Research Journal of Pharmacognosy (RJP) 8(3), 2021: 25–31
Received: 6 Jan 2021
Accepted: 5 Apr 2021
Published online: 14 Apr 2021
DOI: 10.22127/RJP.2021.266106.1660

Original article

Assessment of Molecular Mechanism of Saffron Anti-Stress Property

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Abstract

Background and objectives: There are several documents about protective properties of saffron against stress conditions which refer to the effect of saffron on gene expression pattern of the treated samples. The aim of the present study was determination of the main regulated proteins by saffron extract. **Methods:** Twenty differentially expressed proteins from a published research were investigated via network analysis and assessed to determine the crucial regulated individuals by Cetoscape software. The network was analyzed by network analyzer application of Cytoscape software, and the central nodes were identified. **Results:** Twenty queried proteins were included in a network with 9005 nodes and 11446 edges. Analysis of the network revealed that VCP, SOD1, GRP78 (HSPA5), GRP75 (HSPA9), PRDX1, PHB, COMT, and ATP5H are the central proteins which are regulated by saffron extract. **Conclusion:** Based on the regulated proteins, regulation of mithoconderia and endoplasmic reticulum was identified as the main target of saffron in stress management.

Keywords: bioinformatics; network analysis; protein expression; rat; saffron

Citation: Arjmand B, Khodadoost M, Razzaghi M, Rezaei Tavirani M, Ahmadzadeh A, Rezaei Tavirani S. Assessment of molecular mechanism of saffron anti-stress property. Res J Pharmacogn. 2021; 8(3): 25–31.

Introduction

Role of saffron (*Crocus sativus* L., Iridaceae) as a useful nutrient is discussed in detail by many researchers. Protective properties of saffron against several stress conditions is investigated and confirmed. It is reported that saffron effects on gene expression process and regulates many genes to produce the necessary proteins which are required versus stress. Beside advantages of saffron consumption, there are documents that refer to the disadvantages of saffron consumption. Understanding molecular mechanism of saffron will reveal true aspects about saffron use [1-4].

Proteomics is a high throughput method that is applied to discover protein expression pattern of biological samples. Types of proteins and amounts of proteins expression can be identified by proteomics experiments. Since treatment of living organisms leads to change in protein expression

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pattern, this method is applied widely to detect molecular mechanism of many nutrients such as saffron and coffee. Results of proteomics studies are similar to other high throughput methods that include many proteins whose perfect analysis of findings requires bioinformatics assessment [5-9]. Network analysis based on graph theory, is a bioinformatics approach that is used to interpret proteomes. Proteins as nodes include in a network which elements are connected by edges. Based on the role of nodes on the network (the scale free networks) there are a few numbers of nodes that are known as central nodes [10,11]. Central nodes are discriminated from other node by more connection with the first neighbors and also participation in the shortest paths. It is found that the central nodes of a network are the key elements of network that control main parts of molecular mechanism of the studied condition. Two kinds of central nodes are used frequently in interpretation of network topology including hubs and bottlenecks [12-14]. The hub nodes are determined based on degree value which refer to the amounts of connections between the study node and the first neighbors while the bottleneck nodes are identified based on participation of node in the shortest paths [15]. In the present study, network analysis of saffron effect on gene expression profile of rat liver in response to the ischemia reperfusion injury was assessed to find the critical regulated proteins.

Material and Methods

Ethical considerations

This project is administrated with IR.SBMU.RETECH.REC.1399.625 ethic code by ethics committee of Shahid Beheshti University of medical Sciences.

Plant material and animal study

Method of saffron ethanol extract preparation, animal treatment and proteomics experiments are described in publication of Tai-Long Pan et al. in details [16]. Briefly, six rats were considered as ischemia reperfusion (IR) group and another six individuals were candidate for receiving saffron ethanol extract. Portal vein and hepatic artery of the rats were clamped for 60 min during anesthesia. After removing clamp, animals' livers were prepared after 2 hours of reperfusion for proteomics experiments. The saffron group received 20 mg/kg of saffron extract 2 hours before ischemia.

Network analysis

Two-dimensional electrophoresis was applied to find the differentially expressed protein (DEP) spots. Twenty DEPs were identified that discriminate the two assessed groups.

In this analysis, the 20 introduced DEPs were assessed via network analysis to find the critical proteins which were dysregulated by saffron in response to ischemia. The queried 20 DEPs were imported in "Universal Interaction Database Client" of "network from public databases" port of Cytoscape software 3.7.2 [17]. The "all databases" and the "automatic network merge" options were selected to construct the protein-protein interaction (PPI) network. The network was analyzed by "Network Analyzer" to find the central nodes. Comparing degree values via plot of degree versus nodes names the hub DEPs were identified.

For better understanding, the 20 queried DEPs were included in a network via "protein query" of STRING database [18]. Eighteen DEPs among the 20 individuals were recognized by STRING. Due to weak interactions between the significant DEPs, the network containing a main connected component and 6 isolated proteins was formed. After adding 50 first neighbors, the network including 68 nodes was formed. Results of the two analyzed networks were compared to find common features of both networks.

Since understanding of relationship between the identified bubs requires exploring interactions among them, connection between the determined hub nodes were investigated via STRING database (https://string-db.org).

Results and Discussion

The queried 20 DEPs were involved in a network including 45 connected components via "Universal Interaction Database Client" of "network from public databases" port of Cytoscape software. The network was constructed from 9005 nodes which were connected by 11446 edges. The network was layout based on degree value and the nodes with degree value less than 6 were ignored. Eighty-nine nodes remained which were included mainly in the four main connected components. Among the repeated nodes, only the

node with the largest degree value was considered and also the nodes that were related to the human were excluded. Finally, 56 proteins were determined as the significant elements of the network. To determine hub nodes, degree values of the nodes were plotted (Figure 1). As it is shown in the figure, degree values of 14 nodes did not fit in the trend line. These 14 DEPs were identified as hub nodes; however, VCP and PHB were repeated in the two main connected components. The higher degree values were considered for VCP and PHB and the 12 hub nodes were determined (Table 1). Betweenness centrality and closeness centrality parameters for the hubs were extracted from Cytoscape analysis and are shown in the Table 1.

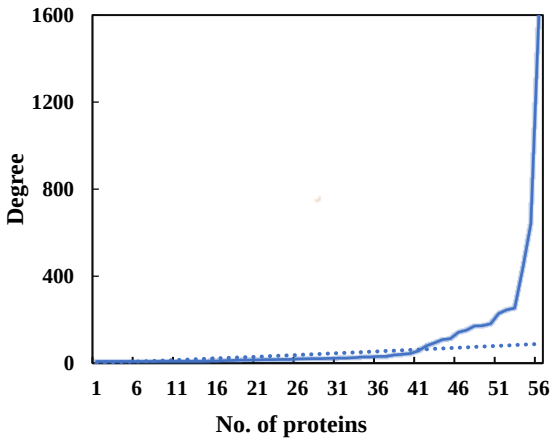

Figure 1. Degree values of the significant elements of the network versus number of proteins; the numbers on the horizontal axis refer to the 56 significant DEPs. The dotted line is the trend line.

The network including the 18 recognized DEPs by STRING and 50 added first neighbors is shown in the Figure 2. The nodes are layout based on degree value; the bigger size and green color refer to larger degree. Centrality properties of the queried hub nodes which are bolded in the Table 1, were extracted from the network that was constructed via STRING and are presented in the Table 2. Interactions between the introduced hub nodes which were determined by STRING database are presented in Figure 3.

Tai-Long Pan et al. investigation indicates that saffron ethanol extract reduces hepatic IR injury via regulation of protein expression. Twenty DEPs are introduced that play significant role in protection against hepatic IR injury [16]. As it is depicted in Figure 1 and Table 1, among the 20 queried DEPs, 8 proteins (including VCP, SOD1, GRP78 (HSPA5), GRP75 (HSPA9), PRDX1, proinhibitin (PHB), COMT, and ATP5H) were determined as hub nodes of the constructed network.

Based on finding from table 1, VCP is a potent hub-bottleneck protein which is characterized by the highest values of degree and betweenness centrality. Hojjat-Allah Abbaszadeh et al. investigation revealed that VCP play a crucial role in response to YAG laser radiation which led to cell cycle regulation [19]. As shown in Figure 2 and Table 2, VCP could not play this crucial role and is located in the 6th row of Table 2.

SOD1 which is the second potent hub in Table 1, is appeared as the third ranked protein in Table 2. Importance of GRP78 and GRP75 is repeated in both analyses. COMt and ATP5H are presented as weak hub proteins in Table 1 and also in Table 2. Finally, both analyses revealed that PRDX1 and proinhibitin can be considered as mild hub proteins.

Table 1. The hub nodes of the analyzed network and the related degree values, betweenness centrality, and closeness centrality

R	Protein name	Degree	BC	CC
1	**VCP**	1601	0.558	0.409
2	**SOD1**	641	0.232	0.349
3	**GRP78**	437	0.229	0.307
4	KAR2	253	0.110	0.199
5	hCG_41332	245	0.086	0.321
6	**GRP75**	228	0.044	0.264
7	**PRDX1**	172	0.056	0.314
8	**PHB**	171	0.044	0.297
9	APOA1	142	0.039	0.307
10	TDPX2	113	0.032	0.296
11	**COMT**	108	0.039	0.309
12	**ATP5H**	93	0.029	0.295

The bolded names refer to the queried DEPs. BC: betweenness centrality; CC: closeness centrality

Table 2. Centrality properties of the bolded queried hub nodes in Table 1 achieved from analysis of Figure 2

R	Name	Degree	BC	CC
1	Hspa9 (GRP75)	48	0.063	0.770
2	Hspa5 (GRP78)	43	0.034	0.728
3	SOD1	37	0.023	0.684
4	PHB	32	0.025	0.651
5	Prdx1	26	0.005	0.609
6	Vcp	22	0.003	0.573
7	ATP5H	14	0.001	0.545
8	COMT	5	0.000	0.459

As it is represented in the Figure 3, there is co-expression relationship between the introduced hubs and the connections are identified mostly experimentally. It seems that there are four central proteins that are responsible to prevent damages from liver ischemia; however, centrality may not correspond to the functional role of proteins.

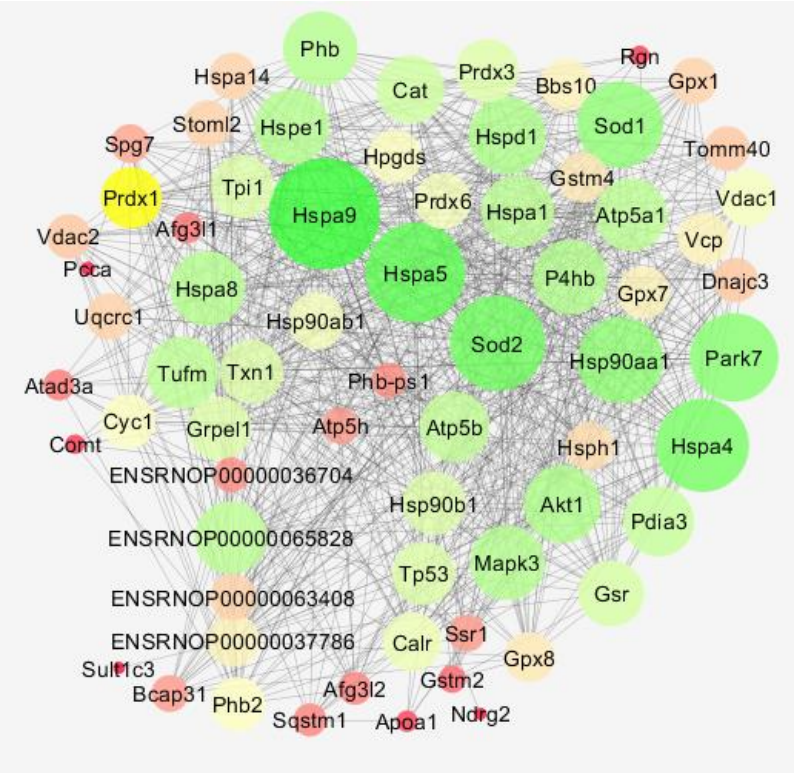

Figure 2. The network including 18 recognized DEPs by STRING database and added 50 first neighbors. The bigger size of nodes and color from red to green refer to increment of degree value.

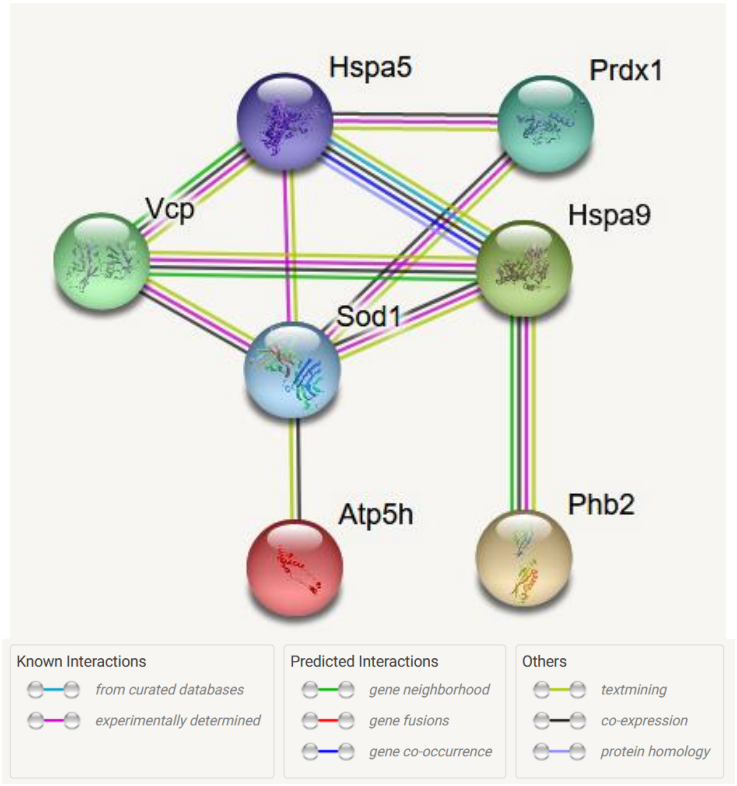

Figure 3. Up: connections between the introduced hub nodes recognized by STRING database; down: types of interactions

These proteins regulated by saffron were identified as VCP, SOD1, GRP78, and GRP75.
Glucose-regulated protein 78 (GRP78) or heat shock 70-kDa protein 5 (HSPA5) is known as one of the main proteins that are responsible for endoplasmic reticulum (ER) stress response [20]. Investigations indicated that different types of disturbances lead to "unfolded protein response" in response to accumulation of the resulted unfolded proteins from many stresses such as viral infections, glucose deficiency, and aberrant calcium ion regulation in ER [21].
Since ER stress can recruit inflammation and inflammation and "unfolded protein response" are known as essential processes in pathogenesis of inflammatory diseases [22], the crucial role of HSPA5 in prevention of protein unfolding is highlighted. Based on the role of HSPA5 in ER stress response, it seems that saffron not only acts as a protective factor against IR injury but also it is an anti-stress nutrient versus different kinds of stresses.
HSPA9 like HSPA5 belongs to the HSP70 family [23]. Association between HSPA9 expression and several diseases such as liver cancer, thyroid carcinoma, inflammatory diseases, and Parkinson's disease is studied and confirmed [24-27]. It can be concluded that regulation of HSPA9 expression by saffron like regulation of HSPA5 expression is an essential process which increases body resistance against stress and diseases.
Cu, Zn-superoxide dismutase (SOD1) is an enzyme which hunts superoxide ions. Research indicated that it is responsible to protect mitochondria against oxidative stress which may lead to different types of diseases such as motor neuron disease and vascular degeneration [28, 29]. Sau et al. reported that there is a correlation between amyotrophic lateral sclerosis and SOD1 mutation [30]. Regulatory effect of saffron on SOD1 expression in response to diabetes is reported by Margaritis et al [31]. Bernadett Kalmar and Linda Greensmith suggested that VCP, HSPA5, HSPA9, and SOD1 can be considered as therapeutic targets in treatment of amyotrophic lateral sclerosis [32].

Conclusion

Our analysis led to introducing VCP, HSPA5, HSPA9, and SOD1 as central regulated proteins by saffron in response to IR injury. The findings indicated that saffron activates protective cellular process against stress condition which includes ER and mitochondria function; however, more investigations considering various dosages of saffron are required to confirm complete concept of findings.

Acknowledgments

This project was supported by Shahid Beheshti University of Medical Sciences.

Author contributions

Mostafa Rezaei Tavirani designed and supervised the study; Babak Arjmand, Mahmood Khodadoost, Mohhamadreza Razzaghi, Alireza Ahmadzadeh and Sina Rezaei Tavirani were involved in data collection and analysis. All authors approved the final draft 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

IR: ischemia reperfusion; DEPs: differentially expressed proteins; PPI: protein-protein interaction; BC: betweenness centrality; CC: closeness centrality; ER: endoplasmic reticulum