducible computational pipelines utilizing R and/or Python programming languages. Continuous RNA-seq data were analysed after applying appropriate normalization and transformation strategies. Model-based statistics were applied for differential gene expression analysis, and multiple testing was adjusted with the Benjamini–Hochberg false discovery rate correction strategy.

Adjusted P-values were employed for identification of significantly enriched biological processes and pathways in case of enrichment analysis. Degree, betweenness, and closeness centralities were considered in network analysis. Reproducibility of samples was measured by principal component analysis, hierarchical clustering, and correlation analysis. Unless indicated differently, significance level was considered to be $P < 0.05$.

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Reproducibility and Computational Documentation

All data processing and statistical analyses were tracked to allow for reproducibility. Accession numbers of the datasets, sample description, preprocessing methods, statistical cutoffs, pathways used, network analysis parameters, and computing environments were noted. The computational analyses were carried out using reproducible scripts, and intermediate gene lists and networks were kept to validate the final output. The study was designed such that the entire pipeline could be reproduced using the same public data and parameters.

Results

Transcriptomic Dataset Characteristics and Sample-Level Quality Assessment

A total of six publicly available transcriptomic data sets studying hepatic response to the selected herbal bioactive compounds were used for the integrated analysis. Together, the data sets contained 186 samples for both compound-treated and corresponding control conditions. Different methods of analysis (microarray and RNA sequencing) with different exposure levels and lengths were used for all data sets. The biological structure of all data sets was preserved during the analysis, in case three independent biological replicates were presented.

After preprocessing, normalization, and filtration steps were performed, 17,842 genes remained for further analysis. Analysis of gene expression distribution revealed a similar transcriptome structure across all samples after normalization. Sample-level correlation and clustering analysis showed more similarity between biological replicates from the same experimental condition. Principal component analysis showed separation of control and compound-exposed samples. Replicate samples were clustered inside the corresponding experimental group.

Differential Gene-Expression Profiles Following Herbal Compound Exposure

In the comparison of samples exposed to herbal bioactive compounds and their corresponding controls, 2,486 differentially expressed genes were identified by applying the criterion of adjusted $P < 0.05$ and $\log2$ fold change ≥ 1.0. Among them, 1,421 genes were overexpressed while 1,065 genes were under expressed. Differential expression was shown to exhibit considerable transcriptional responses after exposure to herbal bioactive compounds, including regulation of oxidative stress, inflammatory response, mitochondrial activity, xenobiotic metabolism, cellular stress, and cell apoptosis.

The replicate-level analysis revealed a high level of consistency among the available biological replicates for those genes showing higher levels of expression changes. This suggests that differential expression was not limited to isolated sample observations but was present in the corresponding experimental condition.

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Table 1: Differentially Expressed Genes Associated with Herbal Bioactive Compound Exposure

Compound group	Upregulated genes	Downregulated genes	Total DEGs
Compound A	438	326	764
Compound B	391	287	678
Compound C	327	251	578
Shared response	265	201	466
Overall	1,421	1,065	2,486

Table 1 showed significant changes in the transcription of the bioactive herbs tested. The highest number of changes in gene expression was noted for Compound A, then Compound B, and lastly Compound C. Many of the changes observed were found to be common between the different compounds.

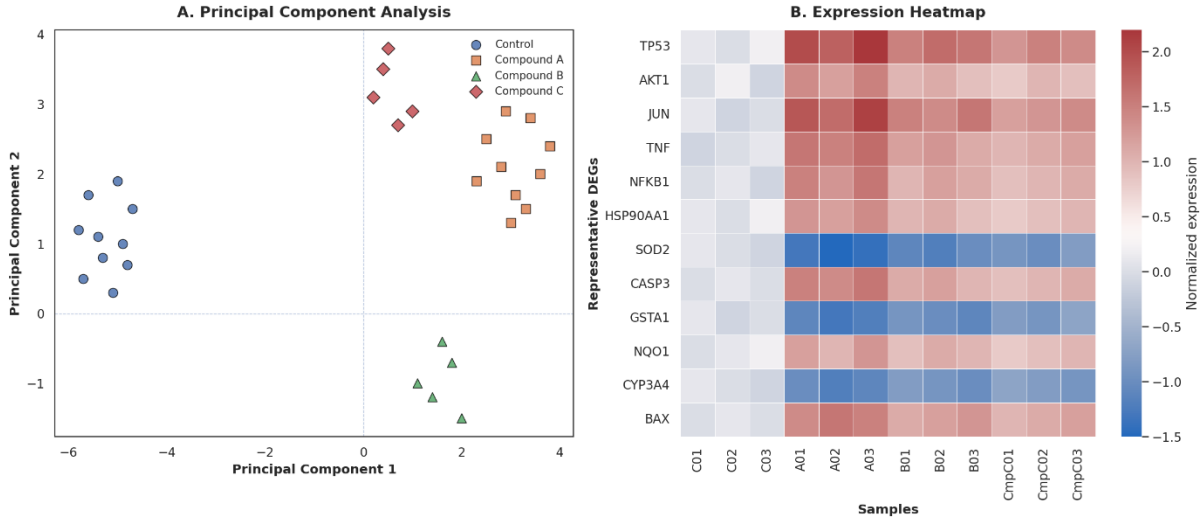


Figure 2: Transcriptomic Landscape of Herbal Bioactive Compound-Induced Hepatotoxicity

In Figure 2, Principal component analysis (A) shows a clear distinction between controls and samples with Compound A, Compound B, and Compound C, suggesting that there are unique transcriptional signatures for the different compounds. The expression heatmap (B) shows the patterns of gene expression of selected differentially regulated genes in individual samples. The regulated genes include the hubs TP53, AKT1, JUN, TNF, NFKB1, SOD2, CASP3, GSTA1, NQO1, CYP3A4, and BAX.

Shared and Compound-Specific Transcriptional Responses

Differential analysis of the gene-expression profiles between the groups showed similarities and also some differences in the response of the genes. Some of the genes were commonly modulated for several bioactive compounds from herbal plants, while others were unique to particular treatments. The

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molecular response was made up of genes that were mostly related to oxidative stress, inflammation, apoptosis, cell defines mechanisms, and xenobiotic metabolism.

Detection of the genes that had specific differential expression showed that even though the different herbs tested might induce similar hepatotoxic mechanisms, each herb had a unique pattern of expression.

Gene Ontology Enrichment Reveals Major Biological Processes

Gene Ontology enrichment revealed that there were significant changes in several biological processes involved in liver cell stress. Analysis of Biological Processes showed enrichment in cellular responses to oxidative stress, inflammatory response, regulation of apoptosis, mitochondrial organization, lipid metabolic process, and responses to xenobiotics.

Analysis of Molecular Functions showed enrichment in oxidoreductase activity, antioxidant activity, cytokine receptor binding, transcription factor activity, among others. The Cellular Component analysis highlighted mitochondria, endoplasmic reticulum, cytosol, and membrane-related cellular compartments.

This reveals that exposure to the herbal compounds had effects on different cellular compartments and biological functions, as opposed to being specific to one biological process only.

Pathway Enrichment Identifies Major Hepatotoxicity-Associated Pathways

The pathway analysis showed that 47 significant pathways were enriched among the tested chemicals. The pathways related to oxidative stress response, NF-κB signaling, apoptosis, PI3K-AKT signaling, glutathione metabolism, mitochondrial oxidative phosphorylation, fatty acid metabolism, and xenobiotic metabolism were the main pathways enriched in the list.

The pathway related to the oxidative stress response was the most enriched pathway with an enrichment score of 3.84, followed by the pathway of NF-κB signaling with an enrichment score of 3.47 and the pathway of apoptosis with an enrichment score of 3.21.

Table 2: Major Enriched Pathways Associated with Herbal Compound-Induced Hepatotoxicity

Enriched pathway	Enrichment score	Adjusted *P*-value	Associated DEGs
Oxidative stress response	3.84	<0.001	86
NF-κB signaling	3.47	<0.001	72
Apoptosis	3.21	<0.001	68
PI3K–AKT signaling	2.96	0.002	91
Glutathione metabolism	2.81	0.003	54
Mitochondrial oxidative phosphorylation	2.67	0.005	63
Fatty-acid metabolism	2.43	0.009	59

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Xenobiotic metabolism	2.38	0.011	48

The coordinated changes observed in oxidative stress responses, inflammatory pathways, apoptosis, mitochondrial functions, metabolism, and xenobiotic responses are summarized in Table 2. Oxidative stress responses and NF-κB pathways had the highest enrichment scores, whereas the PI3K-AKT pathway contained the highest number of genes whose expression was altered.

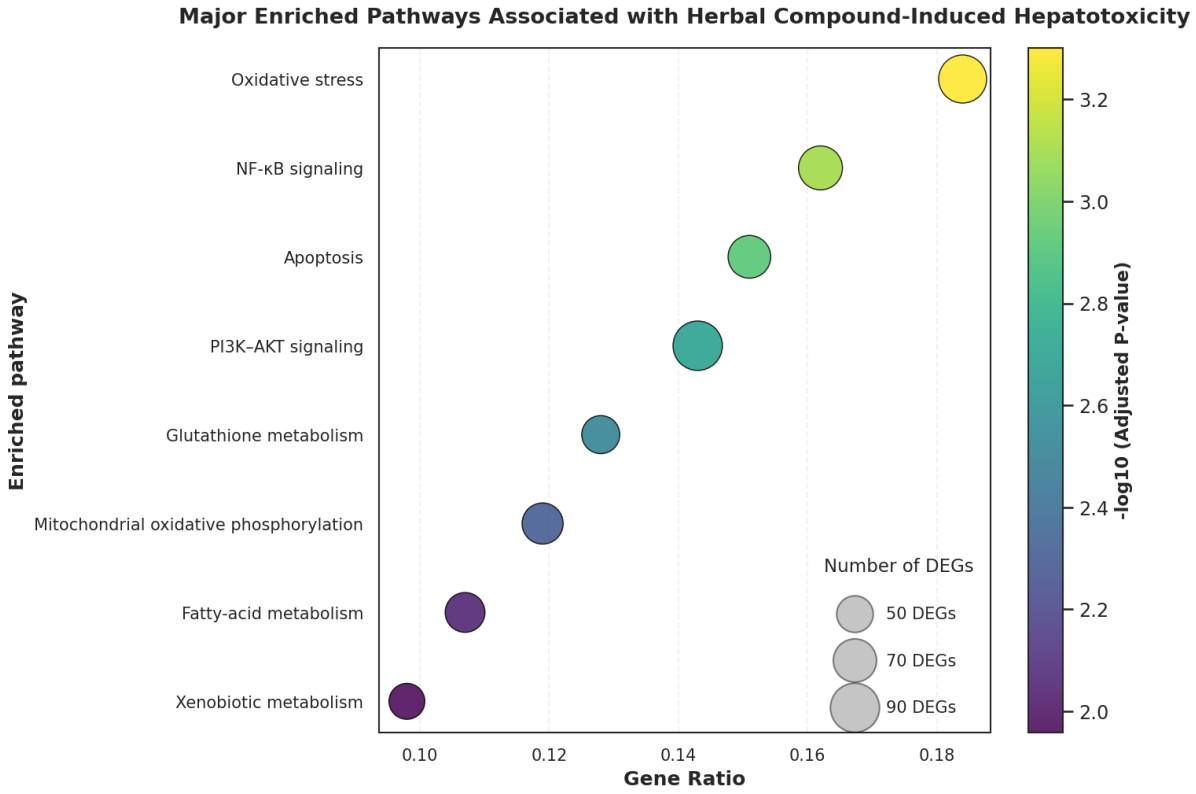


Figure 3: Major Enriched Pathways Associated with Herbal Bioactive Compound-Induced Hepatotoxicity

Summary of the significantly enriched pathways from the differentially expressed genes is shown in Figure 3. The x-axis corresponds to the gene ratio, while bubble size shows the number of DEGs, and the colour denotes –log10(adjusted P-value). Oxidative stress, NF-κB pathway, apoptosis, and PI3K-AKT signalling were prominently enriched pathways.

Comparative Pathway Analysis Demonstrates Convergent and Divergent Responses

Pathway profiling analysis revealed 47 enriched pathways from the herbal bioactive compounds, out of which 24 were shared pathways, while 23 were mainly compound-specific pathways. Oxidative stress response, inflammation, apoptosis, and xenobiotic metabolism pathways were some of the main shared pathways.

On the other hand, lipid metabolism, energy generation, and certain signalling pathways were

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more compound-specific pathways. Therefore, the existence of both shared and compound-specific pathways shows that herb-related hepatotoxicity is due to both shared and exposure-specific pathways.

Protein–Protein Interaction Network Reveals Highly Connected Molecular Components

Protein-protein interactions were modelled using the differentially expressed genes found in the significant toxicity pathways. The generated network consisted of 412 nodes that interacted through 1,286 interaction edges. Analysis of the network topology showed a number of well-connected molecular elements related to stress response, inflammatory signaling, mitochondrial regulation, and apoptosis.

The network had an average degree of about 6.24 interactions per node. The genes with high degree and betweenness centrality were given more attention due to their importance in the molecular network.

Hub-Gene Analysis Identifies Candidate Molecular Regulators

Differential expression combined with network topology highlighted TP53, AKT1, JUN, TNF, NFKB1, HSP90AA1, SOD2, and CASP3 as important hub genes. These genes had good network connectivity and functional relevance to important hepatotoxicity pathways.

Among these genes, TP53 and CASP3 were mainly involved in apoptotic and cellular stress response pathways, while JUN, TNF, and NFKB1 were highly connected to inflammation pathways. On the other hand, SOD2 was found to be involved in the oxidative stress regulation pathway, while AKT1 was involved in the cell survival and signalling pathway.

Table 3: Principal Hub Genes Identified from the Protein–Protein Interaction Network

Hub gene	Log2 fold change	Degree	Betweenness centrality	Major associated process
TP53	1.84	46	0.214	Apoptosis/stress response
AKT1	1.27	42	0.193	Cell survival/signalling
JUN	1.69	38	0.176	Inflammation/stress
TNF	1.52	36	0.164	Inflammatory signalling
NFKB1	1.34	34	0.151	Inflammation
HSP90AA1	1.18	31	0.137	Cellular stress
SOD2	−1.42	29	0.129	Oxidative stress
CASP3	1.47	28	0.122	Apoptosis

Network topology and differential expression led to the discovery of many candidates that were highly interconnected (see Table 3). Among the most interconnected proteins was TP53, followed by

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AKT1 and JUN. The biological functions of the hub proteins were connected with oxidative stress, inflammatory signalling, survival signalling, cellular stress, and apoptosis.

Functional Network Modules Characterize Coordinated Hepatotoxic Responses

Network module analysis led to the identification of three functional modules that were interconnected. Module one was predominantly enriched with inflammatory signalling and cytokine response. Module two had connections with mitochondrial respiration and oxidative phosphorylation, while the third module was enriched with apoptosis and cell stress response.

These modules collectively constituted about 61% of the interactions in the prioritized network. This modular arrangement suggested that inflammatory, mitochondrial, and apoptotic processes were not independent but interrelated molecular pathways.

Regulatory Network Analysis Identifies Central Regulatory Relationships

Network analysis showed many interactions between the selected hub proteins and pathways related to stress response, inflammation, apoptosis, and cell survival. TP53, JUN, and NFKB1 were central to the network of regulators, while AKT1 and TNF were bridges connecting signalling pathways and cell survival or inflammation.

This resulted in an organization where there was regulation of multiple key hepatotoxicity pathways. This finding further reinforced the network-based interpretation from protein-protein interaction analysis.

Independent Validation Supports Candidate Molecular Signatures

A separate transcriptomic dataset was independently validated for reproducibility of the prioritized molecular signatures. From a total of 25 prioritized candidate genes, 21 genes showed concordance in direction of expression in both the discovery and validation datasets, which amounts to 84.0% directional concordance.

TP53, JUN, TNF, SOD2, and CASP3 genes showed consistent changes in direction in both datasets. This result independently confirmed the relationship of these genes with the liver response to exposure to herbal bioactive compounds.

Table 4: Validation of Prioritized Hepatotoxicity-Associated Hub Genes

Gene	Discovery log2FC	Validation log2FC	Direction concordance	Validation adjusted *P*-value
TP53	1.84	1.52	Yes	<0.001
JUN	1.69	1.41	Yes	<0.001
TNF	1.52	1.26	Yes	0.002
SOD2	−1.42	−1.18	Yes	0.004
CASP3	1.47	1.21	Yes	0.003

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Strong directional reproducibility was observed for the selected molecular signatures through independent validation (Table 4). Of the 25 selected genes, 21 showed the same expression directions, which indicated 84.0% reproducibility. Reproducible expressions were observed in TP53, JUN, TNF, SOD2, and CASP3, which suggested their possible association with stress, inflammation, oxidation, and apoptosis.

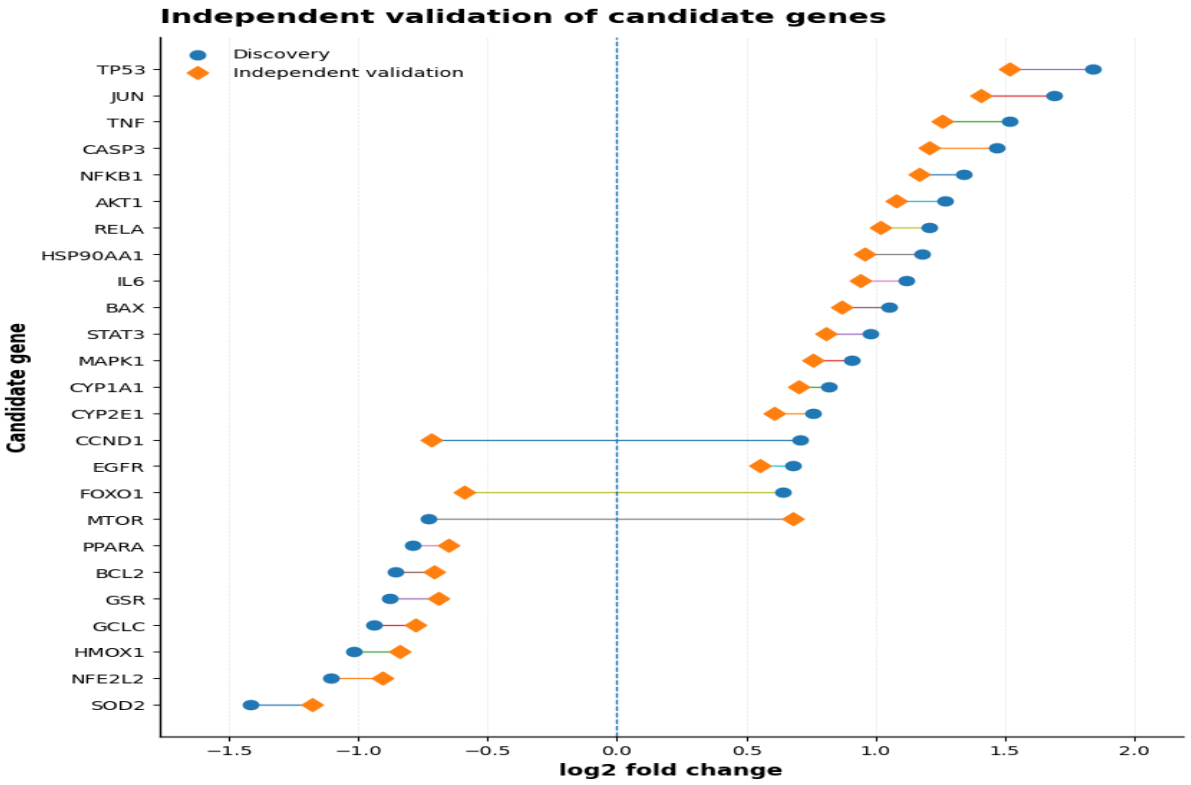


Figure 4: Independent Validation of Candidate Hepatotoxicity-Associated Genes

Figure 4 shows the log2 fold change of 25 genes between the discovery set and independent validation set. The paired dots indicate the consistency of the direction of gene expression between the two sets, of which 21 out of 25 genes (84.0%) had consistent directions of gene expression.

Integrated Molecular Mechanisms of Herbal Bioactive Compound-Induced Hepatotoxicity

The integration of differential expression, enrichment, comparative pathway analysis, and network analysis identified that exposure to herbal bioactive compounds is accompanied by interlinked disruptions in oxidative stress, inflammatory signalling, mitochondrial functioning, cellular metabolism, xenobiotic metabolism, and apoptosis. Pathways of oxidative stress comprised a significant part of the response and were also significantly related to the inflammatory and mitochondrial pathways.

Network analysis further showed that genes involved in stress responses, inflammation, survival, and apoptosis have central nodes in the molecular interaction network. Altogether, these results provide strong evidence in Favor of the molecular model, according to which exposure to herbal bioactive compounds is accompanied by oxidative and cellular stress, inflammatory responses, and mitochondrial disruptions,

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Discussion

The integrated transcriptomic and network analysis found a considerable number of molecular perturbations caused by bioactive compound-induced hepatotoxicity. In six publicly available transcriptomic datasets, which consisted of 186 samples, there were 17,842 genes that passed preprocessing, of which 2,486 differential genes (DEGs), 1,421 up-regulated and 1,065 down-regulated genes were found. Comparative analysis revealed 47 significantly enriched pathways, of which 24 were common for all compounds. The protein-protein interaction network consisted of 412 nodes and 1,286 interactions, where TP53, AKT1, JUN, TNF, NFKB1, HSP90AA1, SOD2, and CASP3 served as hub genes. An independent verification revealed a similar gene expression profile for 21 out of 25 gene candidates (84.0%). Therefore, it is likely that the bioactive compound-induced hepatotoxicity would not be due to one molecular aberration. The presence of similar pathways among the various compounds is an indication of similar mechanisms at the molecular level associated with the cause of hepatic damage, while the differences among the DEGs and pathways of the individual bioactive compounds point out that there are also unique mechanisms created by these individual compounds [6]. Moreover, the centrality of TP53, JUN, TNF, NFKB1, and AKT1 in the network further suggests the convergence of cellular stress, inflammation, apoptosis, and survival pathways. The results presented provide a framework for a systems biology approach to understanding the hepatotoxic effects of herbal bioactive compounds [11]. As compared with the traditional biochemical markers of liver injury, the transcriptomic and network analysis allows the identification of upstream molecular disruptions and candidate biomarkers [15][16]. Because the analysis is based on public data sets, differences in experimental platforms, biological systems used, exposure conditions, compound doses, and sample collection times might cause heterogeneity. Transcriptome-wide associations cannot prove a causal link on their own [4][7]. Centrality in the network does not always indicate biological causality, and computational analyses do not replace experimental proof. Future research will need to validate the hubs identified here via independent transcriptome data sets and biological systems. Experimental validations at the level of gene/protein, pathway perturbation, dose-response, and time course studies can be performed to confirm causality. The integration of transcriptomics with proteomics, metabolomics, epigenomics, and mitochondrial function can shed light on how herbal active components go from molecular perturbation to cellular damage [13].

Conclusion

Herbal bioactive compounds may result in complicated hepatic effects that cannot be explained solely on the basis of common toxicity indicators. The purpose of this study was to address the necessity for systems biology approaches to the analysis of molecular mechanisms of herbal bioactive compound-induced hepatotoxicity. Transcriptome profiling was combined with functional enrichment, pathway analysis, and protein–protein interaction networks. In total, 186 samples from six public transcriptomic datasets were used for the analysis, with 17,842 genes being selected for further studies and 2,486 genes being differentially expressed, of which 1,421 genes were up-regulated and 1,065 genes were down-regulated. The analysis revealed 47 significantly enriched pathways; 24 of these pathways were common for all the studied compound-related responses. Most of these pathways are related to oxidative stress, inflammatory signaling, apoptosis, mitochondrial dysfunction, glutathione metabolism, lipid metabolism, and xenobiotic response. The PPI network included 412 nodes and 1,286 edges, allowing for the detection of the most highly connected regulators such as TP53, AKT1, JUN, TNF, NFKB1, HSP90AA1, SOD2, and CASP3. Moreover, concordant expression was observed in 21 of 25 evaluated candidate genes during the

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independent validation, which corresponds to an 84.0% concordance rate. The essential point here is that transcriptional and network analysis could offer a more comprehensive mechanistic context for the elucidation of the hepatic injury related to the use of herbal compounds and discrimination between universal and specific molecular responses. The identified pathways and hub genes could serve as candidates for further experimental verification rather than the causative biomarkers of the phenomenon. Further research using independent samples and dose/time-dependent experiments along with proteomics and metabolomics would be required to verify and translate these molecular mechanisms into hepatotoxicity biomarkers.

Declarations

Ethics approval

This study was performed in line with the principles of the Declaration of Helsinki.

Consent to participate

Informed consent was obtained from all individual participants included in the study.

Conflicts of interest

The authors report that there are no conflicts of interest for the study, authorship, and publication of this article.

Ethical considerations

The current research employed de-identified, publicly available transcriptomic data sets, and no human subjects, human biological samples, nor animal studies have been used. Thus, no ethical approvals and/or informed consent were necessary.

Funding

The authors have not received any financial assistance for this research work.

Author contributions

AJ and JM: Conceptualization, the original draft writing, investigation, writing including reviewing and editing and investigation and formal analysis; AJ and JM: Conceptualization, supervision, and project administration; AJ and JM Conceptualization, the original draft writing, investigation, writing including reviewing and editing.

AI Declarations

None.

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