



## Investigation of Crucial Affected Proteins in Rat Liver in the Presence of *Scrophularia*

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### Abstract

**Background and objectives:** Radix *Scrophularia* is dried root *Scrophularia ningpoensis* Hemsl. which is used as a drug against several diseases. In the present study, the crucial affected proteins of rat liver in the presence of radix *Scrophularia* has been investigated. **Methods:** The differentially expressed proteins (DEPs) were downloaded from literature. The significant DEPs plus 100 added first neighbors were determined and included in “protein query” of STRING database via Cytoscape software. The network was analyzed and the central nodes among the queried DEPs were identified. The 10 first neighbors of the central DEPs were determined. **Results:** RT1-CE12, Gfer, Serpina3c, Rab13, Rbm14, Ighg Psmb8, COX2, Olr796, Mga, Ugt1a6, Ugt2b, Ebpl, Ugt2b, Igf2r, and Amacr as significant DEPs were analyzed via protein-protein interaction (PPI) network analysis, Ugt1a1 and Ugt2b the two up-regulated proteins were highlighted as the crucial proteins in response to the radix *Scrophularia*. **Conclusion:** Two members of UDP glucuronosyltransferase family; Ugt1a1 and Ugt2b, were pointed as the critical liver enzyme which are dysregulated under effect of radix *Scrophulariae*. Due to crucial role of Ugt1a1 in the liver function, it is suggested that consumption of *Scrophularia ningpoensis* Hemsl. as a traditional medicine required more investigation.

**Keywords:** liver; network; protein; rat; *Scrophularia*

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### Introduction

*Scrophularia ningpoensis* Hemsl. is a medical plants which is used in Chinese traditional medicine as a drug for several diseases such as diabetes, cancer, inflammation, hypertension, and liver diseases [1]. As it is recorded from many years ago, root of this herb is used as a drug to

cure swelling, fever, constipation, laryngitis, pharyngitis, neuritis, rheumatism, sore throat, and arthritis [2]. Radix *Scrophularia* is the dried root of *Scrophularia ningpoensis* and is known as the most commonly used part of the herb traditional medicine [3]. Determining the bioactive

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compounds of *Scrophularia ningpoensis* Hemsl. has attracted attention of researchers [4]. Lu F et al. have administrated an investigation about molecular mechanism of radix *Scrophularia* via integrated metabolomics [5]. Chao, Hung-Hsiang et al. published a case report about liver injury follow long-term consuming of *Scrophularia ningpoensis*.

PPI network analysis is a useful method to explore the main targeted proteins by the probes such as drugs, radiations, and the other induced conditions. In this approach, the queried proteins are interacted by edges to form a network. Each protein plays its unique role in the network. While a limited numbers of proteins which are known as hubs interact to the large numbers of the first neighbors, the other elements of the network may connect to the limited number of first neighbors. Topology analysis provides centrality properties of the nodes. Some important centrality parameters are degree value, betweenness centrality, closeness centrality, and stress [7-10].

PPI network analysis has recently attracted attention of the traditional medicine experts. Effects of many herbal drugs have been studied by many investigators via PPI network analysis. Effect of saffron crocetin on non-alcoholic fatty liver disease, detection of molecular mechanism of ginger on colon cancer treatment, and immunoregulatory mechanism of ginseng leaves effect on lung cancer are examples of many researches which are administrated via PPI network analysis [11-13]. In the present study, proteomic evaluation of effects of radix *Scrophularia* on rat liver which was reported in literature [14] has been analyzed via PPI network analysis to detect the critical targeted proteins by the herbal drug.

## Material and Methods

### Ethical considerations

This project was approved by following ethical code: IR.SBMU.REC.1401017 by Shahid Beheshti University of medical Sciences.

### Data collection

As it is described in the original paper, 10 healthy male Sprague-Dawley rats were received water decoction of radix *Scrophulariae* (dried root of *Scrophularia ningpoensis* Hemsl.) versus 10 control. Liver proteomes of the treated and control rats were compared to find the affected proteins by radix *Scrophulariae*. Seventy six

modulated proteins were introduced as the significant dysregulate individuals [14].

Based on fold change, the significant up and down-regulated proteins were selected for more analysis. The selected dysregulated proteins were included in "protein query" of STRING database via Cytoscape software version 3.7.2 to explore connections between the assessed proteins. Confidence score = 0.4 was considered to form the network. Since most proteins were remained isolated, 100 first neighbors were added to the queried proteins and the network was constructed. The nodes were connected by undirected edges. The network was analyzed by "Network Analyzer" application of Cytoscape. Network was visualized by degree value considering color and size of the related nodes. Four common centrality parameters including degree, betweenness centrality, closeness centrality, and stress were identified for nodes of the network. Hub nodes of the network were determined and discussed. The queried hubs were evaluated via their 10 first neighbors.

## Results and Discussion

Among the 76 dysregulated protein, 15 significant DEPs including; RT1-CE12, Gfer, Serpina3c, Rab13, Rbm14, Ighg Psm8, COX2, Olr796, Mga, Ugt1a6, Ugt2b, Ebpl, Ugt2b, Igf2r, and Amacr were selected to be analyzed (Table 1).

The PPI network was constructed by the queried DEPs. There were poor connections between the interacted nodes (data not shown). When 100 first neighbors were added to the queried DEPs, the network was created with considerable connections and 14 DEPs among the queried proteins were included in the network. The nodes were layout based on degree value and the network was visualized (Figure 1). Since the first and second hubs were two queried DEPs and the third queried DEP were ranked as the 32<sup>th</sup> protein based on degree value, these two top queried proteins and the first added neighbor were pointed as hubs (Table 2). As it is depicted in the Figure 2, 10 first neighbors were added to the two queried hubs. Considering ENSRNOP00000041138 as Cyp3a1, 80% of the added first neighbors belonged to Cyp family. Shen X et al. published a document via microarray-based bioinformatical analyses on the effects of *Scrophularia* on the gene expression profile changes which are companied with proliferation inhibition, apoptosis and anti-

inflammation in the treated cells. They concluded that acetoside is an active compound of *Scrophularia* which induces DNA alkylation [15]. In the present study, the main targeted proteins by radix *Scrophularia* were identified among the large numbers of the dysregulated proteins which were introduced by Lu F et al. [14].

As it is shown in Table 1, 15 significant dysregulated proteins among the 76 introduced individuals were selected for analysis. All dysregulated proteins in the original paper were characterized with p value < 0.05, the selected proteins in our analysis were limited by “fold change” > 1.5. The PPI network was formed by the queried DEPs and the added first neighbors. As it is shown in Figure 1, UDP-glucuronosyltransferase family 1 member A6 and UDP-glucuronosyltransferase family are appeared as the hubs of the constructed network. UDP glucuronosyltransferases (UGTs) belong to a superfamily enzyme which are responsible to metabolize exogenous and endogenous chemicals [16,17]. The top ranked hub among the added first neighbors is Cyp1a1 (Table 2). To find the more related proteins to the queried hubs (UGTs), the 10 first neighbors are shown in the Figure 2. Most of the added first neighbors are members of “Cytochrome P450” family. Cyp3a2, Cyp2c13, and Cyp17a1 are the three dysregulated members of “Cytochrome P450” family that are reported in the original paper. This finding is consistent with

our analysis and confirmed the findings of Lu F et al. [14].

It is expressed that cytochrome P450 enzymes are largely dispersed among living organism. These enzymes are involved in biosynthesis of natural products and degradation of xenobiotics. Metabolism of drug and biosynthesis of steroid are attributed to cytochrome P450 enzymes [18]. Similar to UDP glucuronosyltransferases, the cytochrome P450 members are up-regulated. It seems, there is positive correlation between the expression of these two families of enzymes.

*Styrax japonica* is a medical plant that is used broadly as an anti-inflammatory drug in Korea. Styrylignolide-A has been isolated from this plant and homoeogonol is a lignan derived from this substance. It is reported that homoeogonol removes completely from body via metabolism. Investigations indicate that homoeogonol metabolism is concerned deeply by cytochrome P450 and UDP-glucuronosyltransferase enzymes. This evaluation was performed by using microsomes of human liver, and cDNA-expressed CYP and UGT enzymes [19,20].

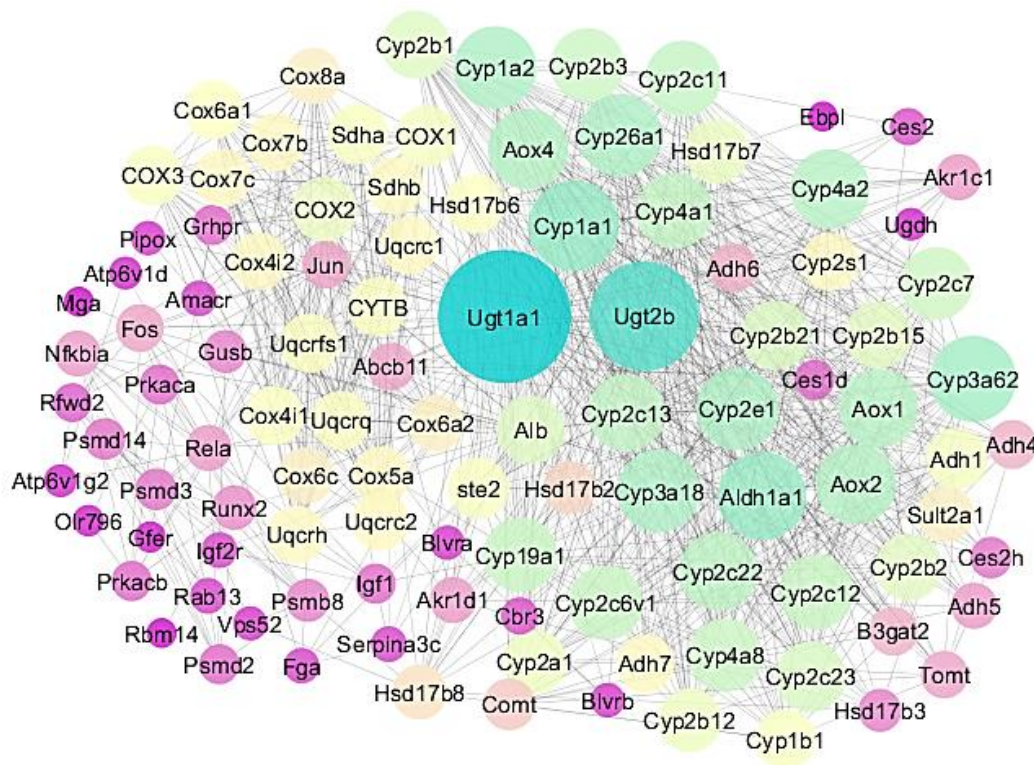
As shown Table 2, Ugt1a1 and Ugt12b are characterized with different centrality parameters. Ugt1a1 relative to Ugt2b is characterized by larger values of degree and betweenness centrality.

**Table 1.** List of significant DEPs (based on fold change) that appeared in the liver of rat in response to *Scrophularia*; data were extracted from published data by Lu F et al [14]

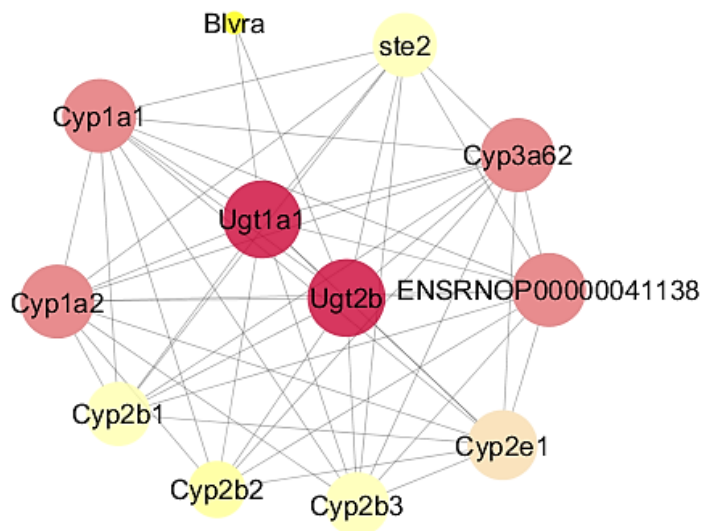
No.	Gene	Description	Fold change
1	RT1-CE12	RT1 class I, CE12	0.47
2	Gfer	FAD-linked sulphydryl oxidase ALR	0.55
3	Serpina3c	Protein Serpina3c	0.55
4	Rab13	Ras-related protein Rab-13	0.61
5	Rbm14	Protein Rbm14	0.63
6	Ighg	Ighg protein	0.64
7	Psm8	Proteasome subunit beta type	0.65
8	COX2	Cytochrome c oxidase subunit 2	1.5
9	Olr796	Olfactory receptor	1.6
10	Mga	Protein Mga	1.6
11	Ugt1a6	UDP-glucuronosyltransferase family 1 member A6	1.7
12	Ugt2b	UDP-glucuronosyltransferase family 2 member B	1.8
13	Ebpl	Emopamil binding protein-like (predicted), isoform CRA_a	1.8
14	Igf2r	Mannose 6-phosphate/insulin-like growth factor II receptor	2.1
15	Amacr	Alpha-methylacyl-CoA racemase	2.6

**Table 2.** Hub nodes of the network, data are ranked based on degree value.

No.	Display name	Degree	Betweenness centrality	Closeness centrality	Stress
1	Ugt1a1	87	0.351	0.779	15898
2	Ugt2b	66	0.093	0.669	8000
3	Cyp1a1	49	0.015	0.585	2378



**Figure 1.** PPI network including the queried DEPs plus 100 first neighbors; neighbors; purple to blue and small to large refer to increasing degree value; confidence score 0.4 was considered.



**Figure 2.** A sub-network including the two queried hubs and the added 10 first neighbors; ENSRNOP00000041138 is Cyp3a1

Stress value was also 2-fold bigger for Ugt1a1 relative to Ugt2b. These findings indicated that Ugt1a1 relative to Ugt2b plays more important

role in the network. Kurita A et al. designed an investigation to evaluate ability of Ugt2b family members to glucuronidate morphine. They

showed difference between Ugt1a1 and Ugt2b family member on glucuronidation of 17 $\beta$ -estradiol. In this document Ugt1a1 was pointed as an important player in human liver [21].

### Conclusion

Among the significant DEPs, UDP glucuronosyltransferase (especially Ugt1a1) is the critical liver enzyme which is up-regulated in the presence of radix *Scrophulariae*. Considering critical role of radix *Scrophularia* in metabolizing exogenous and endogenous chemicals, it can be concluded that consumption of *Scrophularia ningpoensis* as a traditional medicine has intensive effects on liver function. It seems more investigations are required to find suitable protocol to use radix *Scrophularia* as a safe drug.

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### Author contributions

Vahid Mansouri designed the project. Babak Arjmand, Mahmood Khodadoost, Zahra Razzaghi, Mostafa Rezaei Tavirani and Majid Rezaei Tavirani contributed in data collection and preparation of the manuscript.

### Declaration of interest

The authors declare that there is no conflict of interest. The authors alone are responsible for the accuracy and integrity of the paper content.

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### Abbreviations

DEPs: differentially expressed proteins; PPI: protein-protein interaction; UGTs: UDP glucuronosyltransferases