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Original article

Prediction of Coffee Effects in Rats with Healthy and NAFLD Conditions Based on Protein-Protein Interaction Network Analysis

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Abstract

Background and objectives: Non-alcoholic fatty liver disease (NAFLD) is a common liver condition. On the other hand, coffee consumption has shown promising for gastrointestinal diseases. Detection of the most valuable biomarkers of decaffeinated coffee treatment in healthy and non-alcoholic fatty liver disease conditions was the aim of the present study. **Methods:** A previous proteomics study about effect of decaffeinated coffee (1.5 mL daily drinking coffee for two months) on protein expression change of rat liver was selected for protein-protein interaction (PPI) network analysis via Cytoscape v.3.7.1 and the related applications. The most central proteins with regards to a high degree and betweenness centralities in the coffee treatment condition of healthy and NAFLD were then analyzed by ClueGO for biological process (BP) derivation. **Results:** HSPA5, HSPA4, HSPA9, HSPA7, PARK7, HSP90AA1, P4HB, PRDX1, and PDIA3 were introduced as central proteins, which are involved in folding and antioxidant activities. **Conclusion:** There is a complicated combination of the components in coffee; some elements are involved in liver protection against NAFLD and the others are in contrast.

Keywords: non-alcoholic fatty liver disease; protein; protein interaction maps; rats

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Introduction

Coffee is one of the most consumed hot beverages around the world [1]. During the last decades, it has been established that coffee exerts several beneficial effects on human health such as decreasing the risk of cardiovascular diseases, cancer and metabolic diseases including liver disturbances [2]. While, older investigations showed that high consumption of unfiltered coffee are associated with increasing liver enzyme levels [3], recent studies suggest that regular consumption of coffee may act as protective in the development of non-alcoholic fatty acid disease (NAFLD) and fibrosis [4-6]. Coffee includes phenolic compounds and their derivatives (chlorogenic acids), alkaloids (caffeine), diterpenoid alcohols (cafestol and kahweol), carbohydrates, lipids, and volatile and heterocyclic compounds [7]. It has been proposed that the caffeine content of coffee may be critical in the beneficial effects of coffee on liver health

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[8,9]; however, as other caffeine-rich beverages lack similar beneficial effects [10], it has been proposed that compounds such as polyphenols or melanoidins may also be involved in liver effects of coffee [9-11]. Despite diverse efforts to identify compounds and unravel mechanisms involved in the coffee properties, up to now, neither is fully distinguished. NAFLD is one of the most important diseases due to its potential risk for cancer. In fact, it is accounted as a prominent liver cancer causing in the U.S. population [12]. NAFLD has the prevalence of approximately 25% of populations in the world [13]. Diet could be a key factor in NAFLD development and treatments [14]. Epidemiological reports are available concerning NAFLD risk reduction by coffee consumptions in human [15]. This metabolic disease suffers from effective therapy; therefore, exploring underlying molecular mechanisms could be beneficial for understanding and management purposes of this disease [13]. One of the trustworthy molecular analysis is proteomics evaluation of a disease condition. In this way, molecules called biomarkers that are fundamentally changed in expression in a disease condition could be detected. Additionally, this approach could assist understanding the role of applied treatments and affected molecules [16]. Complementary bioinformatics analysis could offer more notion of the introduced biomarkers of the interested condition of the study [17]. Protein-protein interaction network construction as a bioinformatics method provides essential information with regards to protein interactions pattern [18]. Biomarkers with central properties in these interactions could be selected as elements that are more valuable. Previously, a proteomics study demonstrated that coffee alerts the levels of many proteins in healthy and NAFLD rats [19]. Accordingly, we analyzed differentially expressed proteins of samples of healthy and NAFLD rats treated with decaffeinated coffee to achieve information related to the effect of coffee elements on prominent genes. It is possible that a new perspective of coffee consumption would be identified.

Material and Methods

Ethical considerations

Shahid Beheshti University of Medical Sciences approved this research with the code of IR.SBMU.RETECH.REC.1398.061 on 2019-05-05.

Pharmacological design

The original proteomics study [19] was two dimensional electrophoresis based in combination with mass spectrometry to analysis the differential expressed proteins (DEPs) in healthy and none alcoholic fatty liver disease (NAFLD) rats fed with coffee. Twenty-four male Wistar rats were divided in two categories (n=6). The first category includes two groups of fed with (HDF) for three months that after passing one month, one group was treated with decaffeinated coffee and the other one with water. The procedure of treatment was the same for the category of standard diet (SD) that was also consist of two groups.

The fed HFD category was considered as NAFLD rats and the second ones as healthy samples. After a month of feeding one group of the first and the second 2 groups drank decaffeinated coffee and the other water, respectively. Daily dose of 1.5 mL per a rat equal to 2 cups of filtrated coffee per 70 kg weight of a user was applied. Protein composition of liver tissue was analyzed by two-dimensional polyacrylamide gel electrophoresis (2D-PAGE). More explanations of materials and methods are available in the original document of Salomone, F., et al [19]. Since the protecting property of coffee against NAFLD is accepted; in the present study, the differentially expressed proteins (DEPs) in coffee intake groups relative to the SD drank water group which are characterized by equal expression pattern in two HFD groups were considered for further evaluations namely protein-protein interaction (PPI) network analysis.

Protein-protein interaction network analysis

First, Uniprot database (https://www.uniprot.org/) was used for gene name search of identified DEPs and then names were used for network constructions in the Cytoscape v.3.7.1[20]. The first network query (genes without additional neighbors) shows how much the genes are integrated as a map. The second network indicates the interactions between these genes and their surrounded genes. The neighbor genes were selected from STRING database. STRING has an option that selection of numbers of additional genes is possible for the query data.

The second network was then applied for network centrality identification in which the degree (K) and betweenness centrality (BC) as the most highlighted network centrality parameters [21] were considered in this study. Genes with highest K and BC are called hubs and bottlenecks, respectively. In addition, those genes that have the highest score of the mentioned parameters together are hub-bottlenecks [22]. To get more information related to the central nodes, functional analysis in terms of biological process (BP) identification of genes with high K and BC were considered. The application conducting this query was by ClueGO+ CluePedia [23]. This plug-in is provided by Cytoscape and can provide annotations including pathway analysis and gene ontology. Gene ontology (GO) includes molecular function (MF), cellular component (CC), and biological process (BP). GO information for many organisms including *Rattus norvegicus* are available and can be downloaded in ClueGO app. In here, biological process data for the designated genes were assessed through the mentioned application. The statistical criteria are as follow: the adjusted kappa score level threshold for grouping the terms of BPs was set to 0.5. This scoring is from 0 to 1. Number and percentage of genes associated with a term was assigned as minimum of 3 and 4, respectively. This criteria for hub-bottleneck assessments was determined differently as minimum of 2 and 3. P-value correction method was as default (Bonfferoni step down). Moreover, the biological terms that are possessing hub-bottlenecks were then assigned and explored in details.

Results and Discussion

The world coffee consumption has been growing for its suitable taste and beneficial effects on human health. This favorable beverage is rich in caffeine and polyphenolic compounds. These two groups of components along with other constituents play critical role in coffee properties [24].

Building PPI network allows detecting possible highlighted targets of applied treatments such as natural materials. The mechanism by which coffee produces health benefits in NAFLD can be better explained by proteomics as a molecular study and the complementary bioinformatics analysis. In this work, it is aimed to prioritize targets of coffee treatment based on interaction and centrality analysis in a PPI network.

Numbers of 11 spots in coffee treated samples were detected as DEPs in the original study. Among them, there were two proteins with two isoforms, and therefore, nine proteins undergone network analysis in our study. The gene names related to the nine proteins were searched from Uniprot database (table1). The query of nine genes for the genus *Rattus norvegicus* result in two networks, one with only the nine individuals and the second with additional neighbors of 50. This network search was handled by STRING Plug-in. (see figures 1 and 2). Addition of neighbor genes helped individual gene (those not in the connections including Gsta2, Etfa, and Sh3bp5) to get in connection with other query genes in figure 2.

Table 1. Differentially expressed proteins between HFD+water and HFD+coffee for *Rattus norvegicus* (Rat). Pdia3 shows both up-regulation and down-regulation in coffee treatment samples.

Protein name	Gene Name	Protein AN	Coffee treatment
Protein disulfide-isomerase A3	Pdia3	P11598	Up-regulated
D-dopachrome decarboxylase	Ddt	P80254	Up-regulated
Protein DJ-1	Park7	O88767	Up-regulated
Glucose-regulated protein, 78 kDa	Hspa5	P06761	Up-regulated
Peroxiredoxin 1	Prdx1	Q63716	Up-regulated
Electron transfer flavoprotein subunita	Etfa	P13803	Down-regulated
Mitochondrial heat shock protein 70	Hspa9	P48721	Up-regulated
GlutathioneS-transferasea2	Gsta2	P04903	Up-regulated
SH3 domain-binding protein 5	Sh3bp5	Q91Y80	Up-regulated

Using Cytoscape and STRING database, the network analysis indicated that not all the genes were in direct connections and addition of close genes could promote indirect interactions between the query genes. Centrality analysis of the second network shows among DEPs which ones are more dominant in terms of interactions. Moreover, new candidates from additional neighbor genes could be suggested through this analysis. Here, the 20% of highest ranked genes based on degree and betweenness centrality (about 12 genes of the network of 59 nodes) have been assigned in tables 2 and 3, respectively.

Among tables of 2 and 3, there are some common genes that are assigned as hub-bottlenecks with a star in the first table. These genes are Hspa4, Hspa5, Hspa9, Park 7, Hsp90aa1, P4hb, Prdx1, Pdia3, and Hspa1. In each table, five genes belong to the query elements, separately.

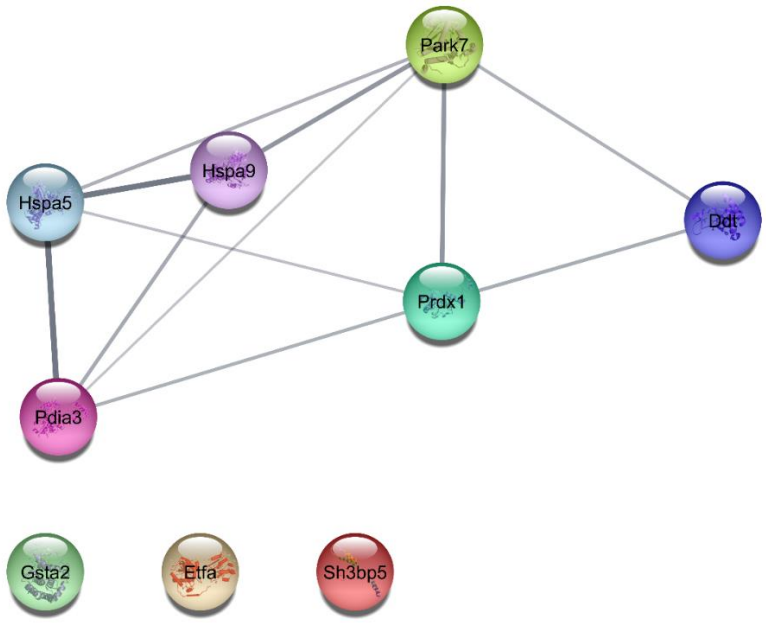

Figure 1. A network of nine genes with 11 links in the first query. Three isolated ones appeared. Confidence score cut off =0.4; maximum additional interactors: 0

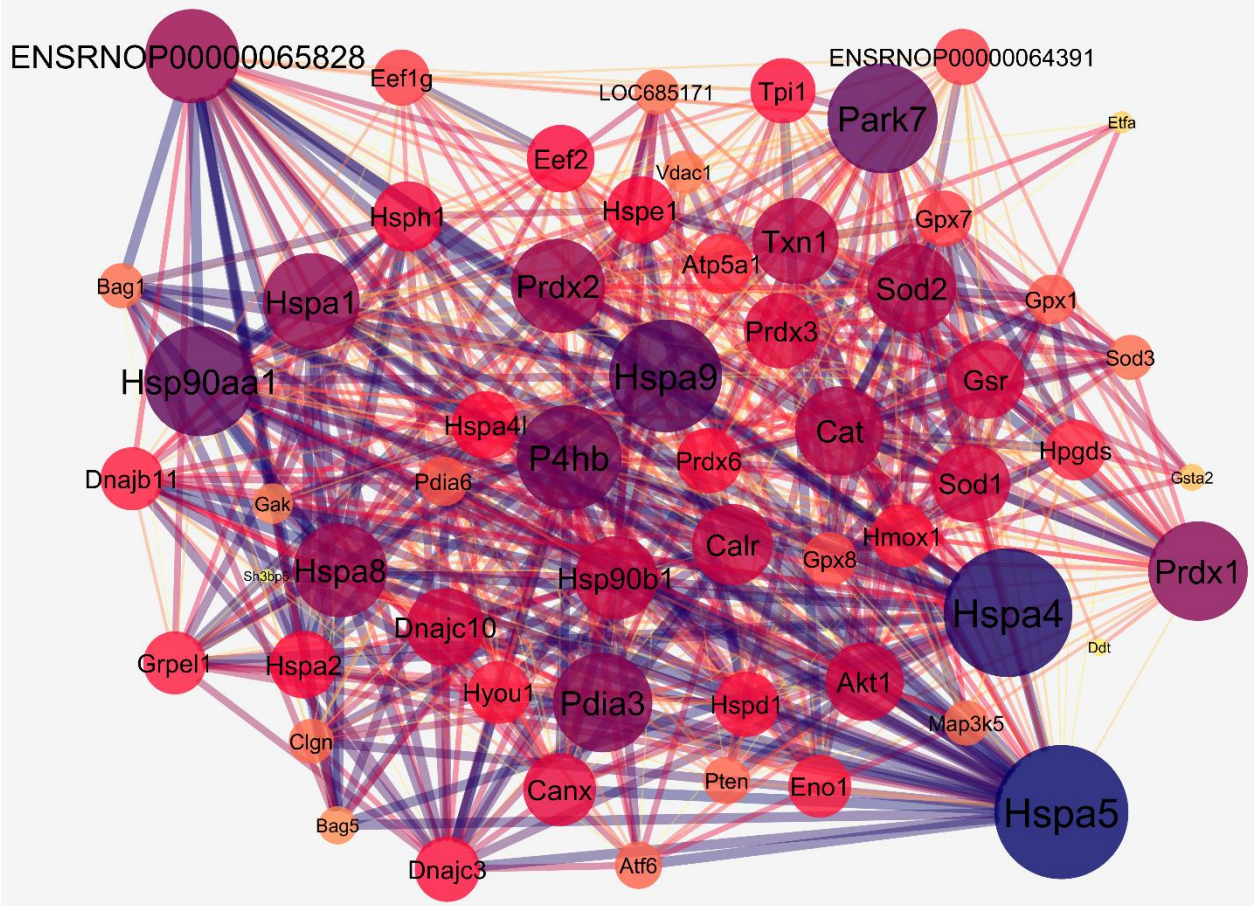

Figure 2. A network of 59 genes with 731 links; confidence score cut off =0.4; maximum additional interactors: 50; the network are labelled, colored, and sized based on degree value. The bigger and darker, the higher the degree value. ENSRONOP00000065828 and ENSRONOP00000064391828 are two uncharacterized proteins.

Table 2. The list of hubs of the second network have been ranked based on K scores. K and BC refer to degree and betweenness centrality. The asterisked ones are common between tables 2 and 3.

Display name	K	BC	Query term
Hspa5*	**50**	0.06	Hspa5
Hspa4*	**48**	0.05	
Hspa9*	**42**	0.03	Hspa9
Park7*	**41**	0.05	Park7
Hsp90aa1*	**41**	0.03	
P4hb*	**39**	0.02	
Prdx1*	**37**	0.03	Prdx1
Pdia3*	**37**	0.02	Pdia3
Hspa1*	**36**	0.02	
Prdx2	**35**	0.02	
ENSRNOP00000065828	**35**	0.01	
Hspa8	**35**	0.01	

Table 3. The list of bottlenecks of the second network that are ranked based on BC scores

Display name	BC	K	Query term
Hspa5	**0.06**	50	Hspa5
Park7	**0.05**	41	Park7
Hspa4	**0.05**	48	
Map3k5	**0.04**	15	
Prdx1	**0.03**	37	Prdx1
Hspa9	**0.03**	42	Hspa9
Hsp90aa1	**0.03**	41	
P4hb	**0.02**	39	
Sod2	**0.02**	33	
Cat	**0.02**	33	
Hspa1	**0.02**	36	
Pdia3	**0.02**	37	Pdia3

The highest score for hubs is 50 and the highest for bottlenecks is 0.06. Prdx2, ENSRNOP00000065828, Hspa8, Sod2, Cat, and Map3K5 are none-common genes. Gsta2, Etfa, and Sh3bp5, the indivuial genes in the figure 1, are neither among the hubs nor bottlenecks. ENSRNOP00000065828 in the hub category is an uncharacterized gene and has sequence similarities to heat shock protein 70 family. Among 9 hub-bottlenecks, there are five query proteins including Hspa5, Hspa9, Park 7, Prdx1, and Pdia3.

Analyzing centrality features of the second network identified genes with prominent role in the network strength. These nodes are both from query genes and added genes from the network construction. More than half of the hub-bottleneck list is enriched with query genes (DEPs). These hub-bottleneck DEPs are Hspa5, Hspa9, Park7, Prdx1, and Pdia3. All of the hub-bottleneck DEPs showed up-regulation in the presence of coffee from the proteomics study [1]. However, the latest one, Pdia3 showed both up-regulation and down-regulation in coffee treatment. While Hspa4, Hsp90aa1, P4hb, and Hspa1 are the other hub-bottlenecks that are not from query elements, they could also have essential contribution in the network built. About 60% of hub list is defined with heath shock protein family (HSPs) members including 70 and 90 KDs. This rate is lower in the bottleneck category, which is equal to 41%. As it is shown in the table 2, hub-bottlenecks include 5 heat shock proteins and Park7, P4hb, Prdx1, and Pdia3. The identified heat shock proteins as hub-bottlenecks are mainly members of Hspa family. Prolyl4-hydroxylase subunit beta (P4hb) is a hub-bottleneck from the added 50 neighbor genes. Significant role of Hspa (Hsp 70) in NAFLD is reported [25]. Based on this document, Hsp 70 is up-regulated in the liver of obese mice. Investigation showed that palmitic acid induces upregulation of Hsp 70 in the HepG-2 cells. As it is reported, increment of total cholesterol and triglyceride are observed after over-expression of Hsp 70. Up-regulation of several lipogenic genes including ACC, FAS, and SCD by up-regulated Hsp 70 gene is highlighted which indicated to promote lipogenesis [25]. A study by Matthew c. Wheeler and Nicholas Gekakis showed that Hsp 90 up-regulation induces increased neutral lipid accumulation in cell line. In this research significant role of Hsp 90 in development of NAFLD has been highlighted [26]. Peroxiredoxin 1 (PRDX 1) the other hub-bottleneck protein is an antioxidant protein which inhibits JNK activation. Role of JNK in NASH pathogenesis is reported by Singh R et al [27-29]. P4hb plays a role as a chaperon and is involved in inhibition of aggregation and misfolding. This protein is known as an oncogene reagent. Findings indicate that P4hb is up-regulated in several cancers such as hepatocellular carcinoma [30]. Role of decaffeinated coffee in protection liver against NAFLD can be interpreted based on presence of antioxidants and the possible similar elements in the coffee.

Biological process (BP) annotations of the hubs and bottlenecks individually were performed by ClueGO+CluePedia plug-ins (figure 3). The BP related to the hubs and bottlenecks are cluster in

the 4 and 7 groups, respectively. More analysis revealed that the hub-bottleneck genes are involved in 2 BPs which are presented in the table 4. In BP analysis, for the first table query (A), Chaperone cofactor-dependent protein refolding, removal of superoxide radicals, regulation of oxidative stress-induced cell death, and cell redox homeostasis were the corresponding terms.

In the second table query (B), hydrogen peroxide metabolic process, chaperone cofactor-dependent protein refolding, response to increased oxygen levels, intrinsic apoptotic signaling pathway in response to oxidative stress, response to cold, response to ischemia, and cell redox homeostasis are identified.

Table 4. The list of significant biological process groups contributing with hub-bottlenecks

Biological process	Hub-bottleneck Name
Chaperone cofactor-dependent protein refolding group	Hspa1a, Hspa4, Hspa5, Hspa9, Hsp90aa1
Cell redox homeostasis	P4hb, Pdia3, Prdx1

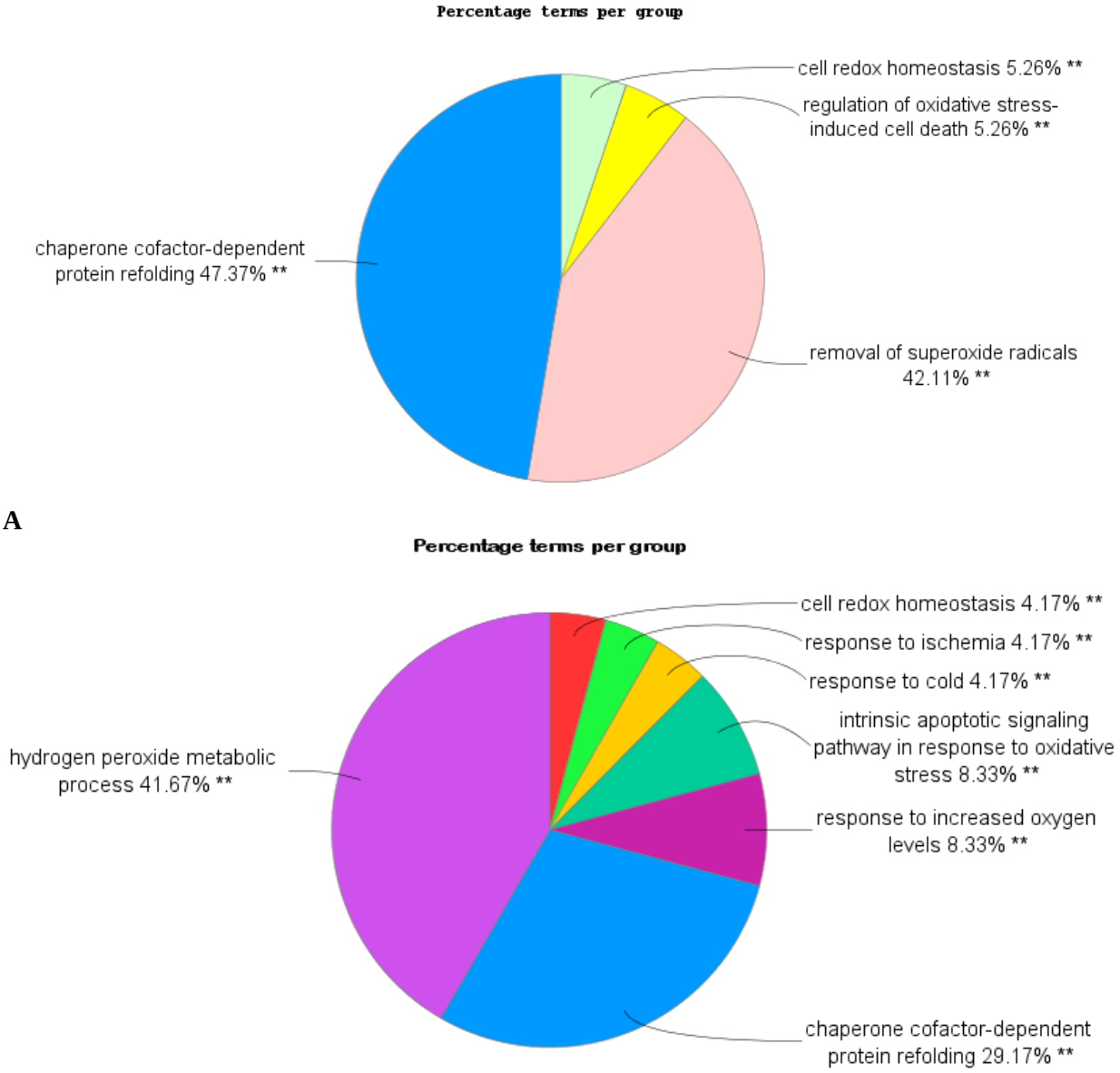

Figure 3. Pie chart of biological processes involved in hubs and bottlenecks, A and B, respectively. The labels are for the most highlighted terms for each groups of terms.

Gene ontology as a useful method to identify role of the genes led to introduce folding and antioxidant activities for the central nodes. As it is mentioned, all identified chaperons are up-regulated and promote NAFLD in the reported samples. This is a complicated finding, because it is confirmed that coffee and caffeine are protectors against NAFLD [31-33]. As it is depicted in figure 3 versus chaperon cofactor dependent protein refolding processes there are removal of superoxide radicals and hydrogen peroxide metabolic processes which act as antioxidants. Percentage of terms that are categorized under name of chaperon cofactor dependent protein refolding are about 49% and 29% for hubs and bottlenecks, respectively. It means that hubs are mainly involved in this process. On the other hand, the bottlenecks are related to the antioxidant and response to stress activities (figure 3-B). It is necessary to consider that decaffeinated coffee contains small amounts of caffeine [34] but this amount is very low to induce protective effects in NAFLD mice as shown in other studies. Recent data suggested that polyphenolics and melanoidins formed during roasting process may also impact health effects of coffee [35]. During an investigation, it has been reported that the effects of both polyphenols and melanoidins extracted from decaffeinated coffee were almost comparable to the protective effects of whole decaffeinated coffee on the development of a high fat diet induced NAFLD [32]; therefore, it could be concluded that different coffee components other than caffeine are involved in the observed effect in this study, but the identification of role of each one is impossible. Some can protect liver from progress of NAFLD and some others promote lipogenic activity. In the case of NAFLD, net effect is to decrease its promotion.

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Author contributions

Homa Hajimehdipoor Participated in plant related parts; Majid Rezaei-Tavirani, Mostafa Rezaei Tavirani, Mona Zamanian Azodi and Zahra Akbari designed and supervised the study, conducted the experiments, and analyzed the data. All authors approved the final version 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

NAFLD: non-alcoholic fatty acid disease; PPI: protein-protein interaction; BP: biological process; DEPS: differential expressed proteins; high HFD: high fat diet; SD: standard diet; 2D-PAGE: two-dimensional polyacrylamide gel electrophoresis; GO: gene ontology; MF: molecular function; CC: cellular component